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Circulating redox status in sheep infected with dermatophytosis: a field study Mostafa A. Saleh*, M. H. Rateb*, Elham A. Abd-Allah**, Ghada A.E. Mohamed*

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ABSTRACT

Dermatophyte infection caused by *Trichophyton verrucosum* has a substantial impact on animal and human health. The present work aimed to determine the reactive oxygen metabolites (ROM), total antioxidant capacity (TAC), lipid peroxides (indicated by malondialdehyde, MDA) and measuring the oxidative stress index (OSI), in addition to investigation of some hematological and trace elements in sheep with dermatophytosis. *T. verrucosum* was diagnosed clinically and mycologically in 12 young sheep with mild infection (1-2 lesions, each with an approximate diameter of 2-4 cm) and 9 with severe lesions (covering more than a quarter of the head and ear), as well as 12 age- and gender-matched healthy control individuals. In comparison to controls, red blood cell count, packed cell volume and hemoglobin did not differ ($P > 0.05$) in the infected group, whereas leukocytic count increased ($P < 0.05$) in the severely infected group accompanied by neutrophilia, monocytosis and eosinophilia. Plasma Zn decreased ($P < 0.05$) in the severely infected group but Cu did not differ ($P > 0.05$) between groups. There was a progressive increase in the ROM in mild ($P < 0.05$) and severely infected group ($P < 0.01$) than the controls. The SOD activity and TAC were higher ($P < 0.05$) in the mild infected group, and lower in the severely infected group ($P < 0.05$). MDA and OSI did not change ($P > 0.05$) in the mild, but increased ($P < 0.01$) in the severely infected group. As a conclusion, sheep with severe infections by *T. verrucosum* exhibits disturbance in the leukogram, and Zn deficiency, associated with enhanced prooxidants and impaired antioxidant potential, suggesting an oxidative stress status.

INTRODUCTION

Dermatophytosis is a mycotic infection of keratinized tissues caused by dermatophytes. *Trichophyton*, *Microsporum*, and *Epidermophyton* are the three genera of dermatophytes recognized based on conidia morphology

(Gnat, et al. 2019). Dermatophyte infection has a substantial impact on animal and human health. It is considered a global problem and is being reported at an alarming pace, particularly in northern Africa because of its specific cultural, economical, and environmental charac-

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teristics, (Nweze and Eke 2016). Despite the existence of effective treatments, its prevalence continues to rise (Gnat et al. 2018). The clinical features of dermatophytosis are mostly determined by the site of infection, the host's immunity, and the species affected (Richardson and Warnock 2012). Clinical findings include patchy, often circular areas of alopecia and scaling, with erythematous peripheral margins (Scott 1975 Sargison et al. 2002).

Trichophyton verrucosum is a dermatophyte that has adapted to cattle as a host (Gnat et al. 2019). It can, however, be transmitted to sheep, where it causes exudative dermatitis with a severe inflammatory response (Sargison et al. 2002). Exoenzymes, especially keratinolytic proteases (Keratinases) produced by *T. verrucosum* to digest hair and keratin, play a key role in mycotic pathogenesis by inducing a stirring reaction at the site of infection, so that a severe inflammatory effect in the host develops and trigger metabolic and stress responses (Martinez-Rossi et al. 2017).

The detection of the invading fungi by the body's immune system augments an influx of neutrophils, monocytes, and dendritic cells to the infection site (Lord and Vyas 2019). Keratinases stimulate non-professional coetaneous immune cells, epidermal keratinocytes, and dermal fibroblasts to produce a broad range of pro-inflammatory cytokines, and inflammatory mediators (Gnat et al. 2019). Accordingly, cascades of molecular actions are enhanced, which enhance free radicals and reactive oxygen species (ROS) production as a major tool for fungal damage (Nakamura et al. 2002 Lord and Vyas 2019).

As a safeguard, the body has a sufficient spectrum of enzymatic and non-enzymatic antioxidants that work together to prevent the ongoing synthesis of prooxidants (Gutteridge and Halliwell 2018). Total antioxidant capacity (TAC) is defined as the sum of all antioxidants' actions and provides useful qualitative data on the dynamic balance between prooxidants and antioxidants (Erel, 2005). Antioxidant defenses, while their effectiveness, have a limited capacity and are susceptible to free radical attacks (Gutteridge and Halliwell

2018).

When there is an imbalance between radical-generating and radical-scavenging activity, oxidative stress (OS) occurs, which can lead to an increase in the synthesis of oxidation products and tissue damage (Lord and Vyas 2019). The oxidation of lipids by reactive oxygen species (ROS) produces a wide range of hazardous chemicals, including malondialdehyde (MDA) as a one of the most dangerous lipid peroxides (Gutteridge and Halliwell 2018). The skin is particularly susceptible to OS, which considered as an integrated part and major component in the pathogenesis of dermatophytosis in humans and experimental models (Ozturk et al. 2013 Barygina et al. 2019). Betul (2020) suggested a possible relationship between antioxidant imbalance, lipid peroxidation, and dermatophytosis disease in cattle.

The trace elements zinc, selenium, and copper are considered to play important roles in the prevention of oxidative damage, as well as the production and function of proteins and lipids (Yildirim et al. 2010 Al-Qudah et al. 2010). At the cellular level, it is well recognized that there is a fine balance between free radical formation and antioxidant defense in healthy situations (Gutteridge and Halliwell, 2018).

Despite significant advances in the knowledge about the causative pathogen (s), the interaction between dermatophytes and the host skin remains poorly delineated (Lord and Vyas, 2019). However, dermatophytosis-induced OS in cattle deficient in the trace elements Zn, Cu and Fe was reported (Yildirim et al. 2010 Al-Qudah et al. 2010). To our knowledge, there are no published reports on the role of OS in the pathogenesis of dermatophytosis in sheep.

The present work aimed to determine reactive oxygen metabolites, total antioxidant potential and the creation of lipid peroxides, in addition to investigation of some hematological and trace elements in sheep with dermatophytosis.

MATERIALS and METHODS: Animals

In this study, *T. verrucosum* was diagnosed clinically and mycologically in 21 young male sheep (5 months of age), as well as 12 age- and gender-matched healthy control individuals. The chosen sheep have all been raised and fed similarly in the eastern part of Assiut governorate, Egypt during October 2021. The animals were free of systemic illnesses and parasite infections after clinical and laboratory diagnosis. The infected sheep were divided clinically into two groups according to the lesion size. The first group showed 1-2 lesions, each with an approximate diameter of 2-4 cm ($n = 12$, mild infection). The second had a larger injury that covered more than a quarter of the head and ear ($n = 9$, severe infection).

Blood sampling

Blood was sampled from the jugular vein in heparinized tubes (10-ml), and divided to two portions. The first was one-ml and used for hematological studies. The second blood sample was centrifuged at $2500 \times g$ for 15 min, at $4^\circ C$ to obtain plasma, which stored at $-80^\circ C$ until biochemical analysis.

Mycological investigations (Rebell and Taplin, 1974)

After clinical examinations and detection of mycotic lesions, ethyl alcohol (70%) was used to clear the skin lesions. To obtain hair and skin tissues for mycological tests, the edges were scraped in a sterile Petri dish. The scraping material was split into two parts. The first was digested in 10% KOH for 15 minutes before being examined under a microscope for spores and hyphae. The remaining material was inoculated into Sabouraud's dextrose agar supplemented with chloramphenicol (0.05 mg/ml), thiamine (0.02 mg/ml), and cycloheximide (0.5 mg/ml), and incubated for 2-6 weeks at $28^\circ C$ and $37^\circ C$. To evaluate the detailed morphology of the isolates, lactophenol cotton blue preparations were used. The colonies' morphology, form, color, consistency, texture, and reverse plate color, as well as other visible qualities, were assessed in this test.

Hematological investigations (Kramer 2000)

Estimation of red blood cell count (RBC), packed cell volume (PCV) and hemoglobin

concentration (Hb) concentration, in addition to the total (TLC) and differential (DLC) leukocytic count were done.

Biochemical analysis

Trace elements zinc and copper:

Plasma copper and zinc were measured with an atomic absorption spectrophotometer (GBC 932 AA; GBC Scientific Equipment, Australia) after wet ashing in perchloric, nitric and sulfuric acids according to Piper and Higgins (1967).

Reactive oxygen metabolites (ROM):

The measurement of reactive oxygen metabolites (ROM) is commonly employed as an indirect approach to quantify the concentration of reactive oxygen species (ROS). Accordingly, the concentration of ROM in plasma was determined using the method described by (Erel 2005). This test uses several types of peroxides in the sample to oxidize Fe^{+2} (as a catalyst) to Fe^{+3} in the presence of xylenol orange in an acidic solution, which binds to Fe^{+3} and produces a color complex whose optical density is measured at 560 nm.

Total Antioxidant Capacity (TAC):

Total antioxidant capacity (TAC) was measured according to the method described by Miller et al. (1993) by using the colorimetric ABTS assay kit (Beyotime Institute of Biotechnology, Haimen, China). TAC was measured in mmol Trolox equivalents per liter.

Superoxide dismutase (SOD):

The SOD activity was examined using the technique of Misra and Fridovich (1972). SOD prevents the conversion of epinephrine to adrenochrome in an alkaline environment. The OD was measured at 480 nm.

Lipid peroxide (malondialdehyde; MDA):

Lipid peroxidation was measured after Placer et al. (1966). The technique is based on the creation of a color complex between lipid peroxidation products and thiobarbituric acid (TBA). The standard used in this test was 1,1,3,3-tetramethoxypropane. The absorbance was measured at 548 nm against a blank of bi-distilled water.

Oxidative stress index (OSI):

The ROM to TAC ratio was calculated as follows: OSI (Arbitrary unit) = (ROM, $\mu\text{mol/L}$ / TAC, $\mu\text{mol Trolox equiv/L}$) $\times 100$ (Abuelo et al. 2013)

Statistical analysis:

The data were analyzed using ANOVA by the SPSS program for Windows (SPSS, Chicago, IL, USA), and presented as mean \pm standard error (SE). Differences between groups were determined by means of t-test to compare the infected cases by the controls. The significance level was set at $P < 0.05$.

RESULTS**Clinical signs**

The infected animals showed no signs of systemic inflammation. Those with severe lesions had a rough coat and varied degrees of dullness. Most of the infected animals had non-pruritic lesions, although some of them showed mild frictions. Scaly grayish areas of alopecia with red to pink exudative dermatitis at the edge were shown on the skin (Fig. 1).

Mycological findings

Based on mycological investigations and identification keys, velvety, button-shaped, creamy colonies were shown (Fig. 2). Lactophenol cotton blue staining revealed chlamydoconidia of *T verrucosum* (Fig. 3). There were no additional fungal infections found, and the *T. verrucosum* strain found in the sheep was identified.

Hematological findings

Hematological indices including red blood cell count (RBC), packed cell volume (PCV) and hemoglobin concentration (Hb) concentration, in addition to the total (TLC) and differential (DLC) leukocytic count are presented in table 1. There were no significant differences in the mean values of the hematological indices of anemia including RBC, Hb and PCV between the infected groups and control animals. The TLC increased in the severely infected group and accompanied by neutrophilia, monocytosis and eosinophilia.

Trace elements results

The mean values of the plasma trace elements, zinc and copper in control and infected sheep are presented in table 2. Plasma Zn concentration decreased in the severely infected group ($P < 0.05$) compared to the healthy controls. On the other hand, the mean value of plasma Cu concentration did not differ significantly between groups.

Oxidative stress indices

The mean values ($\pm se$) of the reactive oxygen metabolites (ROM), superoxide dismutase (SOD), total antioxidant capacity (TAC), lipid peroxide (malondialdehyde; MDA) and oxidative stress index (OSI) in healthy and infected sheep are presented in table 3. Between the three groups, the mean values of ROM concentrations differed significantly ($P < 0.05$). In comparison to the control group, there was a progressive increase in the ROM concentrations in mild, and severely infected group. The SOD activity was higher ($P < 0.05$) in the mild infected group, and lower in the severely infected group ($P < 0.05$) in comparison with the control group. Similarly, the mean concentration values of TAC, where higher in the mild infected group ($P < 0.05$), and lower ($P < 0.05$) in the severely infected group in comparison with the control group. The mean values of MDA concentrations did not change ($P > 0.05$) in the mild infected group, but increased ($P < 0.05$) in the severely infected group compared to the corresponding control values. Similarly, the mean values of OSI concentrations did not change ($P > 0.05$) in the mild infected group, but increased ($P < 0.05$) in the severely infected group compared to the corresponding control values.



Fig 1. Lesions of *T. verrucosum* infection covering most of the skull skin of sheep



Fig 2. Specific colonies of *T. verrucosum* on SDA.

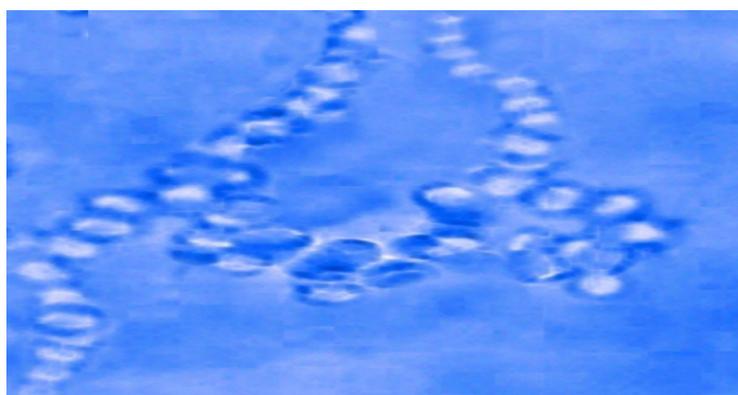


Fig 3. Chlamydoconidia of *T. verrucosum* (x 400).

Table 1. The mean values (\pm se) of red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), total (TLC) and differential leukocytic count in control and infected sheep.

Parameter	Control (n = 12)	Mild infected (n = 12)	Severe infection (n= 9)
RBC ($10^{12}/L$)	8.19 \pm 0.37	8.08 \pm 0.31	7.79 \pm 0.41
Hb (g/L)	97.7 \pm 3.93	96.09 \pm 3.82	95.97 \pm 3.91
PCV (L/L)	0.305 \pm 0.006	0.297 \pm 0.007	0.288 \pm 0.007
TLC ($10^9/L$)	11.73 \pm 0.21	11.78 \pm 0.24	13.02 \pm 0.31*
Lymphocytes %	52.14 \pm 1.42	52.57 \pm 2.32	48.11 \pm 2.19
Neutrophils %	41.65 \pm 1.06	41.04 \pm 0.91	45.01 \pm 0.89*
Monocytes %	3.41 \pm 0.14	3.56 \pm 0.13	3.79 \pm 0.12*
Eosinophils %	2.81 \pm 0.09	2.83 \pm 0.11	3.09 \pm 0.09*

* significantly different from the control at $P < 0.05$

Table 2. The mean values (\pm se) of the plasma trace elements, zinc and copper in control and infected sheep

Parameter	Control (n = 12)	Mild infected (n = 12)	Severe infection (n= 9)
Zinc ($\mu\text{mol}/L$)	8.354 \pm 0.32	8.182 \pm 0.29	7.041 \pm 0.33*
Copper ($\mu\text{mol}/L$)	12.96 \pm 0.52	13.11 \pm 0.49	12.87 \pm 0.55

* significantly different from the control at $P < 0.05$

Table 3. The mean values (\pm se) of oxidative parameters in control and infected sheep

Parameter	Control (n = 12)	Mild infected (n = 12)	Severe infection (n= 9)
ROM ($\mu\text{mol H}_2\text{O}_2$ Equiv/L)	26.14 \pm 1.26	30.18 \pm 1.32*	35.79 \pm 1.64**
SOD (U/mg protein)	4.544 \pm 0.315	5.374 \pm 0.244*	3.717 \pm 0.227*
TAC (mmol Trolox equivalents/L)	1.212 \pm 0.119	1.555 \pm 0.094*	0.948 \pm 0.072*
MDA ($\mu\text{mol}/\text{mg}$ protein)	3.575 \pm 0.4	3.864 \pm 0.478	6.183 \pm 0.711**
OSI (Arbitrary unit)	2.161 \pm 0.12	1.942 \pm 0.151	3.771 \pm 0.112**

ROM: reactive oxygen metabolites, SOD: superoxide dismutase, TAC: total antioxidant capacity, MDA: lipid peroxide (malondialdehyde); OSI: oxidative stress index

*,** significantly different from the control at $P < 0.05$, $P < 0.01$, respectively.

DISCUSSION

Results in the current study indicate an enhanced oxidative stress status in sheep severely infected by *T. verrucosum*. In these cases, there was a disturbance in the leucocytic differentiations accompanied by increased ROM which is coupled by impairment of the antioxidant potential and an enhanced OSI.

Clinical findings of alopecia and exudative dermatitis in the current study agree with the observations of **Sargison et al. (2002)** who found severe inflammatory response sheep

flocks. The observed leukocytosis and disturbed differential leukocytic count in this study agree with the results of **Jameel et al. (2014)** and coincide with the severe inflammatory reactions in the infected sheep (**Gnat, et al. 2019**). However, no statistical difference on the levels of lymphocyte, monocyte, granulocyte, erythrocyte, hemoglobin, hematocrit, and mean corpuscular volume between healthy and dermatophytic calves were reported by **Nisbet et al. (2006)**. This might be related to differences in breeds, the severity of the disease in addition to environmental management condi-

tions (Gnat et al. 2019).

As important components of antioxidant enzymes, trace minerals are necessary at small concentrations in live cells (Underwood and Suttle 1999). Trace element deficiency might predispose trichophytosis in animals by suppressing their immune system and lowering the activity of the antioxidant enzymes that contain copper and zinc as cofactors (Lee et al. 2000).

In the current study, Zn was reduced in severely infected sheep, which lie in agreement with the findings of Nisbet et al. (2006) and Al-Qudah, et al. (2010) who found decreased zinc concentration during dermatophytosis in calves.

One of the most evident consequences of inflammation is the production of pro-oxidant chemical species. Pro-oxidant species production normally begins locally, around the sites of tissue damage or infection, but if the inflammatory response is not properly controlled, it can become chronic (Valacchi, et al. 2018). In the current study, the ROM increased in the plasma of infected sheep. Betul (2020) obtained similar results in cattle. These results may indicate an increased ROS and other peroxides in the plasma, and suggest also an increased concentration of these toxic radicals in the inflamed lesions in the skin. Sener et al. (2019) found oxidative tissue damage in mycotic infections, accompanied by an accumulation of serum peroxide. This provides solid evidence that skin dermatophytes can induce systemic immunological responses, including the probability of systemic inflammation. Prescott et al. (2017) reported that the local induction of immune chemicals in the skin by the fungi may have far-reaching systemic effects on immune regulation.

The enhanced SOD and TAC in mild cases the present study suggests an enhanced antioxidant potential to cope with the enhanced free radical generation. In the severely infected group, these antioxidant potentials were exhausted during neutralization and scavenging free radicals on the long run, where the chronic condition predominates. Valacchi et al. (2012) found depletion of the TAC due to accumula-

tion of ROS in skin diseases. Betul (2020) demonstrated impairment in the TAC in cattle with dermatophytosis.

In the present work, ROM was greater and TAC was lesser in the severely infected group, indicating that OS challenge was involved in *T. verrucosum* infection, resulting in peroxidation of macromolecules, cytotoxicity and pathology. In mycotic diseases, the OSI could provide useful information on the relationship between ROM and TAC so that we could determine of the degree of OS status (Betul 2020). In our study, OSI was more clearly in severely infected sheep and showed the existence of OS. These results suggests a disturbance in the pro-oxidant-antioxidant equilibrium in favor of the pro-oxidant side, leading to potential damage of tissues.

Triggering of free radicals, which primarily attack lipids result in a wide range of reactive toxic by-products, lipid peroxides, the most conspicuous of which is MDA (Baek and Lee, 2016). Increased lipid peroxide markers were increased in cattle with dermatophytosis (Betul, 2020). In the present study, MDA increased in the severely infected group, suggesting an increased lipid peroxidation in the plasma of these cases. This also indicate that high levels MDA is associated with the severity of the disease, which might be a key in understanding the mechanisms of fungal disease.

CONCLUSION

Sheep infected by *T. verrucosum* exhibits disturbance the leukogram especially in severe infection associated with oxidative stress status which effect in the general healthy and lead to decrease production in the sheep

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