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Prevalence of Clinical Dermatophytosis and Oxidative Stress in Cattle

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Abstract: In this study, skin scrapings and hair samples were collected from 50 cattle with clinically suspected of dermatophytosis in the province of Kirikkale, Turkey. Moreover, blood samples were collected from 10 clinically healthy cattle and 37 cattle with clinically suspected of dermatophytosis to investigate the influence of dermatophytosis on some biochemical profiles, lipid peroxidation and antioxidant system. The collected samples were directly examined for fungal elements by direct microscopy. The isolation was made from clinically diagnosed about 50 cattles belonging to Dermatophytosis by 22 (44%). The distribution of isolates was *Trichophyton verrucosum* 19/50 (38%), *Trichophyton mentagrophytes* 3/50 (6%), respectively. The influence of dermatophytosis on plasma Malondialdehyde (MDA), total protein, albumin, uric acid levels, vitamin A and β-carotene, vitamin C levels on serum Cu, Zn and Fe elements, in the erythrocyte Catalase (CAT) and Superoxide Dismutase (SOD) enzyme activity was investigated. There was no significant difference between the control and dermatophitic groups for total protein, albumin, vitamin A and C values. However, plasma MDA, erytrocyte CAT ($p<0.05$) levels and SOD ($p<0.01$) enzyme activity and plasma β-carotene, uric acid and serum Zn, Cu ($p<0.05$), Fe ($p<0.01$) levels in dermatophitic cattles were statistically lower than those of control group. The result of the study revealed that cattle dermatophytosis is highly prevalent in the Central of Anatolia, Kirikkale province. In addition, the effects of lipid peroxidation and antioxidant system have been determined during the pathogenesis of disease.

Key words: Antioxidant status, cattle, dermatophytosis, trichophyton, oxidative stress, prevalence

INTRODUCTION

Dermatophytosis also named Ringworm, a mycotic infection of keratinized tissues causes zoophilic dermatophytes in humans and animals (Efuntoye and Fashanu, 2001; Krakhecke *et al*., 2005; Biberstein and Hirsh, 2004). *Trichophyton verrucosum* is the most common etiologic agent of cattle. *T. mentagrophytes*, *T. rubrum, T. simii* and *Microsporum gypseum* are also caused bovine dermatophytosis (Khosravi and Mahmoudi, 2003; Mitra *et al*., 1998). The etiologic agents of bovine dermatophytosis have been reported in other countries (Khosravi and Mahmoudi, 2003; Cabanes *et al*., 1997; Ranganathan *et al*., 1998). Although, zoophilic dermatophytosis has been reported in a previous study of bovine dermatophytosis in Turkey, there has been a limited number of studies on bovine dermatophytosis (Altay *et al*., 2003; Karapehlivan *et al*., 2007; Ozkanlar *et al*., 2009). The inbalance between prooxidants and antioxidants cause skin diseases (Podda and Grundmann-Kollmann, 2001). The aim of the present study was to determined of the prevalence of dermatophytosis in cattle and other related factors in the province of Kirikkale, Turkey. The study further aimed to reveal the effect of dermatophytosis on some biochemical parameters, lipid peroxidation and antioxidant system.

MATERIALS AND METHODS

Hair and skin scrapings were collected from 10 healthy and 50 cattles with dermatophytosis in Kirikkale province, Turkey during the winter housing season. The affected 50 calves were 2-8 month old were suffering from circle-shaped alopecia and scruff around the area of head and neck. Blood samples were collected into heparinized tubes from the jugular vein of 10 healthy cattles and 37 cattles with dermatophytosis to determine lipid peroxidation and some antioxidant systems. Blood samples were collected non anticoagulant tubes to determine Zinc (Zn), Copper (Cu) and iron (Fe) elements. Blood samples centrifuged at 3000 rpm for 10 min to separate plasma and serum. The plasma and buffy coat layer were removed and the erythrocytes were washed three times with cold Phosphate Buffer Saline (PBS) and

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then frozen at -86°C until analysis. Skin scrapings and brushings were collected from infected sites after cleaning with 70% alcohol. The collected materials were examined for fungal elements by direct microscopy in 10% potassium hydroxide and lactophenol cotton blue mounting fluid and were inoculated on Saboraud Dextrose Agar plates with antibiotics (0.5 mg mL^{-1} cycloheximide and 0.05 mg mL^{-1} chloramphenicol). The plates were incubated at 28 and 37°C. for a month. Multiple inoculations in dublicates were done. The identification of the dermatophyte species was based on their macroscopic and microscopic characteristics of the colonies, special nutritional requirements, urease test and in vitro hair perforation test (Cabanes *et al*., 1997).

Plasma total protein, albumin, uric acid levels were measured in a spectrophotometer (Shimadzu UV-1700, Japan) using commercial kits (TECO, USA). Plasma malondialdehid level was determined according to the method of Moreno *et al*. (2003). Vitamin C (Haag, 1985), vitamin A ve β-carotene levels were determined according to the methods of Suzuki and Katoh (1990). The process of washing of erytrocyte was conducted according to the Winterbourn *et al*. (1975). The detection of hemoglobin level was measured according to the Fairbanks and Klee (1987). Catalase activity and superoxide dismutase activity in erytrocytes were determined according to Aebi (1983) and Sun *et al*. (1988), respectively. Serum Zn (213.857 nm), Cu (327.395 nm) and Fe (238.204 nm) levels were measured in a ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometer) (Varian-Vista) (Lai and Jamieson, 1993).

Statistical analysis: Descriptive statistics and comparison of results were performed using SPSS 13.0 packet programme. Independent-Samples t test was used to compare the parameters between groups. Data were expressed as mean±standard error.

RESULTS

Clinical findings: In clinical examinations of the cattles; scurfy, local alopecic, circle-shaped thick, white, asbestos plaques and erythematous lesions were observed throughout the body mostly in head and neck regions.

Mycological and biochemical findings: In the present study, prevalence rates of cattle dermatophytosis along with some demographic data are shown in Table 1. In the present study, the distribution rate of *Trichophyton* sp. in 50 symptomatic cattles is shown in Table 2. About 19/50 isolates were identified as *Trichopyhton verrucosum*. About 3/50 isolates were identified as *Trichopyhton mentagrophytes*. The effects of cattle dermatophytosis on biochemical parameters, lipid peroxidation and antioxidant system activity affected cattles are shown in Table 3.

Table 1: Dermatophytosis positive prevalence rates in cattles

Variables	No. of positive samples	Total samples	Prevalence (%)
Sex			
Males	6	17	35.2
Females	16	33	48.4
Age			
≤6 months	6	18	33.3
≤6 months	13	32	40.6
Breeding system			
Intensive	0	0	0.0
Semi-intensive	27	50	54.0
Management system			
Conventional	27	50	54.0
Organic	0	0	0.0
Breed			
Holstein	21	39	53.8
Simental	3	11	27.2

Table 2: The distribution rate of *Trichopyhton verrucosum* and *T. mentagrophytes* based on isolation in clinically effected cattles

Host (n = 60)	*T. verrucosum*	*T. mentagrophytes*
Cattle (Control) n = 10	-	-
Cattle (Infected) n = 50	19/50 (38%)	3/50 (6%)
Total no. of isolates		22 (44%)

Table 3: The effects of cattle dermatophytosis on lipid peroxidation, antioxidant systems and some biochemical parameters in cattles

Parameters	Control (n = 10)	Dermatophyte (n = 37)	p-value
MDA (μmol L^{-1})	2.06±0.13	1.44±0.14	*
SOD (U/gHg)	12.71±1.43	6.42±0.40	**
CAT (k/gHg)	0.42±0.03	0.35±0.01	*
Total protein (g dL^{-1})	6.67±0.19	6.98±0.18	-
Albumin (g dL^{-1})	2.85±0.09	2.75±0.07	-
Uric acid (mg dL^{-1})	2.11±0.21	1.67±0.08	*
β-carotene (μg dL^{-1})	37.34±4.36	24.29±2.66	*
Vitamin A (μg dL^{-1})	82.11±9.38	73.71±7.24	-
Vitamin C (μg mL^{-1})	4.00±0.50	3.6±0.300	-
Zn (mg L^{-1})	1.44±0.08	1.15±0.06	*
Cu (mg L^{-1})	0.88±0.04	0.75±0.02	*
Fe (mg L−1)	3.82±0.17	3.04±0.13	**

-: Not significantly, *: $p<0.05$, **:$p<0.01$

There was no significant difference between the control and dermatophitic groups for total protein, albumin, vitamin A and C values. However, plasma MDA ($p<0.05$), erytrocytes CAT ($p<0.05$) and SOD ($p<0.01$) enzyme activity and plasma β-carotene, uric acid, serum Zn, Cu ($p<0.05$) and Fe ($p<0.01$) levels in dermatophitic cattles were significantly lower than those of control group.

DISCUSSION

There were a number studies on the prevalence of cattle dermatophytosis in the world (Khosravi and Mahmoudi, 2003; Mitra *et al*., 1998; Cabanes *et al*., 1997; Ranganathan *et al*., 1998; Ming *et al*., 2006) but the

number of such studies in Turkey is considerably smaller (Altay *et al*., 2003; Karapehlivan *et al*., 2007; Ozkanlar *et al*., 2009). Thus, the present study is import as it provides new data on cattle dermatophytosis in a certain Central Anatolia, Kirikkale province of Turkey.

Khosravi and Mahmoudi (2003) reported the prevalence rates of 85% for *Trichophyton verrucosum*, 15% for *T. mentagrophytes* in cattle with skin lesions. *Trichophyton verrucosum* is known to be more prevalent in cattle because of acquired resistance with increasing age in crowding conditions because of close contacts between animals and in winter season because of high humidity. In the present study, *T. verrucosum* was isolated from 38% of symptomatic cattle and *T. mentagrophytes* was isolated from 6% from of symptomatic cattle in Kirikkale Province. The isolation rate in the present study were similar those of Khosravi's findings in Iran. Mitra *et al*. (1998) indicated that out of 21 clinical cases in cattle, 23.8% were found to be dermatophitic origin in different areas of Utar Pradesh of India. They reported that the culture positive samples yielded one strain each of *Trichophyton mentagrophytes*, *T. rubrum*, *T. simi* and two strains of *Microsporum gypseum*. In the study to the rate of strains were 38% *T. verrucosum* and 6% *T. mentagrophytes*, respectively. It was suggested that the prevalance rates were linked to virulence of prevalant species and geographic areas.

Cabanes *et al*. (1997) reported that *Trichophyton verrucosum* was the only dermatophyte isolated from ruminants in Barcelona, Spain. Ranganathan *et al*. (1998) indicated that 13 *M. gypseum* and 4 *T. mentagrophytes* strains were isolated from 170 bovines (120 cows and 50 buffaloes) in Madras, India. In the present study, the distribution rate in cows was similar to those of the previous studies (Cabanes *et al*., 1997; Ranganathan *et al*., 1998). Ming *et al*. (2006) reported that 20% of 1000 dairy cows were infected with *Trichophyton verrucosum* in a newly established dairy farm in western China. They reported that the western of China was endemic region for *Trichophyton verrucosum* infection and it was determined depending on the region as a result of contact with animal care of the cattle. In the study, the prevalence rates indicated that the Central Anatolia, Kirikkale province of Turkey might be endemic for *Trichophyton verrucosum* and *Trichophyton mentagrophytes* infection.

The skin is constantly exposed to oxidative stress induced by Reactive Oxygen Species (ROS) that are generated both from endogenous sources such as enzyme activity and external pro-oxidant stimuli such as ultraviolet radiation. Reactive oxygen species are generated by cells under certain physiological conditions. Insufficient antioxidant protection or excess production of ROS causes oxidative damage. The balance between oxidative damage and antioxidant enzyme systems appears to determine the physiological and pathological effects of ROS (Koca *et al*., 2004).

Depending on the damage of skin, the free radicals are increased and so that this causes the lipid peroxidation (Koca *et al*., 2004). Karapehlivan *et al*. (2007), reported that the increase to level of MDA because of peroxidative membrane damage. Formation of free radicals and subsequent lipid peroxidation may be caused by dermatophytosis through the production of ROS following skin damage. They suggested that the increase level of MDA in dermatophytic calf blood serum indicated as caused peroxidative process in cell membranes. In the present study, the levels of MDA were found statistically significant higher rate ($p<0.05$).

The skin antioxidant system consists of enzymatic and non-enzymatic antioxidants. Among enzymatic antioxidants, glutathione peroxidase, catalase, superoxide dismutase play a piyotal role. Non-enzymatic antioxidants present in cells are vitamin A, C, E, β-carotene, uric acid and albumin (Fuchs, 1998; Podda and Grundmann-Kollmann 2001; Albers *et al*., 2003). Vitamin levels and antioxidant enzyme activities related dermatophytosis any assesment was not found previous studies. In the present study, SOD ($p<0.01$) and CAT ($p<0.05$) enzymes activities were decreased in erythrocyte. The decreases in the activities at CAT and SOD may be attributed to the consumption of antioxidant enzymes resulted from increasing of the free radicals due to the skin damaging. However, there were some studies that indicate the role of Zn, Cu and Fe included in the structure of SOD and CAT antioxidant enzymes in the pathophysiology of dermatophytosis. It has been demonstrated that Cu, Zn and Fe levels decrease because of stress factors (Podda and Grundmann-Kollmann, 2001; Albers *et al*., 2003). In the present study, Fe ($p<0.01$), Zn and Cu ($p<0.05$) as well as reduced levels reduce in the structure of erythrocyte SOD ($p<0.01$) and CAT ($p<0.05$) enzyme have been observed in dermatophitic cattles. There were important evidence of the influence of Zn, Cu and Fe levels on the pathophysiology of dermatophytosis. Uric acid is an antioxidant and it is present in all tissue and changes in its level may suggest pathology. Uric acid cleans hidroxyl, superoxide and singlet oxygen and peroxyl radicals (Podda and Grundmann-Kollmann, 2001; Briganti and Picardo, 2003). Biesalski and Frank (1995) indicated that albumin and uric acid with vitamin C generates total antioxidant capacity. Karapehlivan *et al*. (2007) uric acid levels may result from changes in the pathology of the disease were reported. Similarly, uric acid level was significantly lower in cattles

with dermatophytosis than control group in the present study. Several studies have proven the efficacy of β-carotene in inhibiting the production of singlet oxygen free radicals (Briganti and Picardo, 2003). However, β-carotene under physiological conditions, tissue oxygen partial pressure in the lower case as a powerful antioxidant that supports immune response is noted (Burton and Ingold, 1984). In the present study, uric acid and β-carotene levels were observed significantly decreased ($p<0.05$) in dermatophitic cattle compared to healthy control because of immunosupression caused by dermatophytosis.

CONCLUSION

As a result, the current report shows that *T. verrucosum* infection may be widespread in cattle in the Central of Anatolia region, more than expected. In addition to the results suggest that there is a possible link between antioxidant imbalance, lipid peroxidation and dermatophytosis. However, further studies are necessary to delineate the pathogenesis of dermatophytosis in this respect. Use of antifungal agents together with antioxidative drugs seems to reasonable in the treatment of dermatophytosis in cattle.

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REFERENCES

Aebi, H.E., 1983. Catalase. In: Methods of Enzymatic Analysis, Bergmeyer, H.U. (Ed.). Verlag Chemie, Weinhem, pp: 273-286.

Albers, R., M. Bol, R. Bleumink, A.A. Willems and R.H. Pieters, 2003. Effects of supplementation with vitamins A, C and E, selenium and zinc on immune function in a murine sensitization model. Nutrition, 19: 940-946.

Altay, G., L. Kökcü, M. Akan and O. Keskin, 2003. The isolation of dermatophytes from domestic animals. Vet. Hek. Mikrobiyol. Derg., 3: 5-8.

Biberstein, E.L. and D.C. Hirsh, 2004. Dermatophytes. In: Veterinary Microbiology, Hirsh, D.C., N.J. MacLachlan and R.L. Walker (Ed.). Blackwell Publishing, Oxford, pp: 273-284.

Biesalski, H.K. and J. Frank, 1995. Antioxidants in nutrition and their importance in the anti/oxidative balance in the immune system. Immun. Infekt, 23: 166-174.

Briganti, S. and M. Picardo, 2003. Antioxidant activity, lipid peroxidation and skin diseases: What's new. JEADV, 17: 663-669.

Burton, G.W. and K.U. Ingold, 1984. â-Carotene: An unusual type of lipid antioxidant. Science, 224: 569-573.

Cabanes, F.J., M.L. Abarca and M.R. Bragulat, 1997. Dermatophytes isolated from domestic animals in Barcelona, Spain. Mycopathologia, 137: 107-113.

Efuntoye, M.O. and S.O. Fashanu, 2001. Fungi isolated from skins and pens of healthy animals in Nigeria. Mycopathologia, 153: 21-23.

Fairbanks, V.F. and G.G. Klee, 1987. Biochemical Aspect of Haematology. In: Fundamentals of Clinical Chemistry, Tietz, N.W. (Ed). 3rd Edn., W.B. Saunders Company, Philadelphia.

Fuchs, J., 1998. Potentials and limitations of the natural antioxidants RRR-alpha-tocopheral L-ascorbic acid and b-carotene in cutaneous photoprotection. Free Radic. Biol. Med., 25: 848-873.

Haag, W., 1985. Praktischen Bedeutung der C Vitamini-bestimmung Beim Rind in Vergangenheit und Gegenwart. Justus Liebig Universitaet, Giessen.

Karapehlivan, M., E. Uzlu, N. Kaya, O. Kankavi, K. Ural and M. Citil, 2007. Investigation of some biochemical parameters and the antioxidant system in calves with dermatophytosis. Turk. J. Vet. Anim. Sci., 31: 85-89.

Khosravi, A.R. and M. Mahmoudi, 2003. Dermatophytes isolated from domestic animals in Iran. Mycoses, 46: 222-225.

Koca, R., F. Armutcu, H.C. Altinyazar and A. Gürel, 2004. Oxidant-antioxidant enzymes and lipid peroxidation in generalized vitiligo. Clin. Exp. Dermatol., 29: 406-409.

Krakhecke, A.G., E. Afonso, J.C. Ferreira and R.C. Candido, 2005. *In vitro* susceptibility testing of *Microsporum gypseum* isolated from healthy cattle and soil samples against itraconazole, terbinafine, fluconazole and topical veterinarian drugs. Mycopathologia, 159: 377-380.

Lai, J.J. and G.C. Jamieson, 1993. Determination of dysprosium in monkey serum by inductively coupled plasma atomic emission spectrometry (ICP-AES) after the administation of sprodiamide injection, a new contrast medium for magnetic resonance imaging. J. Pharm. Biomed. Anal., 11: 1129-1134.

Ming, P.X., Y.L. Xia and G.S. Bulmer, 2006. Outbreak of *Trichophyton verrucosum* in China transmitted from cows to humans. Mycopathologia, 161: 225-228.

Mitra, S.K., A. Sikdar and P. Das, 1998. Dermatophytes isolated from selected ruminants in India. Mycopathologia, 142: 13-16.

Moreno, I.M., A. Mate, G. Repetto, C.M. Vazquez and A.M. Camean, 2003. Influence of microcystin-LR on the activity of membrane enzymes in rat intestinal mucosa. J. Physiol. Biochem., 59: 293-300.

Ozkanlar, Y., M.S. Akta° and E. Kireci, 2009. Mycozoonosis associated with Ringworm of calves in Erzurum Province, Turkey. Kafkas Univ. Vet. Fak. Derg., 15: 141-144.

Podda, M. and M. Grundmann-Kollmann, 2001. Low molecular weight antioxidants and their role in skin ageing. Clin. Exp. Dermatol., 26: 578-582.

Ranganathan, S., S.A.M. Balajee and M. Raja, 1998. A survey of dermatophytosis in animals in Madras, India. Mycopathologia, 140: 137-140.

Sun, Y., L.W. Oberley and L.I. Yaa, 1988. Simple for clinical assay of superoxide dismutase. Clin. Chem., 34: 497-500.

Suzuki, J. and N. Katoh, 1990. A simple and cheap methods for measuring serum vitamin A in cattle using only a spectrophotometer. Nippon Juigaku Zasshi, 52: 1281-1283.

Winterbourn, C.C., R.E. Hawkins, M. Brian and R.W. Carrell, 1975. The estimation of red cell superoxide dismutase activity. J. Lab. Clin. Med., 85: 337-341.