

CLINICAL AND SOME BIOCHEMICAL CHANGES ASSOCIATED WITH THEILERIOSIS IN BUFFALOES

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ABSTRACT

Examination of 101 buffaloes during the period extend from the beginning of March 1999 to the end of February 2000 revealed that thirty four (33.66%) animals were infested with *Theileria*. The present study was aimed firstly to demonstrate the specific clinical findings detected in response to infestation with *Theileria* in buffaloes. The second aim was to record the effect of this intracellular blood parasite on the glutathione redox system and some erythrocytic enzymes which reflect the function of erythrocytes, and also its effect on the electrophoretic pattern of serum proteins, as well as some trace elements concentrations. Clinical examinations of diseased animals revealed rise of body temperature, anorexia, nasal and ocular discharge, enlargement of superficial lymph nodes, dyspnoea, bilateral corneal opacity, progressive anemia and jaundice . The biochemical data of the erythrocytic haemolysate of the diseased group showed highly significant increase in the mean values of the glutathione peroxidase (GSH PX), glutathione transferase (GST) and lactate Dehydrogenase (LDH), while the mean values of reduced glutathione (GSH), glutathione reductase (GR-ase), total superoxide dismutase (t-SoD), Glucose 6- phosphate Dehydrogenase (G 6 PD), and Acetylcholinesterase (ACHE) were significantly decreased. Regarding the alterations in the electrophoretic pattern of serum proteins, there was significant increase in the levels of total proteins, alpha, beta and gamma globulins, meanwhile, the serum albumin was significantly decreased.

On the other hand , serum iron, selenium, copper, manganese and zinc showed significant increase.

INTRODUCTION

Theileriosis is an acute or chronic protozoal disease of animals caused by *Theileria* sp., trans-

mitted by hard ticks and characterized clinically by intermittent fever, enlargement of superficial lymph nodes, nasal discharge, dyspnoea, progressive anemia, jaundice, corneal opacity and ketonuria (Onuma et al, 1997). Theileria are blood parasites multiply by shizogony in the lymphoid tissues and finally invade the red blood corpuscles where they are seen as rod like, round, oval or irregular forms.

This disease is summer spreading as the rate of infection in June, July, and August were the highest than all over the year reaching 34.20, 41.67 and 12.77% respectively (Tutishin and kazakhstan, 1981).

Dhar and Gautam (1979); Sahu et al (1996) and Sandhu et al (1988) Studied the effect of Experimental theileriosis in cattle was accompanied by hypoproteinemia associated with an increase of Alpha and Beta globulins fractions in both acute and subacute stages, while in chronic phases only the Gamma globulin fraction was greatly increased, Dhar and Gautam (1979); Sahu et al (1996) and Sandhu et al (1988).

On the other hand, theileriosis showed an increase of serum iron and copper and this increase usually related to the degenerative process of red blood cells caused by haemoglobinase enzyme of the parasite which is responsible for release of iron and copper (Goldberg et al, 1991 and Watanabe et al, 1998). Selenium, zinc and Manganese act as prosthetic group of the glutathione-dependent enzymes and superoxide dismutase and the increase in their levels indicate the destruction of these enzymes proceeding hemolysis of them (El-sayed and Rady, 1999).

Ashmawy, et al (1994), and El-Sayed and Rady (1999) recorded that, the erythrocytic glutathione peroxidase (GSH - PX) and glutathione transferase (Gst) as well as total superoxide dismutase (T. SoD) were decreased in theileria infested cattle.

Kaniko, (1989) reported that the erythrocytic G - 6 PD, and LDH activities were significantly increased in horses affected with infectious anemia after the development of the disease. Srivastava, et al., (1992) observed that the activities of glycolytic enzymes and transaminases were increased in theileria infected animals.

MATERIAL AND METHODS

Animals : The present study was carried out on 101 buffaloes collected from different localities at kalubia Province during the period extended from the beginning of March 1999 to the end of February 2000. Depending on the clinical picture and blood parasite examinations, these animals were allotted into two groups:

Group I : Consisted of 34 animals suffered from fever, nasal discharge, enlargement of super-

facial lymph nodes and some of them had corneal opacity. Blood examinations of these animals revealed infection with *Theileria*. spp .

Group II : Contained of 67 apparently healthy animals. Blood examination proved that these animals were free from blood parasites. This group was served as control.

Blood sampling : Firstly one drop of blood was collected from the ear vein of each examined animal for preparation of blood smear for parasite examination according to **Pritchard and Kruse (1982)**. Then , two blood samples were collected from jugular vein of both infested and healthy animals. The first sample was collected in to heparinized syringe contained 20 I. U. heparin as anticoagulant for each one ml blood, then gently mixed and rapidly poured in clean, dry and labelled tubes and proceeded for washing of erythrocytes using physiological saline, followed by preparation of erythrocytes haemolysate by Digitonin as described by **Kornburg and Koecker (1955)** for estimation of erythrocytic GSH-PX. (Ec. 1.11.1.9) ,**Chiu, et al (1976)**; GSH, **Sedlak and Lindsay (1968)**; GR- ase. (EC 1.6.4.2), **Bergmayer (1983)**; GST (EC 2.5.1.18), **Vessey and Boyer (1984)**; T. SOD (EC 1.15.1.1) **Misra and Fridovich, (1972)**; LDH, **Smith, et al (1965)** ; G6PD, **Kornburg and Koecker (1955)** and ACHE. **Gingerich and Mia (1981)**. The second sample was collected without anticoagulant for separation of sera which used freshly for electrophoretic study of serum proteins by Agarose gel electrophoresis according to **Alper, (1974)**. The serum iron, selenium, copper, manganese, and zinc were estimated by Atomic absorption spectrophotometer according to **Bauer, (1982)** .

RESULTS AND DISCUSSION

Blood films examination revealed that 34 (33.66 %) out of 101 animals were infected with *Theileria* Species (Table. 1) . Nearly similar results were obtained by **El- Bahy (1986)** . Concerning seasonal prevalence (Table.1), the Peak of infection was in Autumn (51.61%) followed by Summer(40%) and 20% Spring and the lowest was in Winter (6.67%). Similar results were obtained by **Eld et, al (1988)** who recorded that the peak of infestation with *Theileria annulata* in cattle was in Autumn followed by summer . The present data revealed that buffaloes with age of 6 months to 5 years were highly susceptible to theileriosis (54.91%) followed by those over 5 years old (15.79%) and the lowest infestation was for calves less than 6 months . Similar results obtained by **Eld et al (1988)** who recorded (11.11%) in calves under one year and 16.06 % in cattle ranged between 1 to 3 years old . The slight difference may attributed to the species of the animal. Clinical examinations of animals infected with *Theileria* revealed rise of body temperature, anorexia, depression, nasal and ocular discharge, enlargement of superficial lymph nodes, jaundice, dyspnoea and severe emaciation. Some cases showed bilateral corneal opacity. These ani-

mals were heavily infested with ticks. These results were similar to those observed by **Onuma et al (1997)**. The enlargement of superficial lymph nodes usually related to the multiplication of *Theileria* by chizogony in the lymphoid tissues. Nasal discharge and dyspnoea were due to pulmonary oedema. Progressive anemia and jaundice which might be regarded to haemolysis caused by *Theileria*. All these analytical studies are coincided with those of **Radostits et al (1995)**.

The data of table (2) revealed a marked increase in the mean values of GSHPX; GST and LDH, while there are significant decrease in the mean values of GR-ase, GSH, G6PD, TSOD and ACHE in *Theileria* infected animals comparatively with those of clinically healthy animals. The Glutathione redox system is very important in protection of the host cells against oxidative stress and radicals damage. It also plays a role in the protection of phagocytic leukocytes against their own products of oxygen radicals. It consists of reduced glutathione GSH which react with peroxides in the GSHPX to remove toxic substances and radicals, since it possess active sulphhydryl group, **Novak et al (1991)**. As a result of the previous reaction the oxidized glutathione (GSSG) was formed which in turn react with NADPH in the GR-ase to reconstitute reduced glutathione GSH, thus this redox cycle was able to detoxify peroxides and to reduce oxidised thiol group in the proteins and that explain the high significant increase of GSH-PX as mentioned by **Slater, (1979)**.

In this respect the recorded highly significant increase of GST, table (2) may indicated that, the protection of erythrocytes membrane against lipid peroxidation was mediated by one or more glutathione dependent enzymes as illustrated by **Burk et al (1980)**. The recorded significant reduction of GSH and GR-ase were in accordance to the data reported in cattle by **Ashmawy et al (1994)** and **EL-Kattawy, (1995)** which could be attributed to the decreased activities of G6PD which generate reduced NADPH which generate GSH from GSSG under the effect of G-R-ase, **Rotruck et al (1973)**. Regarding the effect of *Theileria* on T-SOD the present data, table (2) revealed a significant decrease for infected animals which might be related to the action of *Theileria* produced radicals inhibiting the red cells superoxide dismutase, **Becuwe et al (1992)**. The recorded significant decrease of G6PD activities table (2) might be attributed to the accumulation of altered forms of the enzyme in the diseased cells that generated less amount of NADPH+H than normal ones, **El-Ghannam (1986)**. The highly significant increase of LDH activities, table (2) were came in accordance to those recorded by **El-Kattawy (1995)**. This elevation might be due to hepatic damage and lesions induced by the parasite, **Niinuma et al (1991)**. The noticed significant decrease of activity of ACHE, table (2) could be related to the blocking of enzyme activity by the Parasite as stated by **Sharma and Tripathi (1985)**. Concerning serum proteins electrophoretic pattern of control as well as infested animals as shown in table (3), total serum pro-

teins and all globulins fractions were significantly increased while the serum albumin fraction was greatly decreased in infested animals. The significant increase of globulins fractions in infected animals was nearly similar to observations obtained by **Dhar and Gautam (1979)** who found that the experimental *Theileria* infection station was accompanied by an increase of alpha and beta globulin fractions, while gamma globulin fraction was increased significantly in chronic phases. The recorded hypoalbuminemia in infected animals may be attributed to the harmful effect of the Parasite on the liver function and consequently reduction of albumin synthesis as mentioned by **Murry, (1978)** Regarding trace elements changes for animals with Theileriosis, table (3) indicated significant increase of serum iron and copper. These changes might be due to the degenerative process of red blood cells caused by haemoglobinase enzyme of the parasite which is responsible for release of copper and iron. **Goldberg et. al, (1991)**. The significant increase of serum selenium, zinc and manganese. table (3) indicated the damage of the glutathione dependent enzymes and superoxide dismutase proceeding liberation of them, as illustrated by **Ashmawy et al (1994)**.

Table 1: Prevalence of Theileriosis among buffaloes.

Season Age	Spring			Summer			Autumn			Winter			Total		
	No. of examined animals	No. of +ve cases	%	No. of examined animals	No. of +ve cases	%	No. of examined animals	No. of +ve cases	%	No. of examined animals	No. of +ve cases	%	No. of examined animals	No. of +ve cases	%
Less than 6 months	9	-	-	6	-	-	7	1	14.29	6	-	-	27	1	3.70
6 months - 5 years	12	5	41.67	18	11	61.11	18	13	72.22	7	1	14.86	55	30	54.91
Over 5 years old	4	-	-	6	1	16.67	6	2	33.33	3	-	-	19	3	15.79
Total	25	5	20	30	12	40	31	16	51.61	15	1	6.67	101	34	33.66

Table 2: The mean values of glutathione redox system; G6PD; LDH; and ACHE in

Parameter Groups	GSH-PX U/g prot	GR - ase U/g prot	GST U/g prot	GSH U/g prot	T-SOD U/g prot	G6PD MU/109 RBCs	LDH UI blood	ACHE X 102 U/L blood
Control (X ± S.E.)	3.696 ± 0.980	0.828 ± 0.084	0.336 ± 0.072	0.792 ± 0.048	13.272 ± 1.400	88.91 ± 3.880	154.18 ± 4.180	39.92 ± 0.630
Infected (X ± S.E.)	7.392** ± 0.348	0.448* ± 0.077	0.840** ± 0.108	0.400* ± 0.020	8.352* ± 0.820	61.120* ± 2.900	231.66** ± 7.120	21.080* ± 0.260

Table 3: The mean values of Electrophoretic pattern of serum proteins and trace elements in clinically healthy (control) and Theileria infected buffaloes.

Parameter Groups	Total Protein (gm/dl.)	Albumin (gm/dl.)	Total Protein (gm/dl.)	Globulin fractions (gm/dl.)				Iron (Ug/dl.)	Selenium (Ug/dl.)	Copper (Ug/dl.)	Manganese (Ug/dl.)	Zinc (Ug/dl.)
				α1	α2	β	γ					
Control (X ± S.E.)	7.980 ± 1.224	4.072 ± 0.670	3.908 ± 0.170	0.432 ± 0.108	0.628 ± 0.071	0.888 ± 0.101	1.960 ± 0.250	323.180 ± 16.340	3.070 ± 0.250	76.000 ± 3.080	0.140 ± 0.125	119.85 ± 4.980
Infected (X ± S.E.)	8.850 ± 0.61	3.160* ± 0.300	5.700* ± 0.290	0.750* ± 0.095	0.971* ± 0.12	1.300* ± 0.109	2.700* ± 0.130	463.530* ± 17.090	7.180* ± 0.189	90.870* ± 4.090	7.860* ± 0.971	193.120* ± 12.70

(X ± S.E.) = mean ± Standard error.

* = Significant at P < 0.05

** = High significant at P < 0.01.

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الملخص العربى

الغيريات الإكلينيكية والبيوكيميائية المصاحبة للثيليريا فى الجاموس

المشركون فى البحث

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قسم انطفيليات - كلية الطب البيطرى جامعة الزقازيق - فرع بنها **

أجريت هذه الدراسة لمعرفة الأعراض الإكلينيكية المصاحبة للثيليريا أنيولاتا فى الجاموس وكذلك لمعرفة نسب الإصابة فى محافظة القليوبية وكذلك أيضاً نسب الإصابة على مدار مواسم السنة وفى الأعمار المختلفة وكان من أهداف هذه الدراسة معرفة بعض تغيرات الدم فى حالة الإصابة بهذا الطفيل وقد تم التركيز على تأثيرها على نظام الجلوتاثيون ريدوكس وبعض خمائر كريات الدم الحمراء وكذلك نط الفصل الكهرسى لبروتينات الدم والعناصر النادرة فى دم الجاموس المصاب بهذا الطفيل والمسجل حقلياً مقارنة بالمستويات فى الجاموس السليم إكلينيكياً وقد أسفرت النتائج الإكلينيكية على وجود تضخم فى الغدد الليمفاوية الظاهرة ووجود عتامة على كلا العينين وكانت الحيوانات المصابة موبوءة بالقراد. وقد شكلت نسبة الإصابة فى محافظة القليوبية حوالى 33.66% وكانت نسبة الإصابة عالية فى فصل الخريف حيث وصلت 51.61% بينما فى فصل الصيف كانت 40% وفى فصل الربيع كانت 20% وكانت أقل نسبة فى فصل الشتاء، حيث وصلت 6.76% وكانت أعلى نسبة فى الإصابة عند أعمار تتراوح بين 6 شهور وخمس سنوات (54.91%) وفوق خمس سنوات كانت 15.79% وكانت أقل نسبة عند أعمار أقل من ستة شهور وبالنسبة لتغيرات الدم كان هناك زيادة معنوية عالية فى مستوى الجلوتاثيون بيروكسيداز والكلوتاثيون ترانسفيراز وكذلك خميرة اللاكتات ديهيدروجينيز بينما كان هناك نقصاً معنوياً فى مستويات الجلوتاثيون المختزل والجلوتاثيون ريدكتيز والسور أوكسيد ديسميوتيز الكلى والجلوكوز 6- فوسفات ديهيدروجينيز والاستيل كولين استراز أما بالنسبة لتأثير الطفيل على نط الفصل الكهرسى لبروتينات الدم فقد سجل نقصاً معنوياً فى مستوى الزلال بينما ازدادت باقى الأنماط الألفا والبيتا والجاما جلوبيولين وكذلك البروتين الكلى وملاحظة مستويات العناصر النادرة فقد وجدت زيادة معنوية فى مستويات الحديد والسليسيوم والنحاس والمنجنيز والزنك وبمما سبق يتضح مدى تأثير هذا الطفيل على العمليات الحيوية الأساسية لكريات الدم الحمراء وكذلك على البروتينات والعناصر النادرة مما يستلزم ضرورة مكافحة هذا الطفيل.