NOTE *Internal Medicine*

Effect of Supplemental Vitamin E on Antibody Titer in Japanese Black Calves Vaccinated against Bovine Herpesvirus-1

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ABSTRACT. We investigated the effect of supplemental vitamin E on antibody titer against bovine herpesvirus-1 (BHV-1) in Japanese Black calves after vaccination with modified live virus. Thirty calves kept at the same farm were studied. They were divided into two groups; fifteen calves received 300 IU/day of vitamin E orally from 1 to 3 months of age (VE Group), and the other fifteen calves did not receive vitamin E supplement (Control Group). BHV-1 modified live vaccine was injected twice to all calves when they were 2 and 3 months of age. Following the vaccination, serum vitamin E concentration and neutralizing antibody titer to BHV-1 were measured over time. VE Group showed higher serum vitamin E at 2, 3 and 4 months of age compared to Control Group ($P<0.05$). The antibody titer in Control Group was the highest at 1 month of age, and it gradually decreased until 4 months of age. VE Group showed increase in antibody titer at 4 months of age resulting in significant difference ($P<0.01$) from Control Group. This study demonstrated that vitamin E supplementation to Japanese Black calves could increase antibody production after the second modified live BHV-1 vaccination.
KEY WORDS: antibody titer, bovine herpesvirus-1, Japanese Black calf, vaccination, vitamin E.

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Young calves have immature immune system [6, 9], demonstrated by fairy low antibody response [8]. Especially, Japanese Black calves are more prone to bovine respiratory disease complex (BRDC) compared to Holstein calves, due to their lower numbers and function of peripheral blood T and B cells, which are responsible for antibody production [11]. Bovine herpesvirus-1 (BHV-1) causes BRDC, and it develops severe symptoms by concomitant infection of bacteria [16]. Therefore, in order to prevent calves from the BHV-1 infection, modified live BHV-1 vaccination has been conducted in Japan. Vitamin E is essential for maintaining functional immune system [7]. Oral administration of vitamin E to calves has proved improvement of cell-mediated as well as humoral immune responses [3, 13, 14]. Our previous study suggested that Japanese Black calves with lower serum vitamin E caused lower antibody response to *Manhaimia hemolytica* vaccination [12]. However, the effect of vitamin E oral administration to Japanese Black calves on antibody response to BHV-1 vaccination has not been elucidated. In order to improve antibody response, we validated the effect of supplemental vitamin E on antibody response in Japanese Black calves vaccinated against live BHV-1.

Thirty Japanese Black calves kept at one farm in Aomori Prefecture were used in this study. The calves, born between spring and fall in 2011, were alternately assigned into two groups; 15 calves received 300 IU/day vitamin E (this dose was determined based on the study by Rajeesh *et al.* [13]) mixed with milk replacer from 1 to 3 months of age (VE Group), and the other fifteen calves receive only milk replacer (Control Group). All calves were fed to meet their nutritional requirements according to the Japanese Feeding Standard for Beef Cattle [1] and managed in the same manner. All calves received commercial modified live BHV-1 vaccine (No. 758-43 strain, Kyoto Biken Laboratories, Kyoto, Japan) at 2 and 3 months of age following manufacturer's instruction. The peripheral blood samples were collected from all calves at 1, 2, 3 and 4 months of age, once for each month, from the jugular vein. Serum was separated by centrifugation and stored at −20°C until analysis.

Serum vitamin E concentration was measured using high performance liquid chromatograph (LC-2000, JASCO, Tokyo, Japan) as previously reported [4]. Modified serum neutralization test of BHV-1 with microtitration system was performed based on the original method by Bitsch [2]. All enrolled calves completed the experiment without dropping out.

Data of serum vitamin E were expressed as mean ± SE. Antibody titers were expressed as geometric mean ± SE. The difference between groups was examined using Student's *t*-test. *P* values less than 0.05 were considered statistically significant.

The serum vitamin E at 1 month of age did not show significant difference between groups (Fig. 1A). The serum vitamin E levels of VE Group at 2 ($P<0.01$), 3 ($P<0.01$) and 4 ($P<0.05$) months of age were significantly higher than Con-

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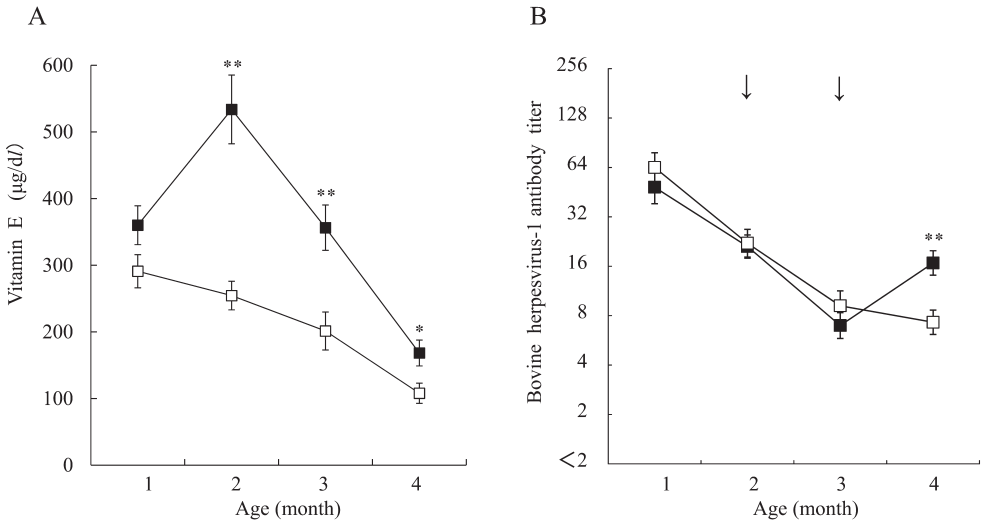

Fig. 1. Change in serum vitamin E concentration (A) and changes in antibody titers to bovine herpesvirus-1 (B). Vitamin E Group (dark square) and Control Group (empty square). Vitamin E data are shown as mean ± SE. Antibody titers are shown as geometric mean ± SE. Arrow indicates bovine herpesvirus-1 vaccination. Asterisk indicates significant difference between groups at the same sampling point (*: $P<0.05$, **: $P<0.01$).

trol Group. The antibody titer against BHV-1 was the highest at 1 month of age for both groups (Fig. 1B). The titer in Control Group decreased gradually after that until 4 months of age. The titer in VE Group was significantly higher than that in Control Group at 4 month of age ($P<0.01$).

Results of present study suggest that increase in serum vitamin E by oral supplementation might increase antibody production after modified live BHV-1 vaccination. The previous study of oral vitamin E administration to calves exhibited stable increase in serum vitamin E [14], which was similar to our present study. In general, young calves have lower number of antibody producing B cells [6], and thus, antibody response is fairly low [9]. Additionally, young calves do not elicit good antibody response to vaccination due to the presence of maternal antibody [10]. A study with Buffalo calves demonstrated that oral administration of vitamin E increased serum vitamin E as well as antibody responses to *Pasteurella multocida* vaccination [13]. Animals with high blood vitamin E showed enhancement in phagocytosis of macrophages and function as well as proliferation of T and B cells [3, 5, 8, 14]. Holstein calves, which received oral vitamin E administration after their birth, exhibited enhanced antibody response to BHV-1 vaccination at 21 weeks of age [15]. Although Japanese Black calves have lower number and function of immune cells [11], oral administration of vitamin E might have improved proliferation and function of immune competent cells in the present study. Thus, they could increase the antibody production when they were vaccinated at 3 months of age.

The results of our investigation confirmed that oral supplementation of vitamin E to Japanese Black calves enhanced antibody production after the second modified live BHV-1 vaccination at 3 months of age. Vitamin E supplementation to young Japanese Black calves seemed to enhance the response to vaccination. In order to reduce the incidence of BRDC in the Japanese Black calves, further studies are needed to clarify how and when oral vitamin E administration improves immune function to enhance antibody production to BHV-1 vaccination and whether oral vitamin E administration improves response to vaccinations against other BRDC causing viruses.

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