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Research Article
Evaluation of Oxidative Stress in Sheep Infested with Ticks and Concurrent Diagnosis of *Theileriosis*

[1]Amira Adel Taha Al-Hosary, [2]Mahmoud Rushdi Abd Ellah and [1]Laila Salah El-Din Ahmed

[1]Department of Animal Medicine (Infectious Diseases), Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt
[2]Department of Animal Medicine (Clinical Laboratory Diagnosis), Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt

Abstract

Background and Objective: Most of the studies related to detection of *Theileriosis* were performed on cattle and buffaloes. Fewer studies were conducted to evaluate the oxidative stress in sheep infested with ticks and/or infected with *Theileriosis*. This study was undertaken to investigate for the presence of *Theileriosis* in sheep infested with ticks and to evaluate the effect of ticks' infestation on blood oxidative status. **Materials and Methods:** A total number of 20 ewes and rams from El-Kharga oasis, El-Wady El-Gaded Governorate, Egypt were subjected to study. Out of them, 13 animals were infested with ticks. Ticks were collected from all infested sheep for identification, furthermore, serum was separated and used for measuring total antioxidants capacity (TAC) and malondialdehyde (MDA) levels. In addition, whole blood samples were collected and were used for molecular diagnosis of *Theileria* infestation. Two specific primers were used for detection of *Theileria* sp. parasites in whole blood. The first primer was specific for *Theileria ovis* and the second one was specific for *Theileria lestoquardi*, both of them amplified the target sequences by using the conventional polymerase chain reaction (PCR). **Results:** Results revealed that ticks were identified morphologically and all of them were related to *Hyalomma anatolicum anatolicum* tick species. PCR assays confirmed the infection with *Theileria ovis* in 2 animals and *Theileria hirci* (*Theileria lestoquardi*) in only one animal. There was a significant increase in serum TAC and insignificant increase in serum MDA level in ewes infested with ticks. Serum of rams revealed insignificant increase in both TAC and MDA levels. **Conclusion:** Tick infestation in sheep is associated with increased blood oxidative stress, on the other hand, ewes seemed to be more susceptible to increase free radical stress than rams.

Key words: Ewes, rams, ticks, *Theileria ovis*, *Theileria lestoquardi*

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Corresponding Author: Mahmoud Rushdi Abd Ellah, Department of Animal Medicine (Clinical Laboratory Diagnosis), Faculty of Veterinary Medicine, Assiut University, 71526 Assiut, Egypt Tel: +201005398773

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sheep is one of the most important domesticated small ruminants in Egypt. The total population of sheep in Egypt is exceeded 4 million heads and is raised mainly for meat production, sheep is greatly affected by tick borne diseases[1]. Ovine theileriosis is considered as one of the destructive diseases affecting small ruminates production in Egypt. There are two species of *Theileria* most commonly detected worldwide. *Theileria hirci* (*Theileria lestoquardi*), is the causative agent of malignant ovine theileriosis and equivalent of tropical *Theileriosis* in cattle and buffaloes[2] and *Theileria ovis* is the causative agent of mild ovine theileriosis. These species considered as non-pathogenic species while both species transmitted by *Hyalomma anatolicum anatolicum*.

Free radicals produced continuously during metabolic processes and participate mainly in immune response, metabolism of unsaturated fatty acids and inflammatory reaction. Free radical excess results in impairment of DNA, enzymes and cellular membranes and may be related to mutagenesis and aging processes[3].

Oxidative stress results when reactive oxygen species (ROS) are produced in concentrations that exceed the capability of the antioxidant system to neutralize[4] and/or from a decrease in the antioxidant defense, which may lead to damage of biological molecules and disruption of normal physiology and metabolism[5]. This condition can lead to the onset of health disorders in animals. Lipid peroxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in body fluids, cells and tissues. Lipid peroxides are unstable compound and when it decomposes it gives a complex series of reactive carbonyl compounds. Polyunsaturated fatty acid peroxides generate malondialdehyde (MDA) during the decomposition[6].

Several studies have reported the presence of oxidative stress in human and animals infected with parasites[7-11] as well as the antioxidant defense mechanism that exists between parasites and the mammalian host[12]. In human allergic inflammatory diseases, such as helminthic infections, the associated influx of eosinophils has been implicated as a primary source of tissue damage[13], possibly via their potent reactive oxygen metabolites production[14]. Fewer studies were conducted to evaluate the oxidative stress in sheep infested with ticks and/or infected with *Theileriosis*. Therefore, this present study aimed to detect of *Theileria* ssp. infection in sheep infested with ticks, morphological identification of these ticks in addition to assess the oxidative stress in the infected sheep.

MATERIALS AND METHODS

Sampling and conventional diagnosis: This study was performed on twenty sheep from El-Kharga oasis, El-Wady El-Gaded Governorate, Egypt, during the period from August-December, 2016. Animals included both sexes (Ewes, n = 13 and rams, n = 7), ages ranged from 1-3 years. Out of them 13 animals (9 ewes and 4 rams) were infested with ticks and 7 animals (4 ewes and 3 rams) were none infested with ticks. The ticks and blood samples were collected from each animal under study.

Two blood samples were collected from jugular vein by sterile sharp needle with wide pore, whole blood samples were collected in clean and dry tube containing EDTA as anticoagulant and used for blood smears and polymerase chain reaction (PCR). Blood for separation of serum was collected in plain tube and used for measuring total antioxidant capacity (TAC) and malondialdehyde (MDA) levels. Three thin blood films were prepared form each animal dried by air and fixed in absolute methyl alcohol for 3 min, staining with freshly filtered, 10% diluted Giemsa stain for 30-45 min then washed with distilled water and examined under oil immersion lens according to Charles[15].

Tick identification: Morphological identification of these ticks was carried out according to ElGhali[16].

Molecular diagnosis: For confirmation of small ruminants *Theileria* species infection two specific primers were used. The first one was specific for *Theileria ovis* and the second one was specific for *Theileria lestoquardi*, both of them amplified the target sequences by using the conventional polymerase chain reaction[17,18]. The forward and reverse primer sequences that were used for detection of *Theileria ovis* included TSsr 170F 5′TCGAGACCTTCGGGT-3′ and TSsr 670R 5′TCCGGACATTGTAA AACAAA-3′. However, primer sequences for *Theileria lestoquardi* included 5′GTGCCGCAAGTGAGTCA-3′ and 5′GGACTGATGAGAAGACGATGAG-3′ as forward and reverse primers, respectively. Specific band for *Theileria ovis* was detected at 520 bp and for *Theileria lestoquardi* at 785 bp.

DNA extraction: The DNA extraction from whole blood samples was carried out according to commercial kits (manufacturer's instructions of QIA amp blood kit, Qiagen, Ltd, UK).

Cycling conditions and electrophoresis: The two PCR reactions were performed separately by using of thermal cycler (Biometera, Germany). The thermal condition for *T. ovis* includes three different steps, the first one for denaturation at 96°C for 3 min, the second step was performed at 94°C for 30 sec, 60°C for 30 sec and 72°C for 2 min and repeated 40 cycles the last step for final extension at 72°C for 10 min on the other hand, *T. lestoquardi* thermal condition included denaturation at 94°C for 1 min and 40 cycles performed at 94°C for 1 min, 60°C for 1 min and 72°C for 2 min and final extension at 72°C for 5 min. The PCR products were electrophoresed on 1% agarose gel stained with ethidium bromide, the visualization was done under UV light and the positive bands were obtained at 520 bp for *T. ovis* and 785 bp for *T. lestoquardi*[17,18].

Serum total antioxidant capacity (TAC) and malondialdehyde (MDA) levels: Total antioxidant capacity (TAC) was determined in serum by using commercial kits supplied from Biodiagnostic Company for diagnostic reagents: Dokki, Giza, Egypt. The determination of the total antioxidant capacity was performed by the reaction of anti-oxidants in the sample with a definite amount of exogenously provide hydrogen peroxide (H_2O_2)[19].

The antioxidants in the sample eliminate a certain amount of the provided H_2O_2. Serum MDA level was estimated using commercially available test kits supplied by Biodiagnostic-Egypt[20].

Statistical analysis: Data from tick infested ewes and rams were compared by means of one way analysis of variance (ANOVA) by using SPSS Statistical Program (SPSS, Chicago, USA). Data were expressed as Mean±SD. Significant differences were determined at $p<0.05$.

RESULTS

Clinical findings and ticks identification: Clinical examination revealed that some of the examined animals were suffering from fever (≥41°C), weight loss but no specific signs of *Theileriosis*. Tick samples were identified morphologically and all of them were related to *Hyalomma anatolicum anatolicum* tick species (Fig. 1, 2).

Conventional and molecular diagnosis of *Theileria*: Giemsa stained blood smears confirmed no real infection in respect to the examined animals but marked eosinophilia was detected in some animals. PCR assays confirmed the infection with *Theileria ovis* in two animals and *Theileria hirci* (*Theileria lestoquardi*) in only one animal (Fig. 3-5).

Fig. 1: *Hyalomma anatolicum anatolicum* tick under the ear base in sheep

Fig. 2: *Hyalomma anatolicum anatolicum* tick inside the ear of sheep

Serum total antioxidants capacity (TAC) and malondialdehyde (MDA) in ewes and rams infested with ticks: There was a significant increase ($p<0.05$) in serum TAC and insignificant increase ($p>0.05$) in serum MDA level in ewes

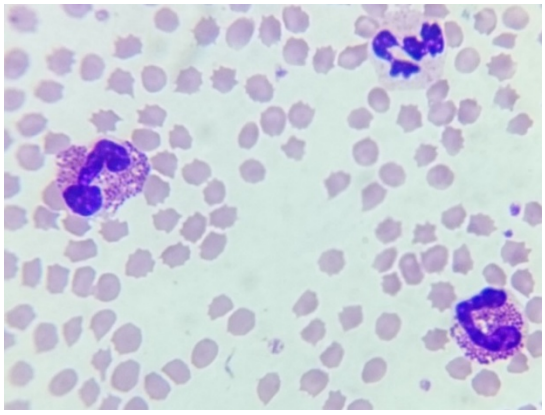

Fig. 3: Giemsa stained blood smears showing marked eosinophils

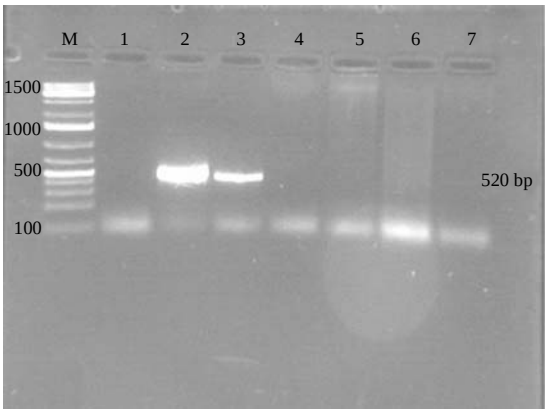

Fig. 4: *Theileria ovis* specific PCR products. Lane M: DNA ladder 100 bp, Lane 1: Negative control. Lanes 4, 5, 6 and 7: Negative, Lanes 2 and 3: Positive PCR with bands at 520 bp

infested with ticks, when compared with healthy ewes. Serum of rams, revealed insignificant increases ($p>0.05$) in both TAC and MDA levels (Table 1).

DISCUSSION

Theileriosis is a progressive lymphoproliferative disease caused by a protozoan parasite. The parasite acts as a serious

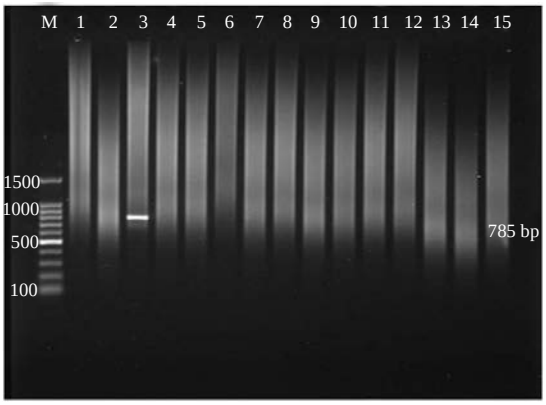

Fig. 5: *Theileria lestoquardi* specific PCR products. Lane M: DNA ladder (100 bp), Lanes 3 positive PCR with bands at 785 bp, Lane 1: Negative control. Lanes: 2, 4-15: Negative

constraint to production in endemic areas[21]. In the present study, the rise in serum lipid peroxidation product (MDA) indicated the occurrence of oxidative stress in *Theileria* infected sheep, which might be attributed to the inability of the antioxidant mechanism to remove the reactive oxygen species. Interaction of ROS with polyunsaturated fatty acids located at the cell membrane results in the formation of lipid peroxidation products in the form of MDA[22]. There is some evidence that oxidative stress and lipid peroxidation incorporate in pathogenesis of *Theileriosis*. An increase in oxidative stress and in lipid peroxidation in blood of cattle infected with *T. annulata* has been reported[22]. However, studying the oxidative stress associating *Theileria* infection in sheep is lacking. Although several studies reported the presence of a negative correlation of blood antioxidant enzyme activities with the level of parasitemia[23, 24]. Grewal *et al*.[25] reported that blood antioxidants activities exhibited significant rise in cattle naturally infected oxidative stress. Also, indicated that ewes were more affected than rams, the latter showed insignificant increases in both TAC with *T. annulata*, they concluded that the increased level of antioxidants during parasitemia could be due to the fact that this enzyme activities are the major mechanism for intracellular destruction of free radicals.

Table 1: Total antioxidants capacity and malondialdehyde levels in ewes and rams infested with ticks

	Groups			
	Ewes (n = 13)		Rams (n = 7)	
Parameters	Healthy (n = 4)	Infested with ticks (n = 9)	Healthy (n = 3)	Infested with ticks (n = 4)
TAC (mmol L^{-1})	0.17±0.06	0.37±0.13*	0.15±0.01	0.19±0.03
MDA (nmol mL^{-1})	2.09±1.77	4.80±2.87	1.19±0.51	3.29±1.26

Data were expressed as mean±standard deviations, *Significant differences were detected at $p<0.05$, TAC: Total antioxidant capacity, MDA: Malondialdehyde, *: Significant ($p<0.05$)

The detection of lipid peroxidation products including MDA in biological samples[26] has been extensively studied[27-29]. Unlike reactive free radicals, aldehydes can persist for a long time and have the ability to diffuse from their site of origin (i.e., membranes) to reach and attack other targets either intracellularly or extracellularly[6]. In this study, the significant increase in serum TAC and the insignificant increase in serum MDA level in ticks infested ewes indicated the increased blood and MDA levels. The variations between ewes and rams in their responses to increase free radical stress might be attributed to other physiological factors in female animals like pregnancy and lactation, which is associated with the release of free radicals inside the body and represent another source for oxidative stress.

CONCLUSION

It could be concluded that tick infestation in sheep is associated with increased blood oxidative stress, on the other hand, ewes seemed to be more susceptible to increased free radical stress than rams. Two species of *Theileria* were detected in investigated sheep, *Theileria ovis* and *Theileria hirci* (*Theileria lestoquardi*).

SIGNIFICANCE STATEMENT

The current study demonstrates the infestation of sheep with *Hyalomma anatolicum anatolicum* tick species. In addition, two species of *Theileria* were detected in investigated sheep, *Theileria ovis* and *Theileria hirci* (*Theileria lestoquardi*). The study also proved the occurrence of increased oxidative stress in sheep infested with ticks.

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