Hematological, Biochemical And Antioxidant System Alterations In Dermatophytosis In Calves

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ABSTRACT

Dermatophytosis is a skin disease of worldwide distribution and responsible for high economic losses in cattle. It is exclusively caused by *Trichophyton verrucosum*. Ten cross- bred calves, 10 – 12 months old, suffering from skin lesions (alopecia and circular circumscribed grayish-white crusty raised foci which mostly found on the head, external ears and neck) and ten healthy control calves were obtained from a private farm in Gharbia governorate. Mycological examination of skin scrapings and hairs from lesions of the affected calves revealed *Trichophyton verrucosum* to be the etiological agent. Blood and serum samples were collected from both groups for hematological examinations, serum biochemical analysis and evaluation of the antioxidant system.

Results of the present work declared that dermatophytosis elicited a significant decrease in the red blood cell count, hemoglobin concentration and packed cell volume. Moreover, the disease produced leukocytosis, neutropenia and lymphocytosis in addition to non significant change in the monocytes, eosinophils and basophiles. A significant increase in the adenosine deaminase (ADA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) reflected the effect of dermatophytosis on the liver function. Evaluation of the antioxidant parameters in affected calves revealed a significant increase in the malonedialdehyde (MDA) and nitric oxide (NO) associated with a significant decrease in the reduced glutathione (GSH) and zinc levels suggesting a possible relationship between the antioxidant system, oxidative stress, lipid peroxidation and dermatophytosis. Moreover, dermatophytosis provoked a significant increase in the serum total sialic acid (TSA) and lipid - bound sialic acid (LBSA) as markers of inflammation and injury in calves.

It could be concluded that dermatophytosis caused a significant hematological, serum biochemical and antioxidant system alterations. Moreover, it is recommended to use antioxidants, as supportive treatment for dermatophytosis.

INTRODUCTION

Dermatophytosis is an infectious disease of animals caused by different species of keratinophilic fungi. It is a major public and veterinary health problem, reported from different parts of the world and cause great economic losses *(1)*. The disease appears to be more common in tropical than temperate climates particularly in countries having hot and humid climatic conditions. Dermatophytosis is exclusively caused by *Trichophyton spp.* and *Microsporum spp. Trichophyton verrucosum*, *Trichophyton mentagrophyte* and *Trchophyton gypseum* which are the common fungi involved in bovine dermatophytosis *(2)*. Dermatophytes are keratinophilic fungi that are able to invade the stratum corneum of the skin and other keratinized structures. The pathogenic interaction between host and fungus are poorly understood *(3)*. Dermatophytes exhibit activity of extracellular enzymes including elastase, keratinase, protease, lipase and phospholipase *(4)*. Although it has been reported that liver tissue can be influenced by some skin diseases such as superfacial dermatitis, metabolic epidermal necrosis or atopic dermatitis *(5)*, enough studies have not been done to investigate the possible effects of

dermatophytosis on liver functions. Moreover, there is scarcity of information on biochemical changes, especially regarding the oxidative stress and antioxidant system during natural dermatophytosis. Therefore, the aim of the present study was to investigate the hematological changes, liver function, oxidative status and the possible relationship between dermatophytosis and other relevant indicators such as total sialic acid and lipid bound sialic acid in cross bred cow - calves.

MATERIAL AND METHODS

Animals

Twenty calves (10-12 month old), 10 healthy (gp 1) and 10 suffering from dermatophytosis (gp 2), belonged to a private farm in Gharbia governorate. Healthy and diseased calves were kept in different barns on the same farm. Skin and complete clinical examination of all calves were carried out including general condition, respiration, heart rate and rectal temperature. Parasitological examination of fecal matter was carried out *(6)* to ensure that the calves were free from the internal parasites.

Mycological examination

The skin lesion was cleaned with a cotton swab soaked with ethyl alcohol, samples were collected by scraping the lesion using sterile scalpel blade and put into sterile Petri dishes. The collected samples were divided into two portions. The first portion was subjected to direct microscopical examination *(7)*. The second portion was used for culture on Sabouroud's dextrose agar media with chloramphenicol and cyclohexidine.The plates were kept at 27°C for 3 weeks. Identification of the colonies was carried out *(8)*.

Blood sampling

Two blood samples were collected from the jugular vein of animals of gps 1&2. The first blood sample was collected in a test tube containing EDTA sodium as anticoagulant and used for hematological examination. The second blood sample was collected in a test tube, left to clot and centrifuged for serum separation and subjected to serum biochemical analysis.

Hematological examination

The RBCs. count, PCV, MCV, MCH, MCHC, total and differential leukocytic count were performed *(6)*. The hemoglobin was measured colorimetrically *(9)*.

Serum biochemical analysis

Serum adenosine deaminase (ADA) was determined *(10)*. AST and ALT activities were determined *(11)*. Lactate dehydrogenase (LDH) *(12)* and gamma glutamyl transferase (GGT) *(13)* were measured. Serum total proteins and albumin were determined colorimetrically using commercial kits (BioMeriux, France). Total sialic acid (TSA) *(14)* and lipid-bound sialic acid (LBSA) levels *(15)* were measured colorimetrically according to the previously detailed method.

Oxidative status

The oxidative status was evaluated by the determination of serum malonedialdehyde (MDA) *(16)*, reduced glutathione (GSH) content *(17)*, nitric oxide (NO) *(18)* and serum zinc by atomic absorption spectrophotometer *(19)*.

Statistical analysis

The obtained data were analyzed by student's "t" test *(20)*.

RESULTS AND DISCUSSION

Dermatophytes are among the most frequent causes of dermatological problems in domestic animals. The superfacial mycosis caused by dermatophytes is reffered as dermatophytosis or ringworm *(21)*. It is generally a cutaneous pathogen which is restricted to the cornified layers of the skin. The clinical signs observed during this study, included the appearance of circumscribed circular areas of alopecia, scaling and crusting. Moreover, multifoccal lesions varied considerably in size. Later, these lesions coalesced forming large irregular lesions. These skin lesions were most commonly found on the face, external ears and neck (figs.1-6).

The infected calves showed a moderate inappetance and discomfort. Similar clinical signs were previously recorded *(22, 23, 24, 25).* The mycological examination of the skin scrapings and hairs revealed that *Trichophyton verrucosum* was the etiological agent. The colonies texture was waxy or glabrous, heaped or flat. Thallus color was white, grey or yellow. The reverse side of colonies was colorless (fig.7). Similar results were previously reported *(22, 23, 26).*

Regarding to the effect of dermatophytosis on hematological parameters, the disease led to a significant decrease in RBCs. count, hemoglobin concentration and packed cell volume in addition to non significant change in the MCV, MCH and MCHC (Table 1).The decrease in erythrogram may be attributed to calves inappetance. Similar results were previously recorded *(23, 27, 28).* Dermatophytosis elicited leukocytosis, neutropenia and lymphocytosis associated with non significant changes in the monocytes, eosinophils and basophiles (Table 1). Similar results were previously recorded *(28).* On the contrary, our results partially disagree with a previous work *(27).* The difference may be attributed to the skin lesion intensity as in this work; calves were more severely affected with dermatophytosis. Dermatophytosis produced a significant increase in the serum ADA activity (Table2). Fungi produce different types of proteolytic enzymes specially keratinases that have a key role in fungal invasion and pathogenesis *(3, 4).* During invasion, the metabolic products of the fungi may penetrate the liver tissue by passing through the skin and blood circulation. An increase in ADA activity has been reported in animals with liver diseases *(29).* It was reported that the increased serum ADA activity in skin leishmaniasis and cattle dermatophytosis may be a reflection of the phagocytic activity of the macrophages. The serum activity of ADA is a marker of cellular immunity as it is elevated in diseases showing a cell–mediated immune response *(30).* Dermatophytosis elicited a significant increase in the serum activity of AST, ALT, LDH and GGT in calves (Table2). Due to intracellular location of AST and ALT in the cytosol, diseases which damage the hepatic cell membrane, lead to their spilling and elevation *(31).* Our results revealed a significant increase in serum LDH and GGT activities in dermatophylic calves which may be point to liver damage. The increase in the liver enzymes in the current study might be induced by metabolic products of the fungi as previously recorded *(24).* Dermatophytosis leads to non significant change in the serum total protein and albumin. Similar results were previously cited *(25).*

Trichophyton verrucosum infection causes oxidative stress leading to alterations in homeostasis. The animal body has an adequate reserve against the production of free radicals, which are produced during metabolism *(32).* However, when the free radicals generation exceeds the antioxidant production capacity, oxidative stress occurs. Recently, there has been considerable interest in oxidative stress caused by the reactive oxygen species and its involvement in disease processes *(33).* It has been well recognized that the defense against the mycotic infections depends upon the oxidative killing of micro-organisms as exhibited during infection with Candida that require the cooperation of several immune cells through candidicidal mechanisms. Formation of free radicals and subsequent lipid peroxidation may be caused by dermatophytosis through the production of reactive oxygen species following skin damage *(25).* The endogenous antioxidant system contains catalase, superoxide dismutase, and glutathione peroxidase and glutathione reductase as enzymatic antioxidants, as well as non - enzymatic antioxidants in skin, including glutathione, uric acid and lipoic acid. The oxidative stress may be monitored using several biomarkers. The significant rise in MDA level and the significant decrease in the GSH level in calves suffering from dermatophytosis may reflect the work of the defense mechanisms against the lipid peroxidation during oxidative stress in dermatophytosis, the high level of MDA in the affected calves indicated the advanced peroxidation process in the cell membrane *(25).* NO plays a role in several biological

processes, including neurotransmission, immune defense and regulation of cell death (apoptosis). Moreover, NO may act as a mediator of inflammatory processes by stimulating the production of proinflammatory eicosanoids. In inflammatory conditions, NO production increases and causes tissue injury by reacting with superoxide to yield peroxynitrite, a powerful toxin *(34)*. In the present study, the significant rise in NO level may have been related to the pathogenesis of dermatophytosis in calves. Similar results were previously obtained *(25)*. Dermatophytosis elicited a significant decrease in the serum zinc level which agrees with previous results *(23, 35)*. Recent studies have reported that the concentration of SA in animals was increased in a number of diseases in which the underlying pathology is tissue damage, proliferation or inflammation *(36)*. Earlier studies recorded a significant increase in the TSA and LBSA level in dermatophytosis. There were significant increase in TSA and LBSA in dermatophylic calves in this study (table2). The rise in TSA and LBSA was attributed to the release of SA from the cell membrane into the circulation. Similar results were previously recorded *(25)*.

It could be concluded that dermatophytosis produced significant hematological alterations and significant increase in liver enzymes activities reflecting liver dysfunction. There were significant increase in the serum MDA and NO, significant decrease in GSH and serum zinc in addition to significant increase in serum TSA and LBSA. Moreover, it is recommended to use antioxidants, as supportive treatment for dermatophytosis.

Table (1). Some hematological parameters (Mean ± SE) in dermatophylic calves compared with the control calves. (n = 10 calves)

Parameters	Healthy control calves	Dermatophylic calves
RBCs. (10^6 / ul)	8.65 ± 0.30	7.12 ± 0.50*
Hemoglobin (gm/dl)	10.16 ± 0.50	8.05 ± 0.20**
PCV %	29.5 ± 0.36	24.12 ± 0.20**
MCV (fl)	34.12 ± 1.12	33.80 ± 1.03
MCH (Pg)	11.7 ± 0.16	11.30 ± 0.52
MCHC (%)	34.77±1.06	33.37±1.12
TLC (10^3 / ul)	9.78 ± 0.2	14.16 ± 0.6**
Neutrophils (10^3 / ul)	2.78 ± 0.81	2.21 ± 0.11*
Lymphocytes (10^3 / ul)	6.35 ± 0.36	10.76 ± 0.64**
Monocyte (10^3 / ul)	0.48 ± 0.06	0.53 ± 0.02
Eosinophils(10^3 / ul)	0.11 ± 0.08	0.14 ± 0.05
Basophil (10^3 / ul)	0.02 ± 0.001	0.03 ± 0.00

* Significant at $P < 0.05$ ** Significant at $P < 0.01$

Table (2). Some serum biochemical and antioxidant parameters (Mean ± SE) in dermatophylic calves compared with the control calves. (n = 10 calves).

Parameters	Healthy control calves	Dermatophylic calves
ADA (U/L)	6.73 ± 0.52	11.18 ± 0.46**
AST (U/L)	21.16 ± 1.18	33.11 ± 0.86*
ALT (U/L)	10.92 ± 0.16	16.26 ± 0.21
LDH (U/L)	501 ± 6.70	589 ± 8.12**
GGT (U/L)	6.63 ± 0.08	10.78 ± 0.32**
Total proteins (g/dl)	7.12 ± 0.80	6.83 ± 0.62
Albumin (g/dl)	4.08 ± 0.50	3.26 ± 0.15
Globulin (g/dl)	3.03 ± 0.12	3.55 ± 0.19
TSA(mg/dl)	49.12 ± 2.16	75.31 ± 4.16**
LBSA (mg/dl)	18.82 ± 1.63	30.16 ± 0.96**
MDA(umol/l)	3.65 ± 0.18	8.82 ± 0.56**
GSH (mg/dl)	73.16±6.23	41.51 ± 2.12**
NO (umol/l)	6.68 ± 1.12	14.56 ± 0.82**
Zinc (microgram/dl)	75.8 ± 4.9	44.3 ± 1.5**

*Significant at P < 0.05 ** Significant at P < 0.01

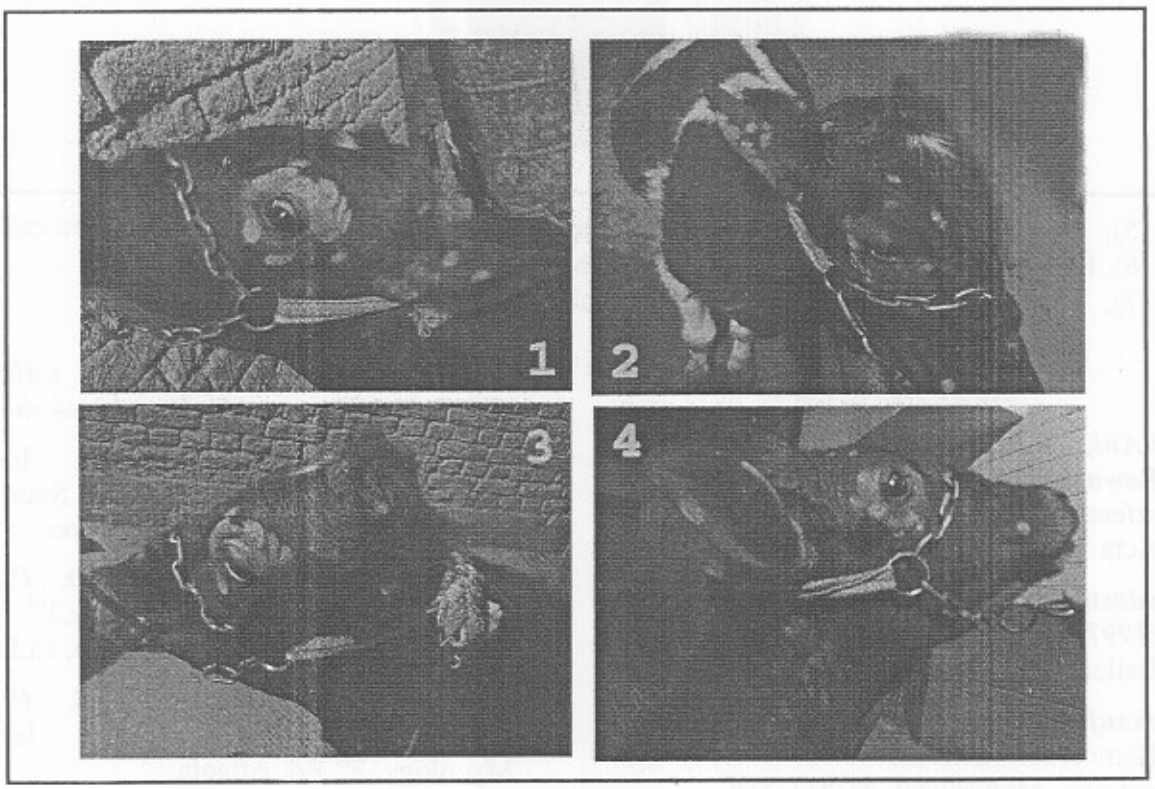

Fig. (1). Ring worm on external ears and around the eyes in cross bred calf.
Fig. (2). Ring worm on the external ears and face in cross bred calf.
Fig. (3). Ring worm on periorbital area in cross bred calf.
Fig. (4). Ring worm on the head, external ear and neck in cross bred c

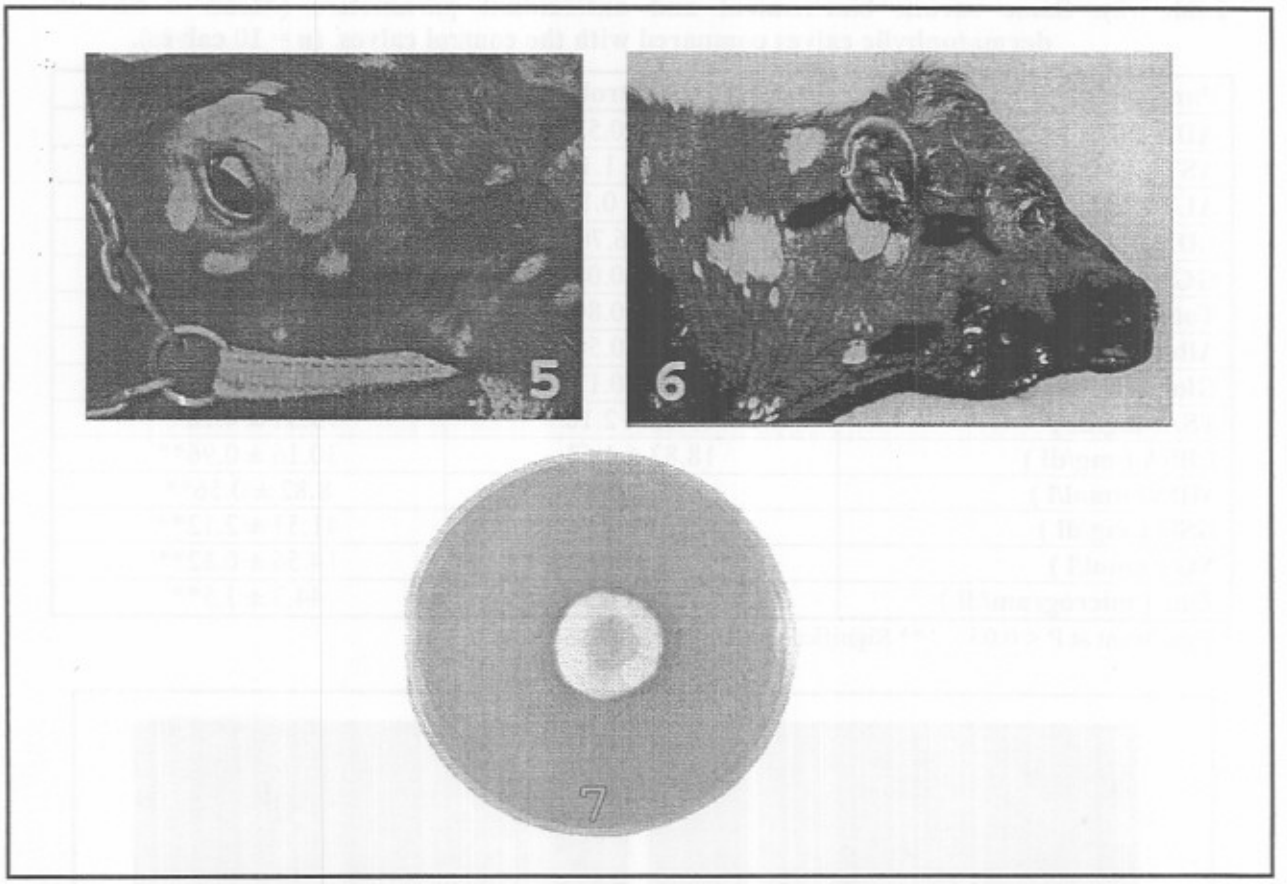

Fig. (5). Ring worms coalesce to each other around the eye and on the head of cross bred calf.
Fig. (6). Ring worms on head and neck of cross bred calf.
Fig. (7). *Trichophyton verrucosum* colony on Sabouroud's dextrose agar.

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الملخص العربى

التغيرات الهيماتولوجية و البيوكيميائية و مضادات الأكسدة في عجول الأبقار المصابة بمرض القراع

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يعتبر مرض القراع من الأمراض الفطرية الجلدية المعدية واسعة الانتشار في العالم و يؤدي هذا المرض إلي خسائر اقتصادية كبيرة في المزارع. اشتملت هذه الدراسة علي ٢٠ عجل أعمارهم ١٠-١٢ شهر منها ١٠ عجول تعاني من إصابات جلدية عبارة عن مناطق محددة و مستديرة خالية من الشعر و عليها بعض القشور و لوحظ أن الإصابات تركزت في مناطق الرأس و الأذن و الرقبة. كما اشتملت الدراسة علي ١٠ عجول سليمة كمجموعة ضابطة للتجربة و التي أجريت بأحدي المزارع بمحافظة الغربية.و قد أظهر الفحص المعملي الفطري لكحتات الجلد و الشعر من الأماكن المصابة في العجول المريضة وجود فطر ترايكوفيتون فيروكوسام كمسبب لهذا المرض. تم تجميع عينات دم و سيرم من كل العجول في المجموعتين. أظهرت نتائج هذه الدراسة أن مرض القراع في العجول قد أدي إلي نقص معنوي في كرات الدم الحمراء و الهيموجلوبين و حجم الخلايا المضغوطة بالإضافة إلي زيادة معنوية في العدد الكلي لكرات الدم البيضاء و الخلايا الليمفاوية و نقص معنوي في خلايا النيوتروفيل. كما أظهر التحليل البيوكيميائي للسيرم وجود زيادة معنوية في إنزيمات أدينوسين ديأميناز وأسبارتات أمينوترانسفيراز و ألانين أمينوترانسفيراز و لاكتات ديهيدروجيناز و جاما جلوتاميل ترانسفيراز مما يعكس وجود تأثير لمرض القراع علي وظائف الكبد. كما أدي المرض إلي عدم تأثر البروتين الكلي و الألبيومين و الجلوبيولين. و بتقييم حالة مضادات الأكسدة وجد أن مرض القراع قد أدي إلي زيادة معنوية في المالونديالديهايد و النيتريك أكسيد ونقص معنوي في الجلوتاثيون المختزل و الزنك في السيرم. كما أدت الإصابة بمرض القراع إلي زيادة معنوية في حمض السياليك الكلي و حمض السياليك المرتبط بالدهون كدلالات للالتهاب في السيرم.

يستخلص من هذه الدراسة أن الإصابة بمرض القراع في العجول قد أدت إلي العديد من التغيرات في صورة الدم و كيمياء السيرم و مضادات الأكسدة . لذا ننصح الأخصائيين الذين يتعاملون مع مثل هذه الحالات أن يضيفوا مضاد للأكسدة كجزء من خطة العلاج لهذا المرض.