Successful Control of Winter Pyrexias Caused by Equine Herpesvirus Type 1 in Japanese Training Centers by Achieving High Vaccination Coverage

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Equine herpesvirus type 1 (EHV-1) is a major cause of winter pyrexia in racehorses in two training centers (Ritto and Miho) in Japan. Until the epizootic period of 2008–2009, a vaccination program using a killed EHV-1 vaccine targeted only susceptible 3-year-old horses with low antibody levels to EHV-1 antigens. However, because the protective effect was not satisfactory, in 2009–2010 the vaccination program was altered to target all 3-year-old horses. To evaluate the vaccine’s efficacy, we investigated the number of horses with pyrexia due to EHV-1 or equine herpesvirus type 4 (EHV-4) infection or both and examined the vaccination coverage in the 3-year-old population and in the whole population before and after changes in the program. The mean (± standard deviation [SD]) estimated numbers of horses infected with EHV-1 or EHV-4 or both, among pyretic horses from 1999–2000 to 2008–2009 were 105 ± 47 at Ritto and 66 ± 44 at Miho. Although the estimated number of infected horses did not change greatly in the first period of the current program, it decreased from the second period, with means (±SD) of 21 ± 12 at Ritto and 14 ± 15 at Miho from 2010–2011 to 2012–2013. Vaccination coverage in the 3-year-old population was 99.4% at Ritto and 99.8% at Miho in the first period, and similar values were maintained thereafter. Coverage in the whole population increased more gradually than that in the 3-year-old population. The results suggest that EHV-1 epizootics can be suppressed by maintaining high vaccination coverage, not only in the 3-year-old population but also in the whole population.

Equine herpesviruses type 1 (EHV-1) and type 4 (EHV-4) are major causative agents of respiratory disease in horses, and EHV-1 infection can also lead to neurological disease and abortion (1). These two viruses have extensive genomic sequence identity and have antigenic cross-reactivity (1). Vaccination is considered to be an important control measure for EHV-1- and EHV-4-induced diseases. Some commercial vaccines have been shown to contribute to protection from EHV-related respiratory disease, neurological disease, and abortion under experimental conditions (2–4). Many reports have described the serological responses of vaccinated horses in the field (5–8), and vaccine use is considered to be partially responsible for a reduction in the incidence of equine abortion (9–12). However, the effect of vaccination on the prevalence of respiratory disease induced by EHV-1 and EHV-4 in the field has not been investigated in detail.

Winter pyrexia caused by EHV-1 epizootics in the training centers of the Japan Racing Association is an important condition that needs to be controlled, because it can delay training schedules and cause horses to be scratched from races (13). The spread of EHV-1 in the training centers may be due to the fact that, in these centers, >2,000 racehorses are kept in close contact and are subjected to stress from cold weather and hard training. In Japan, the horses are introduced into the training centers at the age of 2, and most of them become infected with EHV-1 for the first time during their first winter there (14). In contrast, almost without exception, the horses in the training centers are already infected with EHV-4 before their introduction, because this virus is epizootic on breeding and rearing farms (14). EHV-4 infection among the racehorses in the training centers is generally sporadic and is only rarely associated with pyrexia (14).

During the epizootic of 1994–1995, an inactivated vaccine for EHV-1 (Nisseiken, Tokyo, Japan) was introduced into the training centers as a control measure for the epizootic. Because the majority of the EHV-1-induced pyrexia was observed in the 3-year-old horses (14), the vaccination program aimed to protect this population. The vaccination target was not all 3-year-old horses, but only those that had relatively low antibody levels against EHV-1 antigens. However, the protective effect was not satisfactory, and winter pyrexia was still observed in large numbers of horses after the introduction of the program. In 2009–2010, therefore, the vaccination program was altered, and all horses in the 3-year-old population were included as vaccination targets.

Here, we evaluated the efficacy of the current vaccination program by investigating the EHV-1 epizootic and vaccination coverage in the training centers before and after the program was changed, along with the antibody responses in the vaccinated horses.

MATERIALS AND METHODS

Horse populations. The study horses were trained in either of the two training centers (Ritto and Miho) of the Japan Racing Association. The Ritto training center is in Shiga Prefecture in western Japan, and the Miho training center is in Ibaraki Prefecture in eastern Japan. More than 2,000 racehorses are kept in close contact and are subjected to stress from cold weather and hard training. In Japan, the horses are introduced into the training centers at the age of 2, and most of them become infected with EHV-1 for the first time during their first winter there (14). In contrast, almost without exception, the horses in the training centers are already infected with EHV-4 before their introduction, because this virus is epizootic on breeding and rearing farms (14). EHV-4 infection among the racehorses in the training centers is generally sporadic and is only rarely associated with pyrexia (14).
Racehorses are trained in each center, and about 800 to 1,200 of the horses are replaced with new ones every month. The horses generally stay in the training centers for 1 to 6 months for training and racing. After they leave the training centers, they are usually kept in the other farms for several months for conditioning and then reenter the training centers. The age distributions of the horses in the training centers on 1 January of each year are shown in Fig. 1.

**Period of investigation.** The EHV-1 epizootic usually occurs from December to April, so epizootic periods are designated here as 1999-2000, 2000-2001, and so on to cover this period each year. In the same way, we designated horses that reached the age of 3 years in January as the 3-year-old population, even though they were actually 2 years old at the beginning of the winter. The number of pyretic horses in winter and the estimated number of horses infected with EHV-1 or EHV-4 or both in each month; the total estimated number of pyretic horses infected throughout the epizootic period was thus determined. The statistical significance in the numbers of pyretic horses and the estimated numbers of horses infected with EHV-1 or EHV-4 during the sampling period were also included in the numbers of horses infected with EHV-1 or EHV-4 or both.

**Investigation of the magnitude of EHV-1 and EHV-4 epizootics in the training centers in winter.** The paired serum samples were taken from randomly selected 3-year-old horses that stayed in the training centers throughout the winter. The sera were taken in November, and the postsera were taken in May. The sera were subjected to EHV-1 and EHV-4 gG ELISA, and horses that showed a significant increase in the CF titer (≥24-fold between the paired serum samples) and in the EHV-1 gG ELISA titer were diagnosed as infected with EHV-1. Those with a significant increase in the CF titer, and in the EHV-4 gG ELISA titer were diagnosed as infected with EHV-4. The horses that had a significant increase in the CF titer, no increase in either the EHV-1 or EHV-4 gG ELISA titer, and no history of vaccination during the sampling period were also included in the numbers of horses infected with EHV-1 or EHV-4 or both.

**Administrative coverage of the EHV-1 vaccine.** The vaccination coverages in the 3-year-old population and in the whole population in each training center were calculated as follows. For horses that were present in the training centers on 1 January of each year, the number with a vaccination history (regardless of the number of doses received) in each population was divided by the total number of horses in the corresponding population.

**Serological responses of horses inoculated with EHV-1 vaccine.** In 2010-2011, 3-year-old horses at the Ritto training center were randomly selected. They were inoculated three times with the EHV-1 vaccine at approximately 1-month intervals, in accordance with the current vaccination program. Presera were collected 1 month before the administration of the first dose. Postvaccination sera were collected 1 month after vaccination. The paired serum samples were taken from the horses, and sera were subjected to EHV-1 and EHV-4 gG ELISA. The horses that showed a significant increase in the CF titer (≥4-fold between the paired serum samples) and in the EHV-1 gG ELISA titer were diagnosed as infected with EHV-1. Those with a significant increase in the CF titer, and in the EHV-4 gG ELISA titer were diagnosed as infected with EHV-4. The horses that had a significant increase in the CF titer, no increase in either the EHV-1 or EHV-4 gG ELISA titer, and no history of vaccination during the sampling period were also included in the numbers of horses infected with EHV-1 or EHV-4 or both.

**Vaccination programs.** From 1994-1995 to 2008-2009, 3-year-old horses were tested for EHV-1 antibodies with an enzyme-linked immunosorbent assay (ELISA) kit (Nisseiken) when they were introduced to the training centers. The ELISA was performed in the period from October to April. Horses that showed absorbances of <0.6 were targeted for vaccination. They were inoculated with inactivated EHV-1 vaccine (Nisseiken) (at least 1 dose and at most 3 doses at approximately 1-month intervals). The vaccine contains the formalin-inactivated whole virion of EHV-1 (HH-1 strain) at a concentration of >10^6 TCID_50 50% tissue culture infective dose (TCID_50)/dose and aluminum chloride as an adjuvant. The vaccination term during each epizootic period started in mid-November and finished at the end of April. The horses that entered the training centers before September were not tested by ELISAs due to a budgetary problem and were not vaccinated during the winter. In 2003-2004 at the Ritto training center, the vaccination program was not run properly because insufficient doses of vaccine were supplied.
Pyretic horses with EHV-1 or EHV-4 infection or both in the training centers in winter. First, we investigated how the alteration in the vaccination program had affected the incidence of pyrexia caused by EHV-1 or EHV-4 infection or both. The numbers of pyretic horses and the estimated numbers of horses infected with EHV-1 or EHV-4 or both in each epizootic period are shown in Fig. 2. The number of pyretic horses in each period from 1999-2000 to 2008-2009 ranged from 185 to 367 at the Ritto training center and 105 to 244 at the Miho training center. The mean numbers (± standard deviation [SD]) of pyretic horses in winter in the periods from 1999-2000 to 2008-2009 were 254 ± 54 at the Ritto training center and 166 ± 52 at the Miho training center. The mean estimated numbers (±SD) of horses infected with EHV-1 or EHV-4 or both among the pyretic horses in the same epizootic periods were 105 ± 47 at Ritto and 66 ± 44 at Miho. The highest number of pyretic horses and highest estimated number of infected horses were recorded at the Ritto training center in 2003-2004 at which time the vaccination program was not operated as intended.

In 2009-2010, the first period of the current vaccination program, the numbers of pyretic horses and the estimated numbers of infected horses in the two training centers did not change greatly from those recorded before (Fig. 2). However, reductions in the numbers of pyretic horses and the estimated numbers of horses infected with EHV-1 or EHV-4 or both were apparent in 2010-2011 (the second period of the current vaccination program) and continued in 2011-2012 and 2012-2013 (Fig. 2). The mean number (±SD) of pyretic horses at the Ritto training center in the periods from 2010-2011 to 2012-2013 was 136 ± 15, which was significantly lower than that in the periods from 1999-2000 to 2008-2009 (P < 0.01). The mean estimated number (±SD) of infected horses also decreased to 21 ± 12 (P < 0.01). The same trend was observed at the Miho training center, where the mean number (±SD) of pyretic horses was 78 ± 10 in the periods from 2010-2011 to 2012-2013 (P < 0.01). The mean estimated number (±SD) of infected horses at Miho also declined to 14 ± 15, although this reduction was not statistically significant.

From 2006-2007 to 2012-2013, a total of 284 horses at the Ritto training center and 122 horses at the Miho training center were diagnosed as having fever with EHV-1 or EHV-4 infection or both. At the Ritto training center, 245 (86.3%) of these 284 horses were 3 years old and the remaining 39 (13.7%) were 4 years or older (Fig. 3A). Classification according to the gG ELISA results revealed that 243 cases (85.6%) of fever were due to EHV-1 infection and 12 (4.2%) were due to EHV-4 infection. In 34 cases (12.0%), the virus type could not be identified, although the horses were regarded as being infected with either EHV-1 or EHV-4 or both. At the Miho training center, 100 (82.0%) out of 122 horses were 3 years old, and the remaining 22 (18.0%) were 4 years or older (Fig. 3B). Of these cases, 104 (85.2%) were due to EHV-1 infection, and 6 (4.9%) were due to EHV-4 infection. In 17 cases (13.9%), the virus type could not be identified. Mixed infection with the two viruses was found in some cases at both training centers.

Magnitude of EHV-1 and EHV-4 epizootics in the training centers in winter. To investigate the magnitude of the EHV-1 and EHV-4 epizootics, paired serum samples from randomly selected 3-year-old horses that stayed at the training centers throughout the epizootic period were subjected to gG ELISA for EHV-1 and EHV-4. The magnitudes of the EHV-1 and EHV-4 epizootics in each epizootic period are shown in Fig. 4. The magnitude of the EHV-4 infection ranged from 0% to 8% in the two training centers, and no obvious changes were recorded throughout the investigation periods (Fig. 4). Before the change in the vaccination program, 62% to 68% of horses at the Ritto training center were infected with EHV-1 and 52% to 64% at the Miho training center were infected (Fig. 4). At the Ritto training center, much lower rates of infection with EHV-1 were recorded in 2010-2011 (10%) and 2012-2013 (22%), whereas the rates in 2009-2010 (62%) and 2011-2012 (64%) were almost the same as those recorded previously (Fig. 4A). At the Miho training center, the pattern of fluctu-
ation in infection rates after the change in the vaccination program was similar to that at the Ritto center, although the rates in each period were higher than those at the Ritto center (Fig. 4B).

**Administrative coverage of EHV-1 vaccine in the 3-year-old population and in the whole population.** Next, we investigated the vaccination coverage before and after the change in the vaccination program to estimate its relationship with the reduction in the number of pyretic horses with EHV-1 or EHV-4 infection or both. The coverages of the EHV-1 vaccine in the 3-year-old population and in the whole population of all ages at the training centers in the periods from 2006-2007 to 2012-2013 are shown in Fig. 5. The mean vaccination coverage in the 3-year-old population from 2006-2007 to 2008-2009 was 47.7% at the Ritto training center and 48.0% at the Miho training center. In 2009-2010 (the first epizootic period under the current vaccination program), the vaccination coverage in the 3-year-old population was 99.4% at Ritto and 99.8% at Miho, and similar values were maintained thereafter. The mean coverage in the 3-year-old population from 2009-2010 to 2012-2013 increased to 99.5% at the Ritto training center and to 96.3% at the Miho training center.

The mean vaccination coverage in the whole population from 2006-2007 to 2008-2009 was 50.8% at the Ritto training center and 50.0% at the Miho training center. In contrast to the coverage in the 3-year-old population, that in the whole population increased gradually after introduction of the current vaccination program. In 2009-2010, the coverage in the whole population was 77.9% at Ritto and 79.3% at Miho; by 2012-2013, it had reached 95.7% at Ritto and 95.1% at Miho (Fig. 5).

**Serological responses of horses inoculated with EHV-1 vaccine.** To clarify the serological responses of horses inoculated with the EHV-1 vaccine, VN antibody titers before and after vaccination were measured. The VN titers of vaccinated horses at the Ritto training center in 2010-2011 are shown in Fig. 6. The horses received three doses of the vaccine 1 month apart in accordance
with the current program. The GM titer before vaccination was 31.0. After the first dose, the GM titer increased to 53.7 ($P < 0.05$), but no obvious titer increases were observed after the second and third doses (Fig. 6). The proportion of individual horses with a ≥4-fold titer rise (i.e., the response rate) after the first dose was 20.0%. The response rates after the second and the third doses were 7.6% and 3.8%, respectively (Table 1). After stratification by the prevaccination titers, a higher response rate was observed in horses with low preexisting titers (ranging from <10 to 80) than in those with pretiters of ≥160 (Table 2).

**DISCUSSION**

The magnitude of EHV-1 infection in the training centers varied to some extent among epizootic periods; this fluctuation was probably due to factors such as the age distribution, the history of infection with EHV-1 or EHV-4 or both, the outbreaks on other farms, or the weather. For example, much smaller numbers of pyretic horses than average were recorded in 2000-2001 and 2003-2004 at the Miho training center (Fig. 2), although there were no changes in the vaccination program in these periods. However, we consider that the continuous reduction in the number of EHV-1-induced pyrexias recorded in the three sequential years from 2010-2011 to 2012-2013 at both training centers was sufficient for the evaluation of the vaccine efficacy.

Before the introduction of EHV-1 vaccination in 1994-1995, the numbers of pyretic horses in the epizootic period were 377 at Ritto and 166 at Miho (means from the eight periods from 1986-1987 to 1993-1994; T. Matsumura, unpublished data), although we could not determine what proportions of these cases were caused by EHV-1 or EHV-4 infection or both. In the case of the Ritto training center, the number was much higher than that recorded in the 10 periods after introduction of the vaccination (254 ± 54), whereas there was no obvious change at Miho (166 ± 52). Therefore, the previous vaccination program seemed to be partially effective in reducing EHV-1-induced pyrexia, at least at the Ritto training center. The increase in the number of pyretic horses recorded at Ritto in 2003-2004, when the vaccination program was not operated properly, also seems to corroborate the efficacy of the previous vaccination program.

However, the vaccination coverage seemed not to be sufficient to exert a strong herd effect to control the epizootic. There were two major reasons for the low coverage under the previous program. One reason is that we had believed that vaccination was not required for horses with high ELISA absorbance values, although the relationship between the antibodies detected by ELISAs and the potential to protect the horses from EHV-1-induced diseases was not clear. The second reason was that the horses that had entered the training centers before September were not tested by ELISAs and were not targeted for vaccination, even though some of them were expected to stay over the upcoming winter. This population must have included horses with low antibody levels against EHV-1, although the proportion of such horses was unknown. Hence, the vaccination program before 2008-2009 resulted in coverage of only half of the 3-year-old population; coverage in the whole population was at the same level (Fig. 5).

Horses ≥4 years old were believed to be more resistant to EHV-1 infection and were considered to exhibit clinical signs only rarely, even after infection. In this regard, we expected that vaccination of the whole 3-year-old population might be sufficient to control the EHV-1 epizootic. However, in the first period of the current program, the numbers of pyretic horses with EHV-1 infection were still high, although high coverage in the 3-year-old population was achieved in that period (99.4% at Ritto and 99.8% at Miho). This result indicated that the high vaccination coverage in the 3-year-old population was not sufficient to control the epizootic. A similar example has been found in policies for the control of human influenza virus in most countries, which have focused on protecting groups at high risk for disease complications, including the elderly, pregnant women, young children, and individuals with chronic diseases. However, vaccination of high-risk populations alone is unlikely to reduce the burden of an epidemic, because these groups represent only a fraction of the population among whom the virus spreads (17, 18). In the case of EHV-1 epizootics in the training centers, horses ≥4 years old were also involved, although they accounted for a relatively small proportion of cases (Fig. 3). Therefore, we concluded that this population should also be included in any consideration of vaccination coverage. Increased coverage in the 3-year-old population in one period was reflected in that of the 4-year-old population in the next period; as a consequence, coverage in the whole population gradually increased after the introduction of the current program (Fig. 5). Taking these findings together with our data on the occurrence of EHV-1-induced pyrexia reveals that the coverage in the whole population in 2009-2010 (77.9% at Ritto and 79.3% at Miho) was not sufficient to control the epizootic, but the coverage in 2010-2011 (85.3% at Ritto and 87.8% at Miho) seemed to be sufficient. From these results, we can speculate that the vaccination coverage required for the herd effect was in the range of

**TABLE 1 Numbers of horses that showed significant increases in VN titers against EHV-1 after each vaccination dose**

| Vaccination dose | No. (%) of horses with significant increase in VN titer |
|------------------|--------------------------------------------------------|
| First            | 21 (20.0)                                              |
| Second           | 8 (7.6)                                                |
| Third            | 4 (3.8)                                                |
| Total            | 33 (31.4)                                              |
79.3% to 85.3% of the whole horse population. However, this value might not be applicable to all horse herds, e.g., those on breeding farms and rearing farms. This is because they may differ from the herds in the training centers in terms of factors such as age distribution, geographic location, and magnitude of stress, and such differences may influence their susceptibility to EHV-1 epizootics.

Although extremely high vaccination coverage in the 3-year-old population have been achieved in most of the epizootic periods under the current program, much lower coverage was recorded in 2011-2012 at the Miho training center (88.6%). This was probably because vaccination of horses just before they raced was disallowed by the trainers, who were worried about the vaccine side effects such as fever. However, we were unable to determine why such frequent refusals were observed only in that period.

Although the numbers of pyretic horses with EHV-1 infection were reduced in the periods 2010-2011, 2011-2012, and 2012-2013, the magnitude of the EHV-1 epizootic differed among the periods. That is, the number of pyretic horses with EHV-1 infection sometimes did not reflect the magnitude of the EHV-1 epizootic, because the horses infected with EHV-1 were often asymptomatic. We investigated in more detail the occurrence of pyrexia in each epizootic period and found that in 2010-2011 at both training centers and in 2012-2013 at the Ritto center, EHV-1-induced pyrexias occurred sporadically, and almost no obvious outbreaks were observed (data not shown). In these periods, the magnitude of the EHV-1 epizootic was also extremely low (Fig. 4), and it seemed that virus transmission in the training centers was effectively blocked by the herd effect. In contrast, in 2011-2012 at both training centers and in 2012-2013 at Miho, there were small outbreaks consisting of sequential occurrences of pyrexia, although not as many horses were involved (data not shown). In these periods, the magnitude of the epizootic was almost the same as that observed before the change in the vaccination program (Fig. 4). At these times, the immunity induced by the vaccine did not completely interrupt virus transmission, although disease progression seemed to be mitigated.

The increase in VN titers after vaccination may be one reason for the suppression of the EHV-1 epizootic, because VN antibodies may reduce virus excretion from infected individuals and may reduce the duration and severity of disease outbreaks in populations (19). Vaccination of all 3-year-old horses in the training centers resulted in a phenomenon known as the “law of initial values” or “negative feedback,” whereby there is an inverse association between the prevaccination VN titer and the response rate (7). This was also consistent with previous findings in mares vaccinated with inactivated EHV-1 and EHV-4 vaccines: vaccination responses were seen only in the group of mares that had a lower mean ELISA absorbance at the time of the initial vaccination (8). In that study, the higher response rate in the foals than in the mares also seemed to be attributable to the difference in prevaccination antibody levels between the two cohorts. However, these findings are not sufficient for us to conclude that vaccination of horses with high prevaccination titers is not required for protection, even though VN titers are not likely to rise in this cohort. This is because the antibody level is not the only absolute parameter for predicting clinical protection: cell-mediated and local immunity might also play roles in providing protection (20), although we did not monitor such parameters here. From the perspective of herd immunity, the contribution of repeated vaccinations to suppression of the EHV-1 epizootic was not clear, because the GM titer generally increased only after the first dose. However, repeated vaccinations might be effective in protecting vaccinated individuals directly, because some of the horses showed titer rises after the second and the third doses. The herd effect of vaccination is generally evaluated by the achievement rate, which represents the proportion of individuals with the antibody titers required for protection. Although protective antibody titers have been investigated in various infectious diseases in humans, such as influenza, measles, and rubella, studies have revealed the inappropriateness of using VN titers alone to evaluate the protective effects against EHV-1 (8, 20, 21).

In conclusion, the current vaccination program targeting all 3-year-old horses enabled us to control the pyrexias caused by EHV-1 infection in the training centers. Suppression of the EHV-1 epizootic seemed to require the maintenance of high vaccination coverage, not only in the 3-year-old population but also in the whole population. In addition to appropriate operation of the vaccination program, further monitoring of pyretic horses and serological surveillance is necessary for the continuous control of EHV-1 epizootics in the training centers.

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TABLE 2 Relationship between preexisting VN titer against EHV-1 and its increase after vaccination

| Response                  | Response rate (no.) in horses with prevaccination VN titer of: |
|---------------------------|---------------------------------------------------------------|
|                           | <10 | 10 | 20 | 40 | 80 | 160 | 320 | Total no. |
| Significant increase      | 2   | 9  | 16 | 5  | 1  | 0   | 0   | 33  |
| No increase               | 0   | 9  | 22 | 20 | 9  | 8   | 5   | 73  |
| Total no.                 | 2   | 18 | 38 | 25 | 10 | 8   | 5   | 106 |

In that study, the higher response rate in the foals than in the mares also seemed to be attributable to the difference in prevaccination antibody levels between the two cohorts. However, these findings are not sufficient for us to conclude that vaccination of horses with high prevaccination titers is not required for protection, even though VN titers are not likely to rise in this cohort. This is because the antibody level is not the only absolute parameter for predicting clinical protection: cell-mediated and local immunity might also play roles in providing protection (20), although we did not monitor such parameters here. From the perspective of herd immunity, the contribution of repeated vaccinations to suppression of the EHV-1 epizootic was not clear, because the GM titer generally increased only after the first dose. However, repeated vaccinations might be effective in protecting vaccinated individuals directly, because some of the horses showed titer rises after the second and the third doses. The herd effect of vaccination is generally evaluated by the achievement rate, which represents the proportion of individuals with the antibody titers required for protection. Although protective antibody titers have been investigated in various infectious diseases in humans, such as influenza, measles, and rubella, studies have revealed the inappropriateness of using VN titers alone to evaluate the protective effects against EHV-1 (8, 20, 21).

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