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ORIGINAL ARTICLE

Trace element and cytokine imbalances in calves with dermatophytosis

Han Gyu Lee[1], Ara Cho[1], Ui-Hyung Kim[2], Sihyun An[3], Yoon Jung Do[1], Changyong Choe[1], Are Sun You[1], Young-Hun Jung[1], Seogjin Kang[1], Eun-Yeong Bok[1]*

[1]Division of Animal Diseases and Health, National Institute of Animal Science, Rural Development Administration, Wanju 55365, Korea
[2]Hanwoo Research Institute, National Institute of Animal Science, Rural Development Administration, Pyeongchang 25340, Korea
[3]Agricultural Microbiology Division, National Institute of Agricultural Sciences, Rural Development Administration, Wanju 55365, Korea

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Corresponding author:
Eun-Yeong Bok
E-mail: eybok@korea.kr
https://orcid.org/0000-0002-1045-9670

Bovine dermatophytosis is a highly contagious disease that adversely affects animal growth and reduces meat and milk production. Nutritional deficiencies and immune status are suspected risk factors, but their roles remain unclear. This study investigates the association between trace minerals, vitamins, serum biochemical parameters, and immune-related cytokines with bovine dermatophytosis. Forty calves aged 6~7 months were selected and raised together on the same farm. They were divided into two groups: the control group (n=20) and the infected group (n=20). Serum was collected and analyzed for serum trace minerals, vitamins, biochemical parameters, and cytokine levels. *Trichophyton verrucousm* was isolated from infected calves. The infected calves had significantly lower zinc (P<0.001) and vitamin E (P=0.02) levels and significantly higher interleukin (IL)-6 (P=0.014) and IL-17A (P=0.018) levels. Regarding serum biochemical parameters, glucose (P=0.008) and total bilirubin (P=0.003) levels were significantly higher in the infected calves than in healthy ones. Hyperglobulinemia and high alkaline phosphatase levels were observed in the infected calves, without statistical significance. Our findings suggest the necessity of considering nutritional elements such as zinc and vitamin E for the prevention and treatment of bovine dermatophytosis. Additionally, the observed changes in immune and serum biochemistry factors post-infection may provide a foundation for future research on the host's biological responses to infection.

Key Words: Dermatophytosis, Trace element, Cytokine, Calf

INTRODUCTION

Ringworm or dermatophytosis is a global health concern in humans and animals and is caused by various fungi, including *Trichophyton*, *Microsporum*, and *Epidermophyton*. *Trichophyton verrucousm* (*T. verrucousm*) is one of the most prevalent agents causing bovine dermatophytosis (Rippon, 1989). Bovine dermatophytosis is a highly contagious disease that spreads easily in farm herds and causes continuous economic burden (Dalis et al., 2019; Lee et al., 2023). Risk factors for dermatophyte infection include poor nutrition, young age, the host's immune state, and climate conditions (Al-Ani et al., 2002), with animal nutrition reported to be more important than environmental factors (Al-Qudah et al., 2010).

Nutrition is known to be fundamental for maintaining health in cattle. Specifically, micronutrients such as minerals and vitamins are vital for sustaining the optimal function of immune system (Spears, 2000; Wintergerst et al., 2007). For instance, a lack of zinc (Zn) can lead to keratinization, scaling, and hair loss of the skin due to immune system suppression (Krametter-Froetscher et al., 2005; Nisbet et al., 2006), while supplementing these micronutrients improves immune response, as vitamin E has been shown to boosts

IL-2 production by T cells and strengthens the Type 1 T helper (Th1) cell response (Wintergerst et al., 2007). Moreover, serum biochemistry is used to monitor the nutritional state and overall health of animals (Otter, 2013).

The pathogenesis of dermatophyte infections involves the production of keratin-degrading enzymes, allowing the pathogen to penetrate and proliferate within the keratin layer. This invasion subsequently triggers both innate and adaptive immune responses, leading to release of key cytokines such as interleukin (IL)-1, IL-6, IL-10, IL-17, tumor necrosis factor-alpha, and interferon-gamma (IFN-γ). These mediators activate and migrate the effector cells and play crucial roles in defense against dermatophyte infections (Almeida, 2008; Glowacka et al., 2010).

IL-17 plays a major role in the cutaneous host defense against microorganisms such as fungi (Burstein et al., 2020). IFN-γ produced by Th1 cells activates macrophages and triggers phagocyte stimulation (Almeida, 2008). IL-10 is a cytokine known for its anti-inflammatory properties, which are crucial to maintaining a delicate immune system balance that facilitates infection clearance while minimizing host damage (Moore, 2001; Jankovic et al., 2010).

The associations of nutritional status and immunology with disease resistance are highly complex. Furthermore, the description of nutritional status and immunology in cases of bovine dermatophytosis remains unclear. Therefore, in this study, we aimed to measure the levels of three trace minerals (Zn, Selenium [Se], and Copper [Cu]), two vitamins (vitamins A and E), five serum biochemical parameters (glucose, globulin, alkaline phosphatase [ALKP], gamma-glutamyl transferase [GGT], and total bilirubin [TBIL]), and immune-related cytokines (IFN-γ, IL-6, IL-10, and IL-17A) and their relationship with dermatophytosis in calves.

MATERIALS AND METHODS

Ethics statements

The research was followed the guidelines approved by the Animal Ethics Committee at the National Institute of Animal Science, Republic of Korea (Approval No. NAIS-2020127). The US National Research Council's guidelines for the Care and Use of Laboratory Animals were followed.

Animal experimental design and dermatophytosis diagnosis

In this study, we examined a seasonal breeding cattle farm in Pyeongchang region that breeds more than 100 cattle during the spring and autumn seasons. Forty calves aged 6~7 months were selected and raised together in the same farm under similar management conditions and fed the same diet. The calves were divided into two groups based on a diagnosis of dermatophytosis: the infected group (calves naturally infected with dermatophyte, n=20) and the control group (healthy calves, n=20). The diagnosis was confirmed through clinical examination and microscopic identification. Clinically affected calves were first selected based on visible symptoms, such as scaling and round area of alopecia with a diameter of 0.5~1 cm on the head and neck. For microscopic identification, the lesions on affected animals were cleaned with 70% ethyl alcohol and skin scales were collected by scraping the lesion margins. The skin scale samples were placed in 10% potassium hydroxide solution on a slide and covered. The slide was then examined under a microscope (BX-53-P, Olympus, Tokyo, Japan) at ×10 and ×40 magnifications to identify fungal elements, as previously described by Balikci (2016).

Blood sampling

Blood was collected from the jugular vein in two 8.5 mL

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Serum separator tubes (BD, New Jersey, USA) and centrifuged at 3,000 g at 4℃ 15 min to separate the serum. The serum was stored at −80℃ for further analysis.

Serum trace mineral analysis

Serum levels of Zn, Cu, and Se were measured following the method described by Laur et al. (2020) with modifications. Serum (100 μL) was mixed with 200 μL of 65% nitric acid (Sigma Aldrich, Saint Louis, MO, USA) and 100 μL of hydrogen peroxide (Sigma Aldrich). The mixture was then briefly vortexed. Tubes were heated at 60℃ for 90 min for dissolution. After incubation, samples were cooled by adding 2.1 mL of ultrapure water at room temperature. Sample preparation and analysis were performed using ultrapure water (TNT research, Jeonju, Republic of Korea) with a resistance of approximately 18 MΩ cm^{-1}. Following brief vortexing and centrifugation (at 2,500 rpm for 3 min), trace minerals were analyzed using an Agilent 7900 ICP-MS (Agilent Technologies, Tokyo, Japan) equipped with standard nickel cones and connected to an SPS 4 Autosampler (Agilent Technologies, Tokyo, Japan). All measurements performed in triplicate from each vial.

Serum vitamin analysis

The concentration of serum vitamins A and E was measured using a commensal kit (vitamin A/E, Immuchrom GmbH, Heppenheim, Germany) following the manufacturer's instructions. First, 250 μL of the samples were mixed with 50 μL of internal standard and 500 μL of a precipitation reagent. The mixture was briefly vortexed, left for 30 min at 8℃, and centrifuged at 10,000 g for 2 min. The vitamins were analyzed using high-performance liquid chromatography (Nexera X2, Shimadzu, Kyoto, Japan), and separation was performed on a vitamin A/E column (IC1600 rp, Immuchrom GmbH) at 30℃. The flow rate of the mobile phase was 0.8 mL/min. The determined wavelengths were 300 nm (vitamin E) and 325 nm (vitamin A). All measurements were performed in triplicate.

Serum biochemistry analysis

Five serum biochemical parameters, including glucose, globulin, ALKP, GGT, and TBIL were measured using IDEXX Chemistry (Catalyst One, Westbrook, MA, USA).

Serum cytokine analysis

IFN-γ (Cusabio, Houston, TX, USA), IL-6 (Abcam, Cambridge, UK), IL-10 (Abcam), and IL-17A (Invitrogen, Waltham, MA, USA) were measured using an enzyme-linked immunosorbent assay kit following the manufacturer's instructions. All measurements were performed in triplicate.

Statistical analysis

To compare the infected and control groups, 14 parameters, including three trace minerals, two vitamins, five serum biochemical parameters, and four immune-related cytokines, were evaluated using an independent samples t-test. Statistical analysis was performed using SPSS (version 26' IBM Corporation, Armonk, NY, USA). Statistical significance was set at $P \leq 0.05$.

RESULTS

Mycology

In this study, *T. verrucousm* was observed in the skin of all infected calves. No fungi were observed in the healthy calves.

Serum trace minerals and vitamins

The measurements of serum trace mineral and vitamin concentrations were shown in Fig. 1. The infected calves exhibited significantly lower levels of Zn ($P<0.001$)

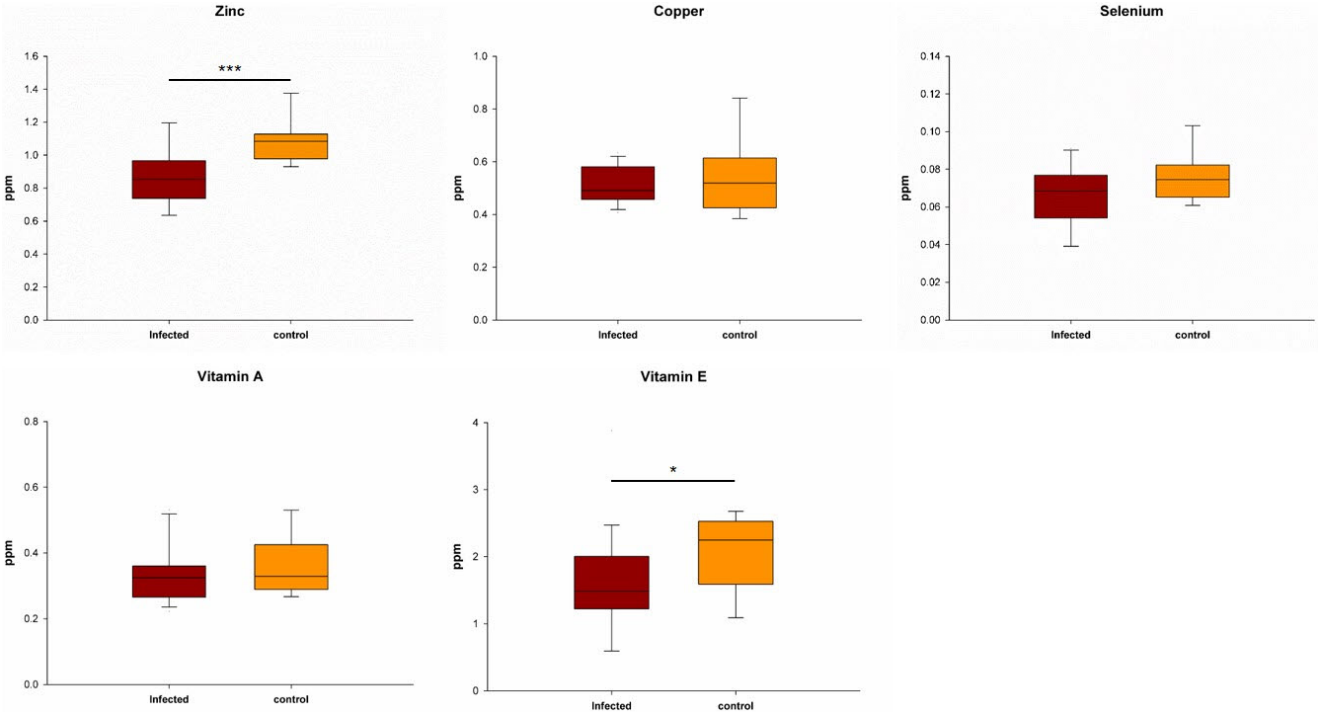

Fig. 1. The concentrations of trace minerals (zinc, copper, and selenium) and vitamins (vitamins A and E) in calves with dermatophytosis and healthy calves.

Table 1. Five serum biochemical parameters in calves with dermatophytosis and healthy calves

Parameters	Infected group	Control group	*P*-value
Glucose (mg/dL)	77.95±4.87	63.15±2.14	0.008
Globulin (g/dL)	3.89±0.08	3.75±0.10	0.298
ALKP (U/L)	214.25±30.84	149.00±9.63	0.061
GGT (U/L)	18.55±1.54	15.45±0.96	0.104
TBIL (mg/dL)	0.23±0.03	0.36±0.02	0.003

Data are presented as mean±standard error of mean (X±SEM). ALKP, alkaline phosphatase; GGT, gamma-glutamyl transferase; TBIL, total bilirubin.

and vitamin E (P=0.02) than the healthy calves. Se, Cu, and vitamin A concentrations were also lower in the infected group than in the control group; however, the difference was not statistically significant.

Serum biochemical parameters

The concentrations of the five serum biochemical parameters are presented in Table 1. The concentrations of glucose (P=0.008) and TBIL (P=0.003) were significantly higher in the infected group than in the control group. Serum globulin, ALKP, and GGT levels were higher in the infected group than in the control group; however, the difference was not statistically significant. Notably, hyperglobulinemia (3.89 g/dL, reference range; 2.7~3.8) and high ALKP (214.25 U/L, reference range; 10~149) levels were observed only in the infected group.

Serum cytokines

To investigate the effect of dermatophytosis on the host's immune state, we analyzed the four immune-related cytokines including IFN-γ, IL-6, IL-10, and IL-17A

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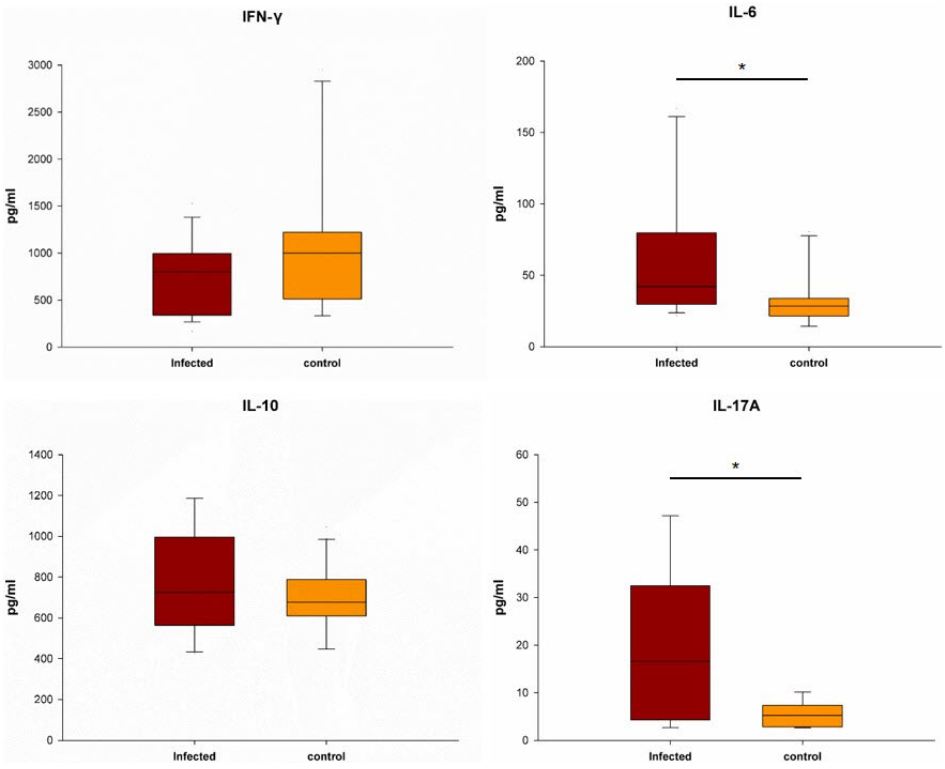

Fig. 2. The levels of four serum cytokines (IFN-γ, IL-6, IL-10, and IL-17A) in calves with dermatophytosis and healthy calves.

(Fig. 2). IL-6, IL-10, and IL-17A levels were higher in the infected than in the control group, with IL-6 (P=0.014) and IL-17A (P=0.018) showing significant differences. The IFN-γ level was lower in the infected group, however, the difference was not significant.

DISCUSSION

Dermatophytosis negatively impacts animal growth, skin health, and meat production (Hameed et al., 2017). Therefore, preventing and managing this disease is crucial to avoiding significant economic losses in livestock. Calves are highly susceptible to diseases because their immune system is not yet fully developed (Chase et al., 2008). The relationship between the deficiency of certain micronutrients and dermatophyte infections is not fully understood; however, micronutrients have been reported to affect various aspects of immunity in cattle (Spears, 2000). Therefore, we analyzed various nutritional factors and immune-related cytokines in calves with dermatophyte infections, comparing them with healthy calves.

We aimed to minimize other factors that may be associated with nutrition, immune state, and dermatophytosis in calves (Al-Ani et al., 2002; Davies et al., 2022). In the present study, we selected a large farm with calves of similar age, raised under management conditions, and fed the same diet similar to those in previous reports (Betul, 2020; Sezer et al., 2021). Furthermore, we selected calves with similar clinical signs, which could be attributed to various factors, such as the host's immune response and fungal virulence (Kojouri et al., 2009). In the present study, only *T. verrucousm*, one of the most prevalent agents causing bovine dermatophytosis, was found in the infected calves, consistent with the findings of previous studies (Kojouri et al., 2009; Betul, 2020).

Micronutrients, including vitamins and trace minerals, are essential for growth, performance, and overall health. In particular, Zn is crucial for immune function and maintaining the integrity of epithelial tissues. As shown in Fig. 1, the infected calves had significantly

lower serum Zn levels, consistent with Al-Qudah et al. (2010). The role of Zn in bovine immunity has been thoroughly investigated in a previous study (Kesler and Abuelo, 2024). Zn supplementation is beneficial in maintaining the epithelial barriers of the skin and mucous membranes. Zn deficiency can lead to skin issues such as alopecia, a rough coat, scaling skin, and lesions (Blackmon et al., 1967). Furthermore, fungal skin infections such as facial eczema, caused by sporidesmin, a fungal toxin, respond well to Zn supplementation (Rickard, 1975).

Vitamin E, Se, and Cu are antioxidants essential for maintaining healthy skin (Wintergerst et al., 2007). In the present study, serum vitamin E levels were significantly lower in the infected group than in the control group. However, Se and Cu levels showed a tendency to decrease in the infected group without reaching statistical significance, which slightly differs from the findings of previous reports in which significant differences were observed between the groups (Al-Qudah et al., 2010). One possible reason for this discrepancy is the clinical state of the infected calves. In the present study, we selected only mildly infected calves based on specific criteria not considered in previous studies. Furthermore, the roles of these antioxidants slightly differ. Vitamin E inhibits the production of nitric oxide and prostaglandin E2, whereas Se and Cu are cofactors of cytoplasmic superoxide dismutase and glutathione peroxidase, respectively, which are both antioxidant enzymes (Lu et al., 2010). Further studies are required to clarify the relationship between antioxidants and trace elements.

Biochemical assays can be conducted to support a suspected diagnosis or indicate the type and severity of a disease. In the present study, liver function parameters were selected to assess calves with dermatophytosis (Otter, 2013). Hyperglobulinemia, elevated ALKP levels, and significantly high glucose and TBIL levels were observed in the infected group. These findings are consistent with those of previous reports (Atakisi et al., 2006; Kabu and Koca, 2018; Davies et al., 2022). Notably, it has been suggested in several studies that liver damage may be associated with toxic metabolic products produced by fungi. However, the relationship between liver damage and dermatophytosis remains unclear.

Cytokines regulate the immune system during fungal skin infections (Paryuni et al., 2020). IL-17A, produced by adaptive Th17 cells and innate lymphocytes, helps clear dermatophytes by inducing the production of various cytokines (tumor necrosis factor and IL-6), chemokines (C-X-C motif chemokine ligand 1 [CXCL1] and CXCL8), and antimicrobial peptides (human beta-defensin 2 and human cathelicidin) (Burstein et al., 2020). In the present study, the infected group exhibited higher IL-6 and IL-17A levels due to Th17-mediated immunity in dermatophytosis. IFN-γ, which is produced by T and natural killer cells, is essential for innate immune responses to fungal infections. It boosts the migration, adherence, and antifungal functions of neutrophils and macrophages. Conversely, IL-10 has a multifaceted role in managing the immune response to fungal infections, mainly by reducing the fungicidal activity of monocytes and neutrophils (Antachopoulos and Roilides, 2005). In the present study, the infected group exhibited lower IFN-γ and higher IL-10 levels than the control group. This suggests that the Th1 response was inhibited, leading to potential immune suppression. Collectively, the decrease in IFN-γ levels, along with the increase in IL-6, IL-17A, and IL-10 levels, suggests that the disease has progressed from the initial cell-mediated immune response to an antibody-mediated response, involving a regulation of both pro-inflammatory and anti-inflammatory pathways, which indicates how the immune system adapts to chronic fungal infections, providing valuable insights into the disease's progression.

The present results suggest that bovine dermatophytosis might be associated with deficiencies in micronutrients such as zinc and vitamin E, which in turn could affect liver-related biochemical markers (glucose and TBIL). Additionally, the findings confirmed that immune factors, including pro-inflammatory cytokines (IL-6 and IL-17A) and anti-inflammatory cytokines (IL-10), play

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significant roles in disease progression. However, to clarify the relationship between dermatophytosis and its risk factor or indicator, supplementation of micronutrients of calf dermatophytosis in large-scale experiments is necessary. Despite these limitations, our findings may help farmworkers to prevent and manage bovine dermatophytosis.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

ORCID

Han Gyu Lee, https://orcid.org/0000-0002-3531-1971
Ara Cho, https://orcid.org/0000-0001-5309-7721
Ui-Hyung Kim, https://orcid.org/0000-0002-2197-5080
Sihyun An, https://orcid.org/0000-0002-1743-1961
Yoon Jung Do, https://orcid.org/0000-0003-3207-3514
Changyong Choe, https://orcid.org/0000-0003-4222-3360
Are Sun You, https://orcid.org/0000-0001-7258-2626
Young-Hun Jung, https://orcid.org/0000-0002-8094-0304
Seogjin Kang, https://orcid.org/0000-0001-7838-9042
Eun-Yeong Bok, https://orcid.org/0000-0002-1045-9670

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