

## EVALUATION OF ANTIOXIDANT RESPONSE MECHANISM IN FATTENING CATTLE CALVES SUFFERING FROM BABESIOSIS IN NEW-VALLEY-GOVERNORATE EGYPT

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### ABSTRACT

Invasion of animal's erythrocytes by *Babesia* parasites causes alteration in antioxidant potential of the red cells so this study was conducted to assess the effect of *Babesiosis* infection on some oxidative stress and antioxidant agent in calf naturally infected with *Babesi* where the study was carried on 70 fattening calves aged in 2-3 years, from different localities in New-Valley Governorate, Egypt. The animal under the study classified into three groups, group 3, include 40 fattening calves naturally infected by *Babesia* and ticks, group 2, include 15 fattening calves infected with ticks only and group 1, include 15 fattening calves clinically and laboratory healthy (control group). The parasitological diagnosis was confirmed by indirect fluorescent technique (IFT) and the diseased calves divided into 4 subgroups according to parasitaemia rates (0.5%, 1%, 1.5%, 2%). The calves under the study were free from internal parasite by clinical and studies. The study revealed a significant increase in oxidative marker (NO and MDA) in the diseased group with *Babesiosis* than tick infected group and control group while the biochemical serum analysis for antioxidant markers revealed an highly significant decrease in all antioxidant parameters under study, SOD, GSH-R, Catalos, Ascorbic acid and TAA, as the fallowing values,  $290.13 \pm 31.21$ ,  $2.01 \pm 0.92$ ,  $0.93 \pm 0.04$ ,  $99.75 \pm 2.47$  and  $0.83 \pm 0.32$  respectively. The study indicated that the *Babesiosis* calves are at greater risk of oxidative damage than other groups studied, hence early diagnosis, treatment and addition of dietary antioxidants are highly encouraged and essential requests for improve of productivity of fattening calves.

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تقييم استجابة الجسم لمضادات الاكسده الدمويه فى عجول التسمين البقرى التى تعانى من البابيزيا فى محافظة الوادى الجديد مصر

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مهاجمة كرات الدم الحمراء للحيوانات بواسطة طفيل البابيزيا يسبب تغيرات فى كفاءة مضادات الاكسدة لهذة الخلايا الحمراء لهذا اجريت هذة الدراسة لتوضيح الاصابة بالبابيزيا على التأثير الاجهادى والانزيمات المضادة للاكسدة فى العجول المصابة طبيعيا. هذة الدراسة اجريت على 70 عجل تسمين تتراوح اعمارهم بين 2-3 اعوام فى محافظة الوادى الجديد بمصر قد قسمت هذة الحيوانات الى مجموعة مصابة طبيعيا بالبابيزيا (40) تم فحصها بعمل مسحة

(Indirect Fluorescence Technique) دموية مباشرة مصبوعة بالجمسا وتم تأكيد الإصابة باختبار سيرولوجي (0, 5%, 1%, 1.5%, 2%) وقد تم تقسيم هذه المجموعة حسب درجة وجود الطفيل الى اربع مجموعات (0, 5%, 1%, 1.5%, 2%) و 15 عجل تسمين مصابة بالقراد فقط و 15 عجل تسمين صحية ظاهريا ومعمليا واستخدمت كمجموعة ضابطة وكل هذه المجموعات كانت خالية من الطفيليات الداخلية وذلك بفحص عينة البراز. هذه الدراسة اوضحت زيادة معنوية فى علامات الاكسدة (NO and MDA) فى المجموعة المصابة بالبببازيا عن المجموعة المصابة بالقراد والمجموعة الضابطة بينما الفحص البيوكيميائى للسيرم اوضح نقص معنوى على فى كل العناصر المضادة للاكسدة تحت الدراسة (SOD, GSH-R, Catalase, Ascorbic acid and TAA) وكانت قيمها كالاتى (290.13±31.21, 2.01±0.92, 0.93±0.04, 99.75±2.47 and 0.83±0.32) بالترتيب هذه الدراسة اوضحت ان العجول المصابة بالبببازيا تحت خطر الاكسدة عن المجموعات الاخرى ولهذا فان التشخيص المبكر والعلاج وكذلك اضافة مضادات الاكسدة الغذائية تعمل على تحسين انتاجية عجول التسمين.

**Key word:** *Fattening calves, oxidative stress, antioxidant parameters and Babesiosis.*

## INTRODUCTION

Poor growth and unthriftiness in fattening calves are considered the widely mentioned complaint among farmers in the world especially in the developing countries as Egypt, where impact of *Babesiosis* is incremented as the most causes for economic losses in the animal production.

Bovine *Babesiosis* is a febrile, tick-borne disease of cattle, caused by protozoan parasites of the genus *Babesia* and generally characterized by extensive erythrocyte lyses leading to hemoglobinuria, jaundice, anemia and death. Tick fever (*Babesiosis*) is an important disease of cattle where the tick *Rhipicephalus. Boophilus* act as a vector for *Babesia* species, Johnson *et al.* (2008), where infection of the host causes a host-mediated pathology and erythrocyte lyses, resulting in fever, hemoglobinuria, anemia and possibility of organ failure, Ahmed *et al.* (2009).

Recently *Babesia* becomes the most widespread parasite due to exposure of 400 million cattle to infection through the world, with consequent heavy economic losses (Collett, 2000 and Kivaria *et al.*, 2007).

The oxidation process in erythrocytes due to the presence of free radicals affects all cell structure, hemoglobin and membrane of erythrocytes and the oxidative hemolytic of erythrocytes can be studied by measuring the variation in the electrical conductivity to investigate the energy gap, Nabile (2003).

Oxidative stress is an imbalance between radical-generating and radical-scavenging activity and resulting in oxidation products and tissue damage, Nabile (2003), it resulting from increase production of antioxidants or from decrease dietary intake, synthesis or increase turn over of antioxidants (Celi, 2010 and Ozbilge *et al.*, 2005).

Oxidative stress is a generally mechanism where by free radicals induce oxidative damage and reduce the antioxidant defiance of biological system, Tsukahara (2007). Over load of reaction oxygen species (ROS) including, superoxid, hydrogen peroxides and hydroxyl radical that exceed the capacity of antioxidant system induce oxidative stress in the body.

Oxidative stress plays an important contributory role in a number of diseases, Zaidi *et al.* (2005). In addition to that, Kelly, (1994) recorded that oxidative stress is a major problem that results from a number of compounding factors such as pulmonary inflammation and the nutritional inadequacies. The present study carried out to throw alight on the effect of infestation of fattening calves by *Babesiosis* on oxidant/antioxidant markers under the environmental conditions in New-Valley Governorate, Egypt.

## MATERIALS and METHODS

### A-Study area:

This study was carried out in New-Valley Governorate (in the western Egyptian desert).

This area is a depression that lies between the Nile, Sudan and Libya with its capital at the Kharga Oasis where the rainfall is almost scarce throughout the year and the ground water is the main source of water.

#### **B-Animals:**

A total number of 70 fattening calves aged (2-3 year) reared in the same condition (feeding and season) and the study was carried in the period from September 2010 to September 2011 and the studied animal classified into three group, 40 fattening calves suffered from *Babesiosis* and ticks group (3) by clinically and laboratory examination and classified into four group according to the degree of parasitism, 15 fattening calves infested with ticks (group 2) only and the rest 15 fattening calves are clinically and laboratory healthy (group 1) and used as a control group.

#### **C-Samples:**

##### **A- Blood samples:**

10 ml of blood were drawn from the jugular vein of each animals in two sterile test tubes as the following:

a. 5 ml of blood collected in test tube containing EDTA and used for thick and thin blood film for microscopically diagnosis of *Babesia* and quantify the degree of parasitaemia by percentage.

b. 5 ml of blood collected in test tube without anticoagulant and used for separation of serum by centrifugation in 1500 rate/minutes for 20 minutes where the separated sera was used for determination of some oxidant and antioxidant parameters.

##### **B- Fecal samples:**

10 gm of fecal samples were taken in clean labeled plastic page directly from the rectum of all animals under the study to excluded the animals positive to parasitic infestation other than *Babesia bovis*.

#### **D-Parasitological analysis:**

1- Thin blood film were prepared and stained with 10% Giemsa stain / 30 minute and examined under oil immersion ( $\times 1000$ ) to observed intraerythrocytic forms of *Babesia* and after examine more than 50 microscopic field of blood films examined. Parasitaemia rate was

quantified and expressed as percentage of infected erythrocytes, Shiono *et al.* (2003 a)

2- Fecal sample were processed as soon as collected from all calves under the study to excluded the animal infected with parasite other than *Babesia* according to Solusby (1986)

#### **F- Serological analysis:**

The diagnosis of acute *Babesia* infection was traditionally based on clinical finding and microscopic examination of thin blood smears .microscopically diagnosis can be difficult due to variable parasitaemia, thus a variety of serological diagnosis are used to detected specific antibodies by indirect fluorescent antibody technique (IFA) was used in this study according to Jonhson *et al.* (1973).

##### **1- Slid antigen;**

preparation make from the blood of high parasitaemia (2%) put on the different slide wells and fixed by acetone in goblin jar and washes three successive time with PBS and the slide dried by Schwarz and keep in deep freeze until used as described by Goff *et al.* (1982).

##### **2- IFA test procedure:**

50  $\mu$ ml of undiluted tested serum added for each slid well and incubated for half hours followed by three successive washing by PBS then added ant bovine conjugated with fluorescence dye (1; 80 dilution) and incubated for 3/4 hours, three successive washing by PBS and finally added the cover on the slid with glycerin and mounted by fluorescence microscope.

#### **H- Biochemical analysis:**

The concentration of oxidant markers including, Malondialdehyde (MDA) which is the biomarker of lipid peroxidation and Nitric oxide (NO) according to Okawa *et al.* (1979). Antioxidant markers including Superoxide dismutase (SOD), according to the method described by Nishikimie *et al.* (1972), while Glutathion-Reduced (GSHR), Catalase and Total antioxidant concentration (TAC) in the serum of diseased calves determined by using commercially available test kites according to the methods described by Beutler *et al.*

(1963), Aebi (1984) and Koracevic *et al.* (2001), respectively while vitamin C determined calorimetrically according to Jagota and Dani (1982).

**K- Statistical analysis:**

Statistical significant difference between subjects was calculated using the students t-test and chi-square (SPSS.). A value of  $p < 0.05$  considered significant.

**RESULTS**

The genus *Babesia* is composed of intra-erythrocytes protozoan parasites of domestic animals clinically characterized by fever, haemoglobinuria and anemia. The blood film prepared from 40 diseased calves showed the presence of piroplasma of *Babesia bovis* in the red blood cells with different parasitaemia as in Table (1). On other hand no piroplasm was detected in the control animals and tick infested animals group.

The fecal analysis for fecal samples of all animals under study indicated that these animals are free from parasite.

The effect of *Babesia* infection in fattening calves on the concentration of some oxidant markers in fattening calves infected with *babesia* is indicated in Table (2), Generally increased in values of oxidant markers as compared to healthy animals especially in Nitric oxide and Malondialdehyde (MDA) in calves infested with *babesia* and ticks as in group (3).

Table (3) revealed of lower values for antioxidant parameters as compared to healthy control group, ASCA, SOD, GSH-R ( $P < 0.01$ ) as well as CAT ( $P < 0.05$ ) and Total antioxidant activity (TAA) where there is marked significant decrease in superoxide dismutase in (3) than (2) and highly significant decrease in Total antioxidant activity in group (3) than group (2), also we found highly significant decrease in Catalase and Ascorbic acid in calves infested with *babesia* and tick infested group (3) than other group.

**Table 1:** Indicated the degree of parasitaemia in diseased calves in the present stud.

Parasitaemia	Parasit /ul of blood	Clinical signs
0.5%	5-20	Number of organism that required for positive thick film(sensitivity) in 5 calves
1%	20-80	The diseased calves may show slid clinical signs(7 calves)
1.5%	80-320	The animals with moderate symptoms(8 calves)
2%	320-1000	Sever clinical symptoms with increase in mortality rate (20 calves)

**Table 2:** Statistical analysis in serum oxidant parameters in animals under study.

Animals group	Healthy control calves (group 1)	Tick infested calves only (2)	Calves infested with babesia and ticks (3)
Parameters			
Malondialdehyde(MDA),mmol/l	2.03±0.7	2.19±0.43	3.21±0.43** □
Nitric oxide(NO) mmol/l	18.56±0.29	19.87±0.45	23.93±1.46* *□

**Table 3:** Statistical analysis of serum antioxidant parameters in the animals under the study.

Animals group	Healthy control calves (1)	Tick infested calves only(2)	Calves infested by ticks and Babesia (3)
Parameters			
Superoxiddismutase(SOD) $\mu$ /l	323.19 $\pm$ 1.96	314.36 $\pm$ 1.98*	290.13 $\pm$ 31.21***
Glutathione-Reduce(GSH-R) mmol/l	5.89 $\pm$ 1.02	2.63 $\pm$ 1.34**	2.01 $\pm$ 0.92**
Catalase $\mu$ /l	2.13 $\pm$ 0.34	1.21 $\pm$ 0.6*	0.93 $\pm$ 0.04***
Ascorbic acid $\mu$ g/l	143.12 $\pm$ 0.51	104.20 $\pm$ 1.8**	99.75 $\pm$ 2.47***
Total antioxidant activity(TAA) mmol/l	2.01 $\pm$ 0.14	1.48 $\pm$ 0.09	0.83 $\pm$ 0.32***

### DISCUSSION

Babesiosis is an important disease of cattle where *Rhipicephalus* (Boophilus) act as a vector for *Babesia bovis* and *babesia bigmina*. Tick fever is transmitted by *ixodid* ticks and infection of the host causes a host mediated pathology and erythrocytes lyses' resulting anemia, hemoglobinuria and possibility of organ failure, Jonhson *et al.* (2008).

Blood parasitic disease of animals is the most important causes for great economic losses especially in the developing countries (Ahmed *et al.*, 2009), where it can affected the reproductive performance of farm animals through impaired growth rate and animal losses by dead and negative energy balance and body losses, Grummer (2007).

Oxidative stress is an imbalance between radical-generating and radical-scavenging activity, resulting in oxidation products and tissue damage, Ahmed *et al.* (2009), it results from increase production of oxidants or from decrease dietary intake, synthesis or increased turnover of antioxidants, Celi. P, (2010) and Ozbilge *et al.* (2005), while Mishara *et al.* (1994) said that the oxidative stress is generally the mechanism where free radical induce oxidative damages and reduce the antioxidant defense of biological system, in addition to that Ahmed (2010), recorded that *Babesia* infection has an additional role in the genesis of anemia and oxidative stress.

In the current study we found marked increase in the values of oxidative markers,

Nitric oxide (NO) and malondialdehyde (MDA) in the fattening affected calves by *babesia* and ticks if compared with healthy control fattening while the change in fattening animals infested with ticks only (Tabel 2), where that agreement with Siemienluk *et al.* (2008) and Xiao *et al.* (2001) where that may be attributed to that Nitric oxide (NO) is produced by a number of different cell type in response to cytokine stimulation and is reported to play in immunological mediated protection against to growing list of protozoan parasite, Rivero (2006), who recorded that there is also evidence that Nitric oxide exerts an important selective pressure on parasites.

It was clear that infected animals in the current study were under oxidative stress as indicated by increase in the malondialdehyde (MDA) and Nitric oxide (NO) in the blood of infected calves where. Halliwell and Chirico (1993) recorded that the increased production of these free radicals lead to augment oxidative stress as evidenced by high levels of erythrocyte lipid per oxidation product while Shado *et al.* (2000) and Goff *et al.* (2002) revealed that bovine babesiosis lead to the production of interleukin 1B, interleukin 12, gamma interferon (IFN-Y). These mediator activate mononuclear phagocytes/ macrophages to realest reactive nitrogen intermediated but Hanafusa *et al.* (1998), reported that while NO production

increased in horse experimentally infected with *B.caballi* and inhibition of NO lead to increased of parasitism and NO may have been a critical effect or molecule of immune defense against the parasite.

In relation to the results of antioxidants markers the results indicated in Table (3), where the inflammatory reactions lead to a decrease in erythrocytes antioxidant parameters in calves infested by *Babesia* and ticks than both calves healthy one and calves infested with ticks only where it revealed of lower values of ASCA, SOD, GSH-R, as well as CAT.

In a number of studies, it has been demonstrated that the cells of hosts infected with different species of parasites, the amount of reactive oxygen radicals which cause lipid per oxidation are increased, thereby causing cell and tissue damage, Sarin *et al.* (1993) and Stocker *et al.* (1986) while Ginsburg and Atamina (1994) and Mishra *et al.* (1994). Recorded that intra-erythrocyte parasites metabolize hemoglobin and produce O<sub>2</sub> which causes oxidative stress. Finally we can concluded that antioxidant systems have a cellular protective action against oxidative stress resulting in cell-organ and tissue damage as a result of *Babesia* invasion in fattening calves while *Babesia* infection induce oxidative stress in the body by reducing the activities of antioxidant enzymes.

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