

# Persistence of virus-neutralizing antibodies in horses inoculated with two doses of a live equine herpesvirus type 1 vaccine with different vaccination intervals

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*The antibody response in horses inoculated with 2 doses of a live equine herpesvirus type 1 vaccine with different vaccination intervals (1 to 3 months) was evaluated with regard to the persistence of virus-neutralizing (VN) antibodies. The durations for which the geometric mean VN titers were maintained significantly higher than those before the first vaccination ( $P < 0.05$ ) were up to 5 months in horses that received the vaccination with a 1-month interval ( $n = 17$ ) and 7 months for those that received it with a 2-month ( $n = 17$ ) or 3-month interval ( $n = 14$  or  $17$ ). The vaccination program with the 2-month interval was the most effective in maintaining VN antibodies for a long duration with the smallest gap of antibody decline between the first and second vaccinations.*

**Key words:** equine herpesvirus type-1, persistence, vaccination interval, virus-neutralizing antibody

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Infection with equine herpesviruses type 1 (EHV-1) induces respiratory illness in young horses, and in winter, it is a major cause of pyrexia in Japanese racehorses [6, 7]. To control epizootic EHV-1 infection in winter, a modified live EHV-1 vaccine (Equi N Tect ERP, Nisseiken Co., Ltd., Tokyo, Japan) has been used in two training centers of the Japan Racing Association (JRA) [5]. Each of these centers accommodates approximately 2,000 racehorses, and the majority of the horses are introduced into the facilities at 2 years old. The vaccine contains a live EHV-1 virus that lacks the *glycoprotein E* gene ( $\Delta gE$ -NIBS strain  $\geq 10^{4.5}$  tissue-culture infective dose<sub>50</sub>/dose) and does not contain an adjuvant, and the manufacturer recommends inoculation of horses with 2 doses of the vaccine intramuscularly with a 3-week interval. At JRA's training centers, 2-year-old horses receive a priming course of EHV-1 vaccination that consists of 2 doses with an approximately 1-month interval,

and 3-year-old or older horses receive 1 annual booster dose [5]. To cover the period from winter to early spring, which is recognized as a potential epizootic season for EHV-1 infection, the vaccination period starts in November and finishes at the end of April [5]. Horses at the training centers are replaced according to their racing and resting schedules, and newly introduced horses receive the EHV-1 vaccine at the earliest opportunity during their stay at the training centers. Although EHV-1 infections have been well controlled in recent years, especially after the inactivated vaccine was replaced with a live vaccine [5], periodic surveillance and update of vaccination programs are vital for continuous disease control.

A previous study showed a strong antibody response in 2-year-old horses vaccinated with Equi N Tect ERP in accordance with the current vaccination program described above [5]. In that report, a significant rise in geometric mean (GM) virus-neutralizing (VN) titers (from 30–38 to 205–220) was observed after the first vaccination, and a similar level of titers (214–260) was maintained at 1 month after the second vaccination [5]. However, the titers at 3 months after the first vaccination, the endpoint of sample collection in that study, were 162–165, which were much lower than the peak values [5]. Therefore, the persistence of VN antibodies raised after the current vaccination program

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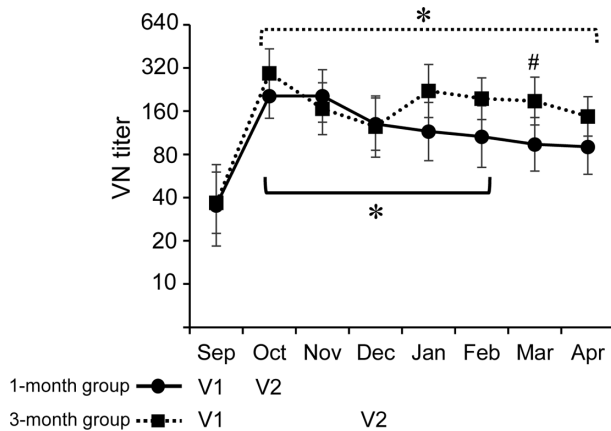
with a 1-month interval was not clear due to the lack of data for the later time point, and time may have been insufficient to cover the epizootic period. In this study, to achieve long-lasting immunity with EHV-1, we explored the potential effect of different vaccination intervals (1 to 3 months) on antibody levels and persistence.

All the experiments were performed according to the Guiding Principles for Animal Experiments issued by the Equine Research Institute, and the ethics of this study were approved by the Equine Research Institute's Committee for Promotion and Research Ethics (identification number 2018-3263-07). One-year-old Thoroughbred horses kept at the JRA's Hidaka Training and Research Center (Urakawa, Japan) were used. They had not received any EHV-1 vaccine before the experiment. In experiment 1, which was conducted in 2018–2019, serum samples were collected from 56 horses when the first vaccination was performed. The VN titer against EHV-1 was measured with a focus-reduction method using Madin-Darby bovine kidney (MDBK, ATCC #CCL-22) cells and EHV-1 strain 89C25p, with an initial starting dilution of serum samples of 1:10, as described previously [3]. The VN titer was the reciprocal of the highest dilution that reduced the number of foci by more than 50% compared with that of the control. Based on the VN titers, horses were allocated to two groups with different vaccination intervals, namely a 1-month group and a 3-month group ( $n=28$ , each), so that both groups had similar levels of GM titers against EHV-1 at the start of the experiment. Likewise, in experiment 2, which was conducted in 2019–2020, 60 horses were allocated to two groups with different vaccination intervals, namely a 2-month group and a 3-month group ( $n=30$  each). They were inoculated twice with Equi N Tect ERP (Nisseiken Co., Ltd.) intramuscularly at the specified vaccination intervals. The first vaccination was performed in September in both experiments, and serum samples were collected at approximately 1-month intervals thereafter until the following April. To exclude horses naturally infected with EHV-1 or EHV-4 during the experiment, the sera were tested with a glycoprotein E1-ELISA [2] and glycoprotein G4-ELISA [4], which detect antibodies raised after infection with EHV-1 and EHV-4, respectively, and not after EHV-1 vaccination. Horses that exhibited no seroconversion in either of these tests were selected for the measurement of VN titers for EHV-1. As a result, 17 horses were subjected to the VN test for both the 1-month and 3-month groups in experiment 1; in experiment 2, 17 were tested for the 2-month group, and 14 were tested for the 3-month group. The VN titers in each group at each time point after vaccination were compared with those before vaccination, using Tukey's honestly significant difference test. To compare titers between groups in the same experiment, a one-way repeated measure analysis of variance was performed

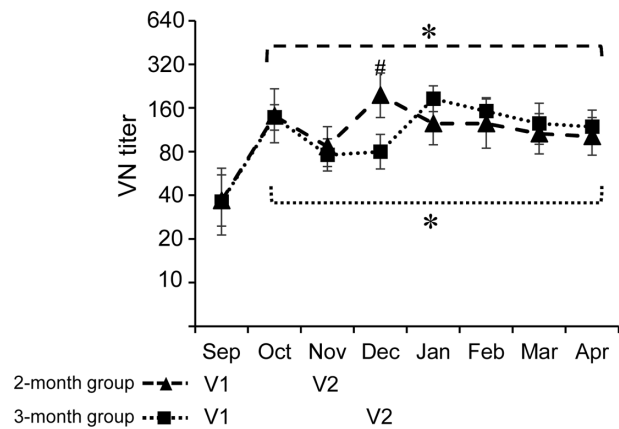
followed by a *post hoc* simple main effect test. The analyses were performed with JMP 15 (SAS Institute Inc., Cary, NC, U.S.A.). A level of  $P<0.05$  was considered significant.

In experiment 1, the horses received 2 vaccinations with either a 1-month ( $n=17$ ) or 3-month interval ( $n=17$ ). Both groups showed similar levels of GM titers before the first vaccination, with the levels rising significantly 1 month after the first vaccination: from 35 (95% confidence interval [CI]: 18–68) to 204 (95% CI: 143–292) in the 1-month group and from 37 (95% CI: 23–60) to 295 (95% CI: 200–435) in the 3-month group ( $P<0.05$ ; Fig. 1). The 1-month group showed a similar VN titer level (204, 95% CI: 134–312) in November, 2 months after the first vaccination, with the level declining gradually thereafter: 130 (95% CI: 86–198) in December, 115 (95% CI: 72–184) in January, 106 (95% CI: 65–174) in February, 94 (95% CI: 61–145) in March, and 90 (95% CI: 58–141) in April (Fig. 1). In contrast, the 3-month group showed an obvious increase in GM titer level after the second vaccination, from 125 (95% CI: 77–205) in December to 222 (95% CI: 145–339) in January (Fig. 1). Although GM titers in the 3-month group declined gradually between February and April from 196 (95% CI: 140–274) to 147 (95% CI: 107–203), they were relatively higher than those in the 1-month group. A statistically significant difference between groups at each time point was detected only in March ( $P<0.05$ ; Fig. 1). The durations for which the GM titers remained significantly higher than those before the first vaccination ( $P<0.05$ ) were up to 5 months and 7 months in the 1-month and 3-month groups, respectively (Fig. 1).

In experiment 2, the horses received 2 vaccinations with either a 2-month ( $n=17$ ) or 3-month interval ( $n=14$ ). Both groups showed similar levels of GM titers before the first vaccination, with the levels rising significantly 1 month after the first vaccination: from 37 (95% CI: 25–55) to 142 (95% CI: 92–217) in the 2-month group and from 36 (95% CI: 21–61) to 138 (95% CI: 113–169) in the 3-month group ( $P<0.05$ ; Fig. 2). The GM titers just before the second vaccination were 87 (95% CI: 63–119) and 80 (95% CI: 61–105) in the 2-month group in November and 3-month group in December, respectively, and these values were slightly lower than those in October (Fig. 2). After the second vaccination, both groups exhibited an obvious booster reaction, with increased titers of 196 (95% CI: 136–279) and 186 (95% CI: 152–227) in the 2-month group in December and 3-month group in January, respectively (Fig. 2). From February to April, the titers in both groups declined gradually, from 125 (95% CI: 85–185) to 102 (95% CI: 76–138) in the 2-month group and from 152 (95% CI: 123–189) to 119 (95% CI: 91–155) in the 3-month group, although they were still significantly higher than those before the first vaccination ( $P<0.05$ ; Fig. 2). Therefore, the VN antibodies in these two groups persisted up to 7 months



**Fig. 1.** Geometric mean virus-neutralizing titers against equine herpesvirus type-1 in horses vaccinated twice, with either a 1- or 3-month interval, in experiment 1. Horses in the 1-month group ( $n=17$ , solid line with circle) received equine herpesvirus type 1 (EHV-1) vaccinations in September (V1) and October (V2), and those in the 3-month group ( $n=17$ , dotted line with square) received EHV-1 vaccinations in September (V1) and December (V2). Error bars, 95% confidence interval. \*Titers are significantly higher than that in September in the same group ( $P<0.05$ ). #Significant difference in titers between groups at the same time point ( $P<0.05$ ).



**Fig. 2.** Geometric mean virus-neutralizing titers against equine herpesvirus type-1 in horses vaccinated twice, with either a 2-month or 3-month interval, in experiment 2. Horses in the 2-month group ( $n=17$ , dashed line with triangle) received equine herpesvirus type 1 (EHV-1) vaccinations in September (V1) and November (V2), and those in the 3-month group ( $n=14$ , dotted line with square) received EHV-1 vaccinations in September (V1) and December (V2). Error bars, 95% confidence interval. \*Titers are significantly higher than that in September in the same group ( $P<0.05$ ). #Significant difference in titers between groups at the same time point ( $P<0.05$ ).

(Fig. 2). A significant difference between groups in terms of GM titers at each time point was detected only in December ( $P<0.05$ ), and this probably reflected the different timing of the second vaccination (Fig. 2).

The VN antibody is considered to play a role in reducing virus excretion during EHV-1 infection and may reduce the duration and severity of disease outbreaks in horse populations [1]. However, unlike the immunity to equine influenza virus and some other pathogens, the protective level of antibody titer against EHV-1 infection is not known. Therefore, in this study, the persistence of immunity was evaluated based on the period during which the GM titer was significantly higher than before the first vaccination. As observed in experiment 1, horses that received vaccination with a 1-month interval did not exhibit an obvious booster response after the second dose, although the antibody titer was maintained in the short term at a level similar to that before inoculation with the second dose. The antibody level declined to the baseline by March, 6 months after the first inoculation (Fig. 1). In contrast, vaccination with a 3-month interval was effective in maintaining a significantly higher antibody titer until April, the end point of the experiment, than that before vaccination (Fig. 1). When we compared the 2-month and 3-month intervals in experiment 2, there was no difference in the persistence of the VN antibodies, and horses in both groups maintained significantly higher

titers until April than those before vaccination (Fig. 2). Therefore, vaccination programs with a 2- or 3-month interval were superior to one with a 1-month interval in terms of the persistence of VN antibodies. Additionally, in the light of the shorter period with a temporal decline in VN antibodies before the second vaccination, a 2-month interval would be more beneficial than a 3-month interval.

In conclusion, for EHV-1 vaccination using Equi N Tect ERP, the program with 2 vaccination doses with a 2-month interval was the most effective in maintaining VN antibodies for a long duration with the smallest gap of antibody decline between the first and second inoculations.

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