



Relationship between coccidiosis and lipid peroxidation level in blood of cattle.

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Abstract

Summary: Bovine coccidiosis is one of the most common diseases in livestock, which leads to large economic losses in addition to high mortality for animals, and despite the large number of studies on this disease, studies on the biochemical and physiological effects of this parasite are still limited, especially the effect of parasite infection on some variables Blood in cows, and from this standpoint the current study was conducted, which included measuring the levels of malondaldehyde (MDA), and glutathione(GSH). As the study included a number of adult cows (40) and a number of young calves 2-3 months old (35 calves). A stool sample (5 grams) and a blood sample (10 cm) were taken from each animal. The affected animals were diagnosed through the detection of the visible symptoms on animal and stool examination The stool was laboratory treated through an examination, using floatation and direct smear methods to find ovarian cysts of the parasite, and the percentage of infection was determined using the modified McMaster method, and then biochemical tests were performed on blood samples of both infected and healthy cows. The color test method was used to measure the level of malondialdehyde and the modified Almanns method to measure the level of glutathione in plasma. The results of the current study showed that infection with the parasite increased lipid peroxidation in the blood plasma of both calves and cows, respectively (4.3423 ± 1.0238 , 7.4918 ± 1.4232) compared with normal cows and calves (2.3423 ± 0.465 , 2.9537 ± 1.063), while it led to a decrease in glutathione level in Both of the infected cows (7.3041 ± 2.8213) compared with healthy cows (11.2322 ± 2.2628), and there was no significant difference in the level of glutathione between infected and uninfected calves.

key words :Coccidiosis, Cattle, Glutathion ·lipid peroxidation , Mosul.

العلاقة بين الكوكسيديا ومستوى بيروكسيد الدهون في دم الماشية.

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الملخص :

يعتبر كوكسيديا الابقار احد اكثر الامراض الشائعة في الماشية والذي يؤدي إلى خسائر اقتصادية كبيرة اضافة الى الوفيات العالية للحيوانات , وعلى الرغم من كثرة الدراسات عن هذا المرض , الا ان الدراسات المتعلقة بالتاثيرات البايوكيميائية والفسلجية لهذا الطفيل لازالت محدودة , خاصة تاثير الاصابة بالطفيل على بعض متغيرات الدم في الابقار , ومن هذا المنطلق اجريت الدراسة الحالية واتي تضمنت قياس مستويات المألوندايالدهايد , والكلوتاثيون , اذ شملت الدراسة عدد من الابقار البالغة (40) وعدد من العجول الصغيرة باعمار 2-3 اشهر (35 عجلا) , اخذ من كل حيوان عينة براز (5 غم) وعينة دم (10 سم) , شخضت الحيوانات المصابة من خلال الكشف عن الاعراض الظاهرة على الحيوان وفحص البراز .

بعد اخذ العينات عولمت مختبريا من خلال فحص البراز باستخدام طريقتي الطفو والمسحة المباشرة للعثور على الاكياس البيضية للطفيل , وحددت شدة الإصابة باستخدام طريقة الماكستر المعدلة , ثم اجريت الاختبارات البايوكيميائية على عينات الدم لكل من الابقار المصابة والسليمة , اذ استخدمت طريقة الاختبار اللوني لقياس مستوى المألوندايالديهايد وطريقة المان المحورة لقياس مستوى الكلوتاثيون في البلازما .

اظهرت نتائج الدراسة الحالية ان الاصابة بالطفيل ادى الى زيادة بيروكسدة الدهون في بلازما الدم لكلا من العجول والابقار على التوالي (1.0238 ± 4.3423 , 1.4232 ± 7.4918) مقارنة مع الابقار والعجول السليمة (0.465 ± 2.3423 , 1.063 ± 2.9537) بينما ادى الى انخفاض في مستوى الكلوتاثيون في كلا من الابقار المصابة (2.8213 ± 7.3041) مقارنة مع الابقار السليمة (2.2628 ± 11.2322) , كما لم يظهر فرقا معنويا في مستوى الكلوتاثيون ما بين العجول المصابة وغير المصابة .

الكلمات المفتاحية: داء الكوكسيديا , الابقار , بيروكسدة الدهون , الكلوتاثيون , الموصل.

The study results showed an increasing MDA units in the infected cows plasma ($4.3423+1.0238$) and infected calves ($7.4918+1.4232$) as compared to the non-infected ones ($2.3423+0.465$) & ($2.9537+1.06301$). Whereas the infection of that

parasite lead to decreasing GSH of the infected cows plasma (7.3041+2.82138) as compared to the non-infected ones (11.2322+2.26283). No big difference of MDA units could be found with the little calves despite the low GSH of the infected ones (7.1167+2.1895) as compared to the non-infected ones (5.7817+2.6865).

Key words: Cattle, Age , lipid peroxidation, Glutathione, Mosul.

Introduction

Protozoa are one cell creatures that can attack a lot of animals. There are 65000 types of free living protozoa some of which can attack both humans and animals [1].

This study deals with the diagnosis of the attack of one of the main and widespread types of such the creatures, coccidiosis, which is caused by certain types of protozoa (Eimeria sp, Eimeriidae, and has two other types: Eimeria sp and Esospora), [2]. Many livestock like cows and sheep can be attacked by such the disease during their lives and they can live with such the parasite in the sense that the main symptoms only appear when the infection is so severe or when the immune system of the animal is not that strong enough due to malnutrition, stress or being infected by some diseases [1,3].

Cows coccidiosis is one of the common cattle diseases that can attack cows and calves. Its symptoms are simple in cows whereas they are severe in little calves (less than a year) result in diarrhea and weakness [4]. Such the disease leads to high economic losses in the world (four million dollars annually) as a result of low quality meat and low weigh of the animal producing it in addition to its increasing sensitivity to other diseases. Such the disease is intracellular that attacks the intestinal membranes of poultry [5]. Generally, the symptoms can be simple and not apparent

that can lead to difficulties of early diagnosis [6]. The main symptoms of severe infection in cows and little calves are bloody or non-bloody diarrhea, decreased appetite, anemia, dehydration and low weigh [7]. This disease is really unique and can attack cattle severely, leading to death cases in little calves which result in high economic losses due to the need for effective treatments [8]. Generally, there are certain types of *Eimeria* that attack cows[9,10, 11]. Certain studies showed that the spread of such the parasite in cows depends on some factors: living areas, nutrition, age, sex and pond system. Clearly it was found that the parasite can attack cattle effectively in closed living areas as compared to open ones. Also, this parasite can highly attack in pond watered animals. Besides, the parasite can highly attack living areas with high number of cattle. The attack of this disease is intermittent in cattle, especially during moist times though some cases can be in summer, summer coccidiosis, and winter coccidiosis and it could last long based on crowded living areas, stress and limited water resources [12].

The parasite life cycle is complex, beginning with oocyst that comes out with the animal stool resisting hard circumstances for long times. Next comes the sporulate that are swallowed by hosts and spread as sporozoites that will pass to stomach cells creating merozoites, the latter penetrate the intestine membranes making macrozoites and microzoites that will be released and make zygote. The zygote will make new generations of oocyst to repeat the life cycle [13].

Generally, the attack comes from the sporulate that are available in water and food of the cattle and the biggest destruction of the host body can result from the multiple divisions of *Eimeria* near the intestine, a matter that leads to the decay of inner intestine cells [14].

Many studies around the world showed that diseases can lead to balance trouble between anti oxidation levels in blood and free radicals levels[15] and also that various infected cases as well as old age lead to increasing blood free radicals levels [16]. Such the balance can face certain trouble due to certain diseases, physiological ones, liver ones, inflammations, parasitic and cancer ones [16]. Getting such the physiological and parasitic diseases leads to high oxidation and so high lipid oxidation of not saturated lipid acids in cells membranes [17]. Oxidation effort can be defined as the lack of balance between free radicals quantities and anti-oxidation of the body, a matter that leads to destroying the structure of the cells [18].

The oxidation effort usually happens in multiple infected cases in animals and the increase of that lead to the increase of active Oxygen production (Reactive oxygen species) as well as the free radicals of the animal body which in turns lead to peroxidation of lipids and the destruction of the body cells [19].

Free radicals are defined as small molecules or atoms of high activity since they have non-double electrons. They had been expected to be derived from types of Reactive oxygen species (ROS). However, recent studies showed that they also have types of Reactive Nitrogen species (RNS).

Generally, the free radicals may be produced naturally in the body through the respiratory series of energy ATP in the mitochondria or through cellular oxidating under controlled circumstances like enzymes and its activities, Xanthin oxidase, nicotinamide adenine dinucleotide phosphate oxidase (NADPHoxidase) and Myocytes. It also plays a vital role in the immune system of the body within Myeloperoxidase, in the sense that it is available in Neutrophils and is a Monocysts and eosinophils [20,21].



Such the radicals are poisonous molecules of oxygen and capable of destroying all parts of body cells since it can enter into ten thousands of reaction series that lead to the destruction of DNA molecule, a matter that causes trouble in embryos and early old age [22].

Despite the fact that the existence of such the free roots of high concentration, they take part in many physiological processes of the body, like the enzyme activity, the immune reactions of the body, in the sense that they play an important role in the natural and acquired immunity, as well as a role of supplying anti-oxidation which is related to the immunity which contradict the lack of immunity that decreases within the age [18].

Materials and methods of the study

The recent study includes 40 cows and 35 little calves of 2-3 months old selected randomly from Ninawah plains between May 2019 and February 2020. A blood sample is taken from each animal together with stool samples.

Collecting samples

1 . Stool samples

An amount of 5gm of stool is taken directly from the anal cavity of the animal and protected in clean glass containers.

2 . Blood samples

An amount of (5ml)blood is taken from each animal and kept in tiny glass pipes which contain Ethylenediaminetetraacetic acid (EDTA) ,to get the plasma, then we kept them freezing.



Lab Tests

After viewing the symptoms of the infected animals like general weakness, bloody diarrhea and no interest in feeding, stool samples are taken to be tested microscopically using both direct smear and floating ways to examine the ovarian cysts. The infection level is to be determined by Assis et al, 2012 that depends on counting the number of those ovarian cysts in the sample in the slide, then calculating the cysts numbers per 1 gm stool multiplied by 10 and counting them by the microscope [23].

Biochemical Tests.

A . Malondialdehyde(MDA) level measuring in blood of Cattle.

The method is based on the principle of the chemical reaction between thiobarbutyric acid with the final product of lipid peroxidation(MDA) in the blood, within an acidic medium that can be obtained by adding TCA to the reaction solution, after that ,the solution is placed in a water bath for 15 minutes at 100 to complete the reaction ,then samples are cooled, and the filtrate is separated using centrifugation at 3000 rpm for several minutes, and then the absorbance is measured at a wavelength of 532 nm using Spectrophotometer-EMC LAB German origin [24].

B . Glutathione level measuring in blood plasma.

This is modified Elman's method which includes the reaction of Ellmans reagent 5,5-dithiobis-2-nitrobenzoic acid(DNTB) with cows plasma Glutathione leading to making a yellowish compound the absorption of which is measured by using



spectrophotometer instrument. The strength and depth of the color depend on Glutathione concentration in the sample [25].

Results

The recent study results show that the little calves infection percentage is 42.11% whereas it is 30.16% in cows.

The impact of infection on Malondialdehyde (MDA) level in Cattle blood .

The recent study results show a clear increase of malondialdehyde MDA concentration in infected cows plasma (4.1635±1.02388) as compared to non-infected ones (2.3423±0.4650) at (P < 0.05).

The impact of coccidiosis parasite on MDA level in little calves blood plasma.

The recent study results show a clear increase of MDA concentration in infected calves plasma (7.4918±1.4232) as compared to the non-infected ones (2.9537±1.0630) of P < 0.05.

MDA umol/L	Groups			
	A (11)	B (14)	C (17)	D (23)
	7.4918±1.4232	2.9537±1.06307	4.1635±1.02388	2.3433±0.4650



Table 1: The impact of coccidiosis parasite on MDA level in little calves and cows blood plasma.(A=Infected Calves , B=Control Calves , C=Infected Cow , D=Control Cow) .

The impact of the infection on Glutathione level in Cattle Blood.

The recent study results show a clear decrease of GSH levels in infected cows (7.3041+2.82138) as compared to non-infected ones (11.2322+2.26283) of $P < 0.05$

The impact of coccidiosis parasite on GSH level in little calves blood plasma.

The recent study results show no clear difference in GSH level between infected calves with coccidiosis parasite (5.7817+2.6865) and non-infected ones (7.1167+2.18950). Glutathione concentration decreased. However, it shows no clear function.

GSH <i>umol/L</i>	Groups			
	A	B	C	D
	11.2322±2.26283	7.3041±2.8213	7.1167±2.18950	5.7817±2.6865

Table 2: The impact of coccidiosis parasite on GSH level in little calves and cows blood plasma.(A=Control Cow ,B=Infected Cow ,C=Control Calves ,D=Infected Calves).



A= control cows, B= non-infected cows, C= non-infected calves, D= infected calves

The impact of age variable on MDA level for both non-infected cows and calves

The recent study results show no clear difference of MDA levels in cows plasma (2.3423+0.4650) as compared to little calves (2.9537+1.0630) in blood plasma of $P < 0.05$.

The impact of Age variable on MDA level in blood plasma for both infected and non-infected Cattle.

The recent study results show high MDA concentration in infected calves plasma (7.4918+1.4232) as compared to infected cows (4.1635+1.02388).

MDA umol/L	Infected		Non-infected	
	Cow	Calves	Cow	Calves
	4.1635±1.02388	7.4918±1.4232	2.3423±0.4650	2.9537±1.0630

Table 3: The impact of age variable on MDA level in blood plasma for both infected and non –infected cattle.

The impact of Age variable on Glutathione level for both non-infected and infected Cattle.

The recent study results show a clear increase of Glutathione level in cows plasma (11.2322+2.2628) as compared to its level in blood plasma of little non-infected calves (7.1167+2.18950) of $P < 0.05$.



The impact of age variable on Glutathione level in the blood plasma of both infected cows and calves with coccidiosis parasite.

No clear difference of GSH level in blood plasma of infected cows (7.3041 ± 2.8213) as compared to its concentrations in infected calves plasma (5.7817 ± 2.6865) could be seen.

GSH <i>umol/L</i>	COWS		CALVES	
	infected	Non-infected	Infected	Non-infected
	7.3041 ± 2.8213	5.7817 ± 2.6865	11.2322 ± 2.2628	7.1167 ± 2.1167

Table 4: The impact of age variable on GSH level in blood plasma for both for infected and non –infected cattle.

Malonedialdehyde (MDA) concentrations

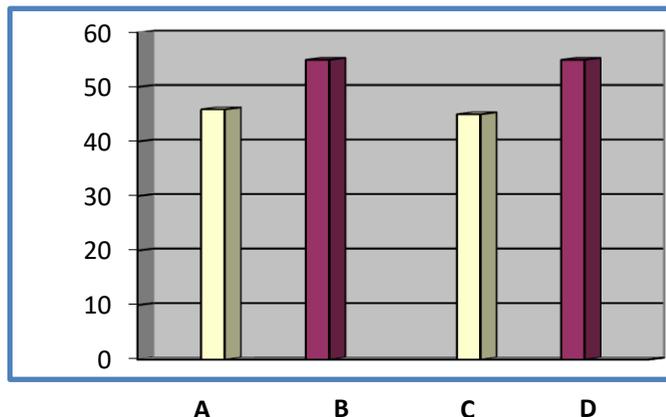


Diagram 1: The impact of Coccidiosis parasite infection on MDA level in both cattle and little calves plasma. (a.Non- infected calves 7.4918 , b. Infected calves 2.9537, c. Non-infected cattle 4.1635 , d. Infected cattle 2.3423).

Glutathione(GSH) concentrations

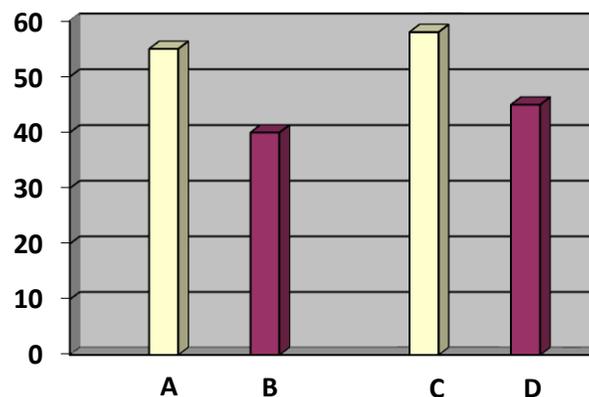


Diagram 2: The impact of Coccidiosis parasite infection on GSH level in both cattle and little calves (a. Non-Infected calves 5.7817 , b. Infected calves 7.1167 ,c.Non-infected cattle 7.3041 , d. Infected cattle 2.3423).

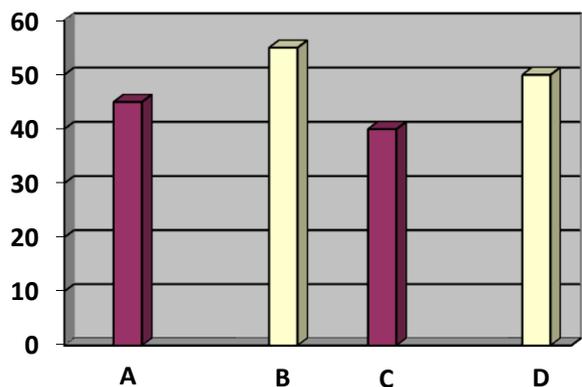


Diagram 3: The impact of Age variable on MDA level of cattle. (A.Non-infected calves 7.4918 , B. . Non-infected cattle 4.1635 ,c.Infected Calves 2.9537 ,d.Infected cattle).

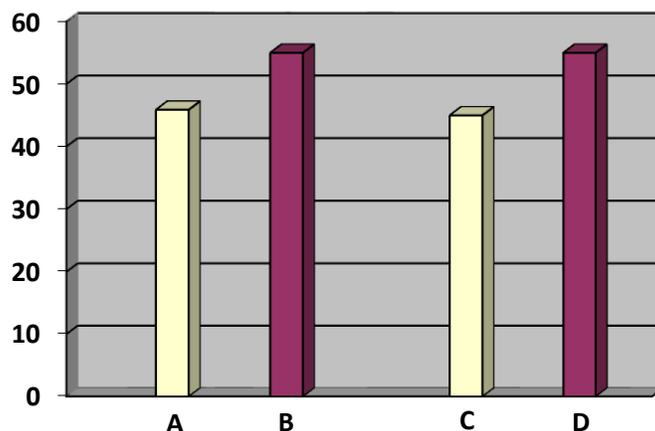


Diagram 4: The impact of Age on GSH level in cattle. (a.Non-infected calves 5.7817 , b.Non-infected cattle 7.3041, c. Infected calves 7.1167 , d. Infected cattle 2.3423).

Discussion

This study shows that the coccidiosis infection percentage in calves was higher than the one in cows, 42.11% & 30.16% respectively. The number of the infected cases increases in the closed and crowded areas. Although this study has not dealt with the type of coccidiosis, the infection is diagnosed and the percentage is checked in both

cows and calves in addition to determining its level. Then blood measurement done for Malondialdehyde and Glutathione in the plasma of the infected and non-infected cows and calves showing that the infection with cows coccidiosis leads to some changes on blood measurements as the increase of the infected cases in both cows and calves increases MDA level in the infected animals blood as compared to those non-infected ones, a similar study was done in Turkey [9] which also shows a clear increase of MDA levels and breathing speed in the blood of the infected cows with coccidiosis parasite as compared to non-infected ones. Also, it shows that a clear decrease on the reaction of anti-oxidation of the animal. The recent study agrees with another one done by [27, 5] which showed that the infection of coccidiosis parasite in both cows and buffalos led to increasing MDA levels and decreasing the anti-oxidation levels, like catalase, super oxidase, Glutathione,

Generally, it was seen that getting infected with different parasitic diseases lead to an increase of the free radicals and lipid peroxidation in the host body, also the free radicals quantity and its increase in the host body depends on a number of elements: nutrition type, the parasitic infection level, the destructive impact of the parasite as well as the parasite type. The infection of parasites like *Eimeria* spp causes oxidation effort increase in the host body and so increasing lipid peroxidation [28].

Besides, it was seen in other studies done on other animals, like poultry, that getting infected with coccidiosis parasite results in a clear increase of MDA level in the infected poultry as compared to non-infected ones in addition to a clear decrease of anti-oxidation enzymes activities [29].



In another study done on cattle, it was seen that getting infected with coccidiosis parasite leads to the increase of lipid peroxidation and so decreases the levels of anti-oxidation. This is due to a number of elements among which is the incapability of the infected cells with parasitic diseases to produce suitable amounts of anti-oxidation as well as the increase of oxidation effort on cells membranes as a result of high existence of free roots in them [30].

Furthermore, it was seen in this study that there was a clear decrease in Glutathione levels of both infected cows and calves as compared to the non-infected ones. That is similar to other studies mentioned previously which in turn showed that the parasitic infection leads to a clear decrease of anti-oxidation levels.

This study also includes determining the impact of age variable on the levels of both MDA as a final outcome of lipid peroxidation and GSH (anti-oxidation) in the sense that MDA and Glutathione levels of non-infected cows and calves do not show a clear difference between them. However, when infected with coccidiosis parasite, there is a clear increase of MDA level in little calves plasma as compared to infected cows that can be seen. On the other hand, no clear difference is seen of GSH levels between infected cows and calves with coccidiosis parasite. As for the non-infected cows and calves, the results showed a clear increase of GSH concentration for the cows as compared to little calves.

The increase of the free roots as well as the levels of lipid peroxidation in the little calves in addition to the decrease of Glutathione levels are due to the reason of having an immature immune system in little calves [6] and this is the same reason that leads to the existence and increase of certain diseases of little calves as compared to cows. [31] finds out that many of the stomach and parasitic diseases are of high



levels in little animals as compared to older ones and this was due to a number of reasons: the immature immune system, not getting such the diseases before and the lack of resistance to those diseases in little calves.

Similarly, the study of [32] showed that the newborn calves have high MDA concentration as compared to older animals and a low anti-oxidation level on the other hand which is due to the creation of oxidation effort during the birth giving of little calves. Also, in a relevant study [10] in Iran, there was seen a clear relation between the percentage of coccidiosis infection and the strength of both symptoms and age impact in the sense that the older infected cows show no symptoms.

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