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Peripheral Blood Leukocyte Subpopulation of Dairy Cows with Digital Dermatitis and Effect of Hoof Trimming with Antibiotic Treatment

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ABSTRACT. In the present study, 30 cows were used to evaluate the changes in the peripheral blood leukocyte subpopulation of dairy cows with digital dermatitis (DD) following hoof trimming and antibiotic treatment. The cows were divided into two groups; 18 cows (DD group) had DD on both hind feet, and 12 cows (control group) had four feet with no clinical abnormalities. The DD group was further divided into two groups based on the treatment; the antibiotic group (8 cows) was treated with only 2% lincomycin liquid spray once daily for 3 days, and the trimmed group (10 cows) received trimming of hooves as well as treatment with 2% lincomycin liquid spray. The plasma cortisol concentration was significantly higher in both DD groups before treatment than in the control group, and it decreased significantly after hoof trimming in the trimmed group. The number of $CD3^+$, $CD4^+$, $WC1^+$ and $CD21^+$ cells in both DD groups before treatment was significantly lower than that of the control group. The number of $CD3^+$, $CD4^+$, $WC1^+$ and $CD21^+$ cells in the trimmed group increased after treatment. These results indicated that cows with DD suffer from stress and reduced number of T and B cells. Treatment of DD with both hoof trimming and 2% lincomycin liquid spray was effective for reducing the stress and bringing the immune cell number back to the normal range.

KEY WORDS: cortisol, dairy cow, digital dermatitis, hoof trimming, leukocyte subpopulation.

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Hoof diseases cause severe economic losses due to premature culling of affected animals, decreased milk production, weight loss, reduced fertility (delayed pregnancy and weak estrus) and the high costs of treatment [3, 4, 6, 7, 16, 20]. It is also a problem regarding animal welfare [1]. Digital dermatitis (DD), which is a hoof disease, has become a principal cause of lameness in dairy cows [6, 22]. DD is highly contagious, and spirochetes as well as several other bacteria have been isolated from DD lesions [19, 22]. Dissemination of DD is affected by several factors, such as age, barn environment, pollution of footbaths, hoof trimming and immune status [7].

Recently, footbaths, intramuscular injection and topical application of antibiotics have been used to treat DD [8, 11]. However, it has been it is reported that footbaths generally have no effect on DD [18]. Intramuscular injection of oxytetracycline, erythromycin and ceftiofur is useful for treatment of DD [14]. In other reports, topical application of erythromycin, oxytetracycline and lincomycin has been shown to be effective for treatment of DD [6–8]. Hoof trimming decreases hoof diseases and stress by balancing the loads of weight-bearing limbs [15]. We previously reported that hoof trimming is efficient in reducing overall stress and in maintaining the nutritional condition of dairy cows after parturition [2]. The purpose of this study was to assess the influence of DD on leukocyte subpopulation and to evaluate the effectiveness of a DD treatment using hoof trimming and an antibiotic.

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MATERIALS AND METHODS

Thirty Holstein dairy cows at one commercial dairy farm in Japan were used in the present study. The cows were housed indoors throughout the year in a tie stall barn; the average milk yield per cow year was 9,363 kg, and the average herd size was 132 cows. This study was conducted from October 2006 until March 2007. Cows more than 150 days after parturition were used in order to exclude the influence of parturition, and the animals were divided into two groups; 18 cows (DD group) had DD on both hind legs, and 12 cows (control group) had four legs with no clinical abnormalities (Table 1). All cows were diagnosed in a hoof trimming chute, and DD was defined as scores 1–3 using the scoring system developed by Manske [9]. BCSs were calculated according to previously reported methods [2]. The DD group was randomly divided into two subgroups based on the treatment; the hooves of 8 cows (antibiotic group) were treated only with 2% lincomycin liquid spray once daily for 3 days, and both hind hooves of 10 cows (trimmed group) were trimmied and treated with 2% lincomycin liquid spray once daily for 3 days. All cows had all four of their hooves trimmed between 6 and 7 months before this study, and no infectious disease requiring clinical treatment was detected by a veterinarian during routine clinical examinations throughout the examination period. Clinical examinations

and blood sampling were performed on day 0 (= the day of first hoof treatment), day 7, day 28 and day 56 in the DD group, and the cows in the control group were examined only once on day 0. Both the antibiotic and trimmed group were in the healing process on day 7. Blood samples were used to measure the plasma cortisol concentrations and to analyze the peripheral blood leukocyte subpopulation. Functional hoof trimming was performed by one veterinary practitioner to maintain a hoof thickness from the sole of 5 mm at the apex and 8 mm at the heel. Blood samples were collected between 10 and 12 am on the day of sampling from the caudal vein using syringes. Blood in the syringe was immediately divided into two tubes, one containing heparin Na and the other containing dipotassium-EDTA. Plasma samples obtained from tubes with heparin Na were used to measure the plasma cortisol concentration. The plasma cortisol concentration was analyzed using the enzyme immunoassay reported by Ando [2] with minor modifications using anti-cortisol-3 (Cosmo Bio Co., Tokyo, Japan) and goat anti-rabbit IgG (Chemicon International, Temecula, U.S.A.). The intra- and interassay coefficients of variation of this assay were 3.5% and 4.6%, respectively. Blood samples containing anticoagulant dipotassium-EDTA were used to measure total white blood cell (WBC) counts using a blood cell counter (PC607, ERMA, Germany) and to analyze the leukocyte subpopulation as described by Ohtsuka [12]. In order to analyze the surface antigens on peripheral blood WBCs, red blood cells were lysed using 0.83% ammonium chloride. After washing with PBS, WBCs were finally resuspended in cold phosphate buffered saline (PBS). WBCs (1×10^6) were then incubated with a primary monoclonal antibody at 4°C for one hour. The monoclonal antibodies against CD3 (clone MM1A, made of mouse IgG, VMRD, Pullman, WA, U.S.A.), CD4 (clone CACT138A, made of mouse IgG, VMRD), CD8 (clone BAT82A, made of mouse IgG, VMRD), WC1-N3 (clone CACTB32A, made of mouse IgG, VMRD), CD21 (clone GB25A, made of mouse IgG, VMRD), MHC classII (clone CAT82A, made of mouse IgG, VMRD) and CD14 (clone MY4-FITC, made from mouse IgG, Beckman Coulter, Inc., Hialeah, Florida, U.S.A.) were used. These monoclonal antibodies are known to react with these bovine markers [12]. In order to detect these marker-positive cells, cells were incubated with phycoerythrin (PE)-labeled anti-mouse IgG (Durham, NC, U.S.A.) at 4°C for 30 min. After washing the cells with PBS, the samples were analyzed by flow cytometry (FACScan, Becton Diskinson, San Jose, CA, U.S.A.) using the CellQuest software (Becton Dickinson, San Jose CA, U.S.A.).

Statistical analysis was performed using repeated measures two-way ANOVAs for each sampling time in order to determine differences, and values of $P<0.05$ were considered to be significant using the Tukey-Kramer test. The StatView statistical software package (Abacus Concepts, Inc., Berkeley, CA, U.S.A.) was used for all statistical analyses.

RESULTS

The plasma cortisol concentrations on day 0 in the antibiotic and trimmed groups were significantly higher than that of the control group ($P<0.05$, Fig. 1). The plasma cortisol concentration showed no changes after treatment in the antibiotic group. In contrast, the plasma cortisol concentration in the trimmed group showed a significant decrease on day 7 compared with day 0 and remained low thereafter ($P<0.05$).

In the antibiotic group, the numbers of $CD3^+$ and $WC1^+$ cells on days 0, 7, 28 and day 56, $CD4^+$ cells on days 0, 7 and 28 and $CD21^+$ cells on days 0 and 7 were significantly lower and the numbers of MHC $classII^+$ $CD14^+$ cells on days 28 and 56 were significantly higher than those of the control group ($P<0.05$, Figs. 2 and 3). There was no difference in the number of $CD8^+$ cells between the antibiotic and the control groups. In the trimmed group, the numbers of $CD3^+$ and $CD4^+$ cells on day 0, numbers of $WC1^+$ cells on days 0, 28 and 56 and numbers of $CD21^+$ cells on days 0, 7 and 28 were significantly lower than those of the control group ($P<0.05$, Figs. 2 and 3). The numbers of $CD3^+$ and $CD4^+$ cells on day 56 and $WC1^+$ cells on day 7 in the trimmed group showed a significant increase compared with day 0 ($P<0.05$). The number of $CD21^+$ cells in the trimmed group showed a significant increase on day 56 compared with day 28 ($P<0.05$). There was no difference in the number of $CD8^+$ and MHC $classII^+CD14^+$ cells between the trimmed and control groups.

DISCUSSION

In this study, we examined the peripheral blood leukocyte subpopulation to assess the influence of DD on immune cells. The cows in the DD group showed higher concentra-

Table 1. Comparison of age, body weight, body condition score and days after parturition of the groups on day 0

	DD group		Control group
	Antibiotic group	Trimmed group	
Number of cows	8	10	12
Age (years)	3.7 ± 0.4	4.1 ± 0.4	3.1 ± 0.3
Body weight (kg)	625.3 ± 34.2	626.4 ± 24.4	605.2 ± 16.5
Body condition score	2.5 ± 0.1	2.8 ± 0.2	2.9 ± 0.1
Days after parturition	199.0 ± 37.4	232.1 ± 46.9	224.0 ± 47.0

All data are expressed as means. ± se.

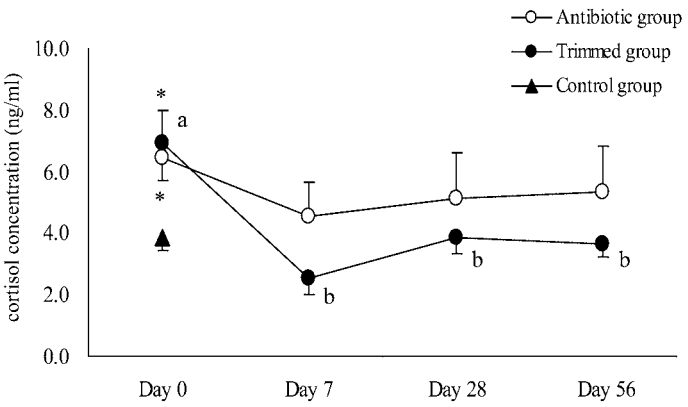

Fig. 1. Changes in the cortisol concentrations after treatment. *: Significant difference compared with the control group ($P<0.05$). a-b: Significant difference between samplings within a group ($P<0.05$).

tions of plasma cortisol than those in the control group. This indicated that the cows with DD were suffering from inflammation and stress associated with the hoof lesions. It has been reported that dairy cows under stress have decreased leukocyte function with high cortisol and catecholamine secretion [12]. In agreement with a previous report [12], the numbers of $CD3^+$, $CD4^+$, $WC1^+$ and $CD21^+$ cells in the DD group were significantly lower in the present study. These results indicate that the cows with DD had stress associated with hoof inflammation that decreased leukocyte function. However, the relationship between the duration of DD and leukocyte function was not clear in the present study. Further investigation is required to determine the influence of duration of DD and severity of symptoms on leukocyte function.

We also evaluated the effects of 2% lincomycin liquid spray and hoof trimming on DD based on the plasma cortisol concentration. The plasma cortisol concentration of the trimmed group showed a significant decrease from day 7 onward. In contrast, there was no significant difference in the plasma cortisol concentration of the antibiotic group after treatment. Because the hoof lesions appeared to be in the healing process on day 7 in both the antibiotic and trimming groups, the decrease of the cortisol concentration in the trimming group might have been due to stress reduction by balancing the weight load. MHC $classII^+$ $CD14^+$ cells are considered to be activated monocytes, which increase during the inflammatory response [5]. The number of MHC $classII^+$ $CD14^+$ cells in the antibiotic group was significantly higher on days 28 and 56 than on day 0 in the present study. These results suggested that treatment of DD with 2% lincomycin liquid spray might have had a temporary effect in reducing the inflammatory response, but inflammation recurred due to the presence of imbalanced weight loading. Therefore, hoof trimming together with 2% lincomycin liquid spray is a more effective treatment for DD in reducing stress resulting for the inflammatory response.

$CD4^+$ T cells are classified as either Th1 or Th2 type according to their cytokine secretory pattern [10]. Th1 cells induce secretion of IL-2 and INF-γ and are involved in cellular immunity [21]. On the other hand, Th2 cells induce secretion of IL-4 and IL-10 and are involved in humoral immunity. Furthermore, it has been reported that $WC1^+$ T cells are closely linked to the production of IFN-γ in cattle [17]. In the present study, the numbers of $CD3^+$ and $CD4^+$ T cells on day 56 and $WC1^+$ T cells on day 7 in the trimmed group were significantly higher compared with day 0. These increases in the number of $CD3^+$, $CD4^+$ and $WC1^+$ T cells might reflect improvement of cellular immunity due to a reduction of stress resulting from hoof trimming together with the antibiotic treatment. The number of $CD21^+$ cells, which are considered to be B cells, were significantly increased on day 56 in the trimmed group. Dairy cows with malnutrition have low numbers of B cells during mid and late lactation [13]. These results suggested that treatment of DD with hoof trimming an antibiotic might have induced an increase in the number of B cells as a result of improvement of nutritional status under nonstressful conditions.

In conclusion, it is clear that the cows with DD were suffering from stress associated with hoof inflammation and that they had decreased peripheral blood leukocyte numbers. After treatment of DD with 2% lincomycin liquid spray alone, clinical improvement was observed, but it had little influence on the leulocyte subpopulation. Treatment of DD with hoof trimming and 2% lincomycin liquid spray was effective in reducing the stress and bringing the immune cell numbers back to a normal level.

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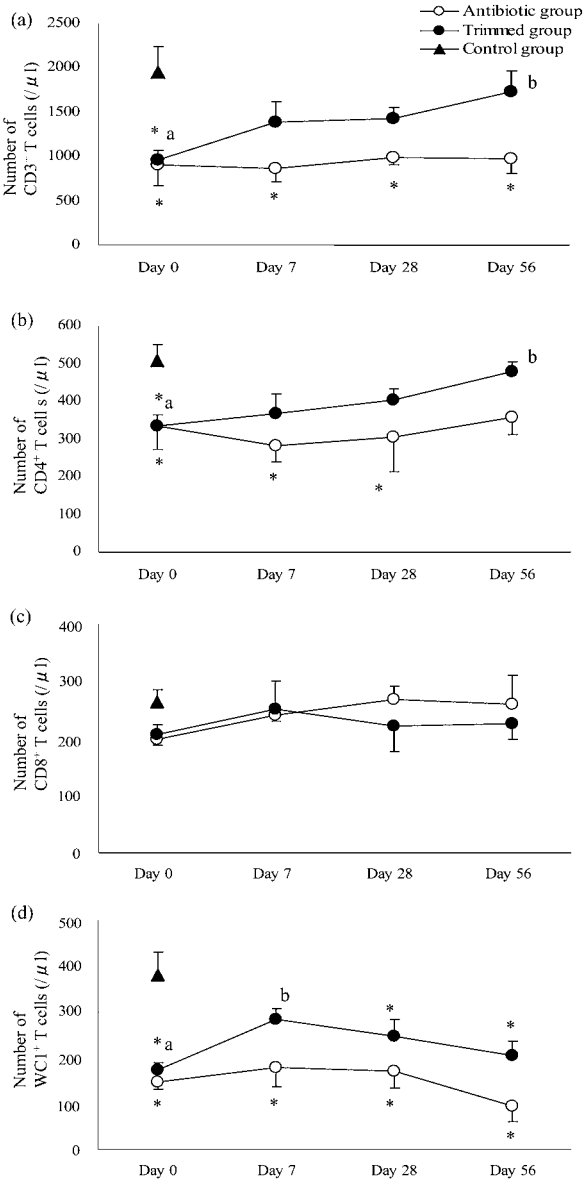

Fig. 2. Changes in the numbers of $CD3^+$ cells (a), $CD4^+$ cells (b) $CD8^+$ cells (c) and $WC1^+$ cells (d) after treatment.
*: Significant difference compared with the control group (P<0.05).
a-b: Significant difference between samplings within a group (P<0.05).

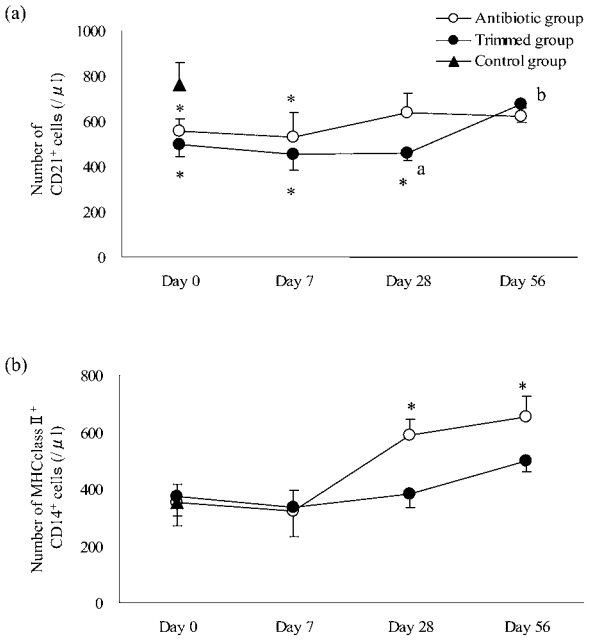

Fig. 3 Changes in the numbers of $CD21^+$ cells (a) and MHC class II^+ $CD14^+$ cells (b) after treatment.
*: Significant difference compared with the control group (P<0.05).
a-b: Significant difference between samplings within a group (P<0.05).

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