





HEMATOLOGICAL AND BIOCHEMICAL INVASTIGATION IN BOVIN BABESIOSIS AND THEILERIOSIS

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ABSTRACT

Fifteen out of 75 native cattle 5-7 years old from different localities at Sharkia Provence were used to study hematological changes in Babesia and Theileria infestation verified with the clinical signs during period from May 2009 to the end of April 2010. Animals were allotted into three groups (n=5/each), 1st group were healthy cattle free from blood parasite (control), 2nd group were suffering from babesiosis and 3rd group were suffering from theilerosis. Clinical examination included body temperture, rate of respiration and ruminal movment. Two blood samples were collected from each animals for determination of erythrogram and leukogram changes in whole blood and some biochemical parameters in seprated sera. The results revealed that body temperture and respiration rate were significantly increased in cattle suffering from babesiosis and thelariosis while ruminal movement were significantly decreased. Hematological picture revealed a significant reduction in RBCs, HB and PCV in cattle infected with eitheir babesiosis or theileriosis. Babesiosis induce significant reduction in MCV, MCH and MCHC (microcytic hypochromic anaemia) while theileriosis induced increase in MCV and decrease in MCH and MCHC (macrocytic hypo-chromic anaemia). Bovine infected with babesiosis and thelariosis revealed leukopenia, neturopenia and eosinopenia accompanied with lymphocytosis, monocytosis and basophilia compared to that of control group. The result of biochemicaly analysis showed that cattle infected with babesiosis and theileriosis revealed significant reduction in total protein, albumin, globulin, cholesterol, Triglycerides and glucose levels associated with significant increase in the activities of liver enzymes (AST, ALT, alkaline phosphatase) and total bilirubin. It could be concluded clinical signs namely red brown to coffee urine (in babesiosis), enlargement of superfacial lymph nodes, corneal opacity and macrocytic hypochromic anaemia (in theileriosis) in addition to hematological picture (microcytic and macrocytic hypochromic anaemia in babsiosis and theilorosis, respectively) could be useful for easily diagnosis of blood parasites

KEY WORDS: Biochemical, Blood Parasite, Hematological, Theileriosis

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1.INTRODUCTION

arasitic infection as blood parasite constituent a major problem in cattle breeding due to severe economic losses and lowered vitality which lead to decrease of animal production and increase the susceptibility to bacterial and viral infections [16]. The main clinical signs in the animals infected with blood parasite were fever (41.0–41.5°C), congested mucous membranes,

anorexia, nasal discharge, lacrimation, haemoglobinuria, anemia, respiratory distress and eye lesions beside presence of ticks on the animal body [6]. Theileriosis and babesiosis cause severe economic losses and have effect on the immune status of the body [57].

Babesiosis is a tick-borne hemolytic disease of domestic and wild mammals caused by intraerythrocytic protozoan parasites of the genus Babesia [15]. Babesiosis is endemic in tropical and subtropical regions as Egypt [14]. Babesiosis is initiated by tick-borne transmission of the sporozoites, which subsequently invade host red blood cells in the infected animals [62]. It is mainly transovarian transmitted by ticks [34].

Theileriosis is an important parasitic disease of animal in Egypt which causes great economic losses in animals and their products. Theileria species is a protozoan parasite of cattle and domestic buffaloes, transmitted by ticks of genus Hyalomma and causes a disease named theileriosis [36]. Theileriosis have high morbidity and mortality rate in cattle [9].

Diagnosis of blood parasite in acute cases is mainly based on clinical signs of the infected animals which confirmed by microscopic examination of a thin blood film stained with Giemsa stain [53]. However, expertise in piroplasm microscopy is required in subclinical or chronic infections because parasitaemias are often extremely low and Theileria piroplasms may be difficult to be found in stained blood smears or otherwise may be missed [2].

For these reasons, the present study was planned to determine hematological picture and with protein profile, to confirm clinical signs for clinical diagnosis of babesiosis and theileriosis.

2. MATERIALS AND METHODS

2.1. Animals

The study was conducted on 75 native cattle of 5-7 years old from different localities at Sharkia Provence in the period from May 2009 to the end of April 2010.

2.2. Blood smears

Blood smears were prepared from ear vein, left to dry, fixed in methanol and

stained by freshly prepared Geimsa stain for 45 minutes to look for parasites [53].

2.3. Experimental Design:

Fifteen cattle out of seventy five native cattle which divided into three equal $groups, 1^{st} \quad group \quad healthy \quad cattle \quad as$ control,2nd group cattle suffering from 3rdgroup cattle and babesiosis suffering from theilerosis. Clinical examination body temperature, rate of respiration and ruminal movement were done, Two blood samples were taken from each animal by jugular vein puncture, 1st sample was taken in tub contain EDTA for determination of erythrogram, leukogram and blood indices (Mean Corpuscular Volume Mean corpuscular hemoglobin and Mean Corpuscular Haemoglubin Concentration) according to (26), meanwhile 2nd sample was taken in centrifuge tube to obtain clear serum for estimation of total proteins [12], and globulin albumin [13] mathematically, transaminases (AST, ALT) [47], alkaline phosphatase (27), glucose [54], cholesterol [46] and triglycerides [48].

2.4. Statistical analysis:

The obtained data were statistically analyzed according to [42].

3. RESULTS AND DISCUSSION

Clinical investigations (table 1&2) revealed that cattle suffering from babesiosis were showed clinical symptoms, including fever, anorexia, congested mucous membranes, with presence of tick on the animal body and presence of hemoglobinuria in cattle suffering. Same clinical signs were reported previously [18] where cattle suffering from babesiosis show

fever, anorexia, congested mucous membranes and dark brown to coffee urine and this brown urine may be due severe haemolytic process associated with the presence of Babesia within red blood cells. Cattle theilerosis infected with showed enlargement of the superficial lymph nodes beside anorexia, congested membranes. Clinical observation of the cattle infected with thelaria species were in agreement with the findings of other studies [40, 59] who stated that the main clinical signs in buffaloes infected with Theileria were pyrexia (40.5–41.5°C), slight nasal discharges, enlargement of superficial lymph nodes, salivation, decreased milk yield, and respiratory distress.

The results of the hematological investigation in cattle suffering from babesiosis and thelariosis revealed significant reduction in erythrocytic count, haemoglobin content, packed cell volume%. Microcytic hypochromic anaemia was occurred to

significant reduction in MCV and MCHC in case of babesiosis, while in theileriosis there was macrocytic hypochro- mic anemia, increase in MCV and decrease in MCHC table (3). These results were identical to that recorded formerly [10] in cattle suffering from babesiosis. Changes in erythrogram picture might be due to destructive effect of the parasites on erythrocyte [44, 58] in cattle infected with babesiosis. Also, Homer et al. that [24] mentioned protozoan intraerythrocytic parasites induced lyses of the infected RBC, which resulting in severe clinical symptoms, such as anemia, fever, and hematuria. Microcytic hypochromic anaemia observed in cattle infected with babesiosis could be attributed to intravascular haemolysis of red blood cells in this field [41]. Besides, theileriosis induced general anemia with fall in total erythrocytic count, packed cell volume% and hemoglobin content [17, 49].

Table 1 Incidence of Babesia and Theileria infection in cattle in different localities at Sharkia Province based on blood film examination.

			No. of cattle			Babesiosis		theileriosis	
City		he	healthy diseased		seased	N T		N . 0/	0.4
•	Total number.	No	%	No	%	No	%	No	%
Abu-Kabeer	12	10	83.33	2	16.67	2	16.67	-	-
Kafr-Sakr	10	9	90	1	10	-	-	1	10
Menia-lkamh	13	9	69.23	4	30.77	1	7.69	3	23.08
Abo-Hamad	14	12	85.71	2	14.28	1	7.14	1	7.14
Ibrahimia	15	14	93.33	1	6.67	-	-	1	6.67
Blibas	11	9	81.82	2	18.18	1	9.09	1	9.09
Total	75	63	84	12	16	5	6.67	7	9.33

Table 2 Clinical examination of animals (Body temperature, respiratory rates & ruminal movment of healthy and diseased cattle (n= 5)

D	Healthy cattle	Dise	ased
Parameters		Babesiosis	Thelariosis
Body temperature (°C)	38.6±0.62	40.54±0.52*	40.90 ± 0.55*
Respiratory rate/min	22.17±1.93	32.20±2.04**	31.19±1.96**
Ruminal contraction/2min	4.39±0.25	2.52±0.41**	2.79±0.56*

^{* =} P < 0.05 and ** = P < 0.01

Our observations were in accordance with those documented previously [1, 33, 45] stated that Theileria induce significant decrease in packed cell volume, total erythrocytic count and hemoglobin content. Besides, theileria calves induced macrocytic hypochromic anaemia [52]. This might be attributed to the toxic metabolites of theileria species which have harmful effect on bone marrow and interfere with the process of erythropoiesis [21]. In the present study, it has been shown that bovine babesiosis and theilariosis evoked leukopenia, neturopenia and accompanied eosinopenia with lymphocytosis, monocytosis basophilia compared to that of control group (Table, 4). Also, there was significant increase in monocytes in Babesia infection due to their role as active mediators in the innate immune response [11]. Formerly it mentioned that babesia disintegrates the leukocytes resulting in leukopenia Γ29, 37]. Our results were in accordance with that recorded [15] previously mentioned that monocytosis in animals infected with babesiosis species occurred as a mean of body defense against infection. The increased number of lymphocytes in our result was previously reported [8]. Similar findings were reported

formerly [38, 40] as a significant increase in lymphocyte counts was fund in buffaloes with theleriosis. In this field, former authors [35, 52] mentioned that theileria infection induced significant decrease leukocytic count. This finding was supported by earlier observations [43, 51] found that leukopenia, neturopenia and eosinopenia accompanied with monocytosis lymphocytosis, basophilia in cattle infected with Theileria. The obtained data were in accordance with those previously obtained [50] who mentioned that in cattle infected with theileriosis induced significant elevation in lymphocytes and decrease in total leukocytic count. Benjamine [8] postulated that the lymphocytosis was marked during the formation of antibodies in response to antigens and also during theileria infection.

The obtained of biochemical analysis reported in the table (5) showed significant decrease in serum total protein, albumin and globulin levels while A/G ratio was insignificantly decreased in cattle infected with babesiosis or theileriosis .These results were in consistence with that illustrated in buffaloes[3, 37]. Earlier studies [19, 31] stated that babesiosis in dogs induced hypoalbuminemia.

Table 3 Erythrogram of healthy and diseased cattle (n=5)

Groups	$R.B.C.(10^6/mm^3)$	H.B (gm %)	P. C.V. (%)	MCV (fl)	MCH (Pg)	MCHC (%)
Control	8.06 ± 0.89	13.22±1.18	34.72±113	43.08±0.44	16.40±0.66	38.0±0.42
Babesiosis	$4.52\pm0.92^*$	$9.09 \pm 1.34^*$	27.17±2.18*	40.20±0.51**	13.47±0.59*	33.51±0.83**
Thelariosis	$5.47\pm0.61^*$	10.18±1.22*	28.61±0.33*	56.16.±2.94**	14.95±0.17*	26.6±1.48**

 $^{^*}$ = P < 0.05 and ** = P < 0.01.

Table 4 Erythrogram of healthy and diseased cattle (n=5)

Groups	$R.B.C.(10^6/mm^3)$	H.B (gm %)	P. C.V. (%)	MCV (fl)	MCH (Pg)	MCHC (%)
Control	10.27±0.17	3.12 ± 0.22	3.23±0.21	1.32 ± 0.10	1.65±0.15	0.95±0.12
Babesiosis	$9.75\pm0.16^*$	$1.93\pm0.35^*$	$3.88\pm0.19^*$	$1.80\pm0.17^*$	$0.90\pm0.26^*$	$1.24\pm0.03^*$
Thelariosis	$9.70\pm0.19^*$	$1.59\pm0.42^{**}$	$3.90\pm0.20^*$	$1.64\pm0.12^*$	$1.19\pm0.13^*$	$1.33\pm0.10^*$

 $^{^*}$ = P < 0.05 and ** = P < 0.01.

The changes in the protein picture in animals suffering from babesiosis and theileriosis could be due to decrease protein production as a result of deprivation of diet protein resulting from anorexia and fever accompanied Babesia species and Theileria species infection [28]. The decrease in serum total protein in cattle infected with Babesia species or Theileria species might be due to decrease in albumin and globulin as a result of liver insufficiency [30]. Similarly, former 39] recorded studies [22, Babesiosis and theileriosis has harmful effects on the hepatocytes leading to disturbid hepatic functions decreased synthesis of albumin accompanied by an increase albumin catabolism [38]. Also, decrease in protein picture might be due to distructed RBCs and its excretion in urine as albuminuria occurs during disease [23]. Our results revealed significant increase in the liver enzymes (AST-ALT and alkaline phosphates) and total bilirubin in cattle suffering from babesiosis or thalariosis as presented in table (6). These results were supported by that obtained before [5, 61, 56]. Our Also, babesiosis in dogs was found to induce a significant increase in serum activity of AST and ALT [19]. Also, Singh et al. [55] who

mentioned that liver enzymes (AST, ALT and alkaline phosphates) and bilirubin were significantly total increased in calves infected with theileriosis. On other hand [7] stated that the elevation in liver enzymes in babesiosis may be due to the hepatic damage and lesions induced by the parasite during its multiplication in the blood followed by disturbed liver function. Elevated liver enzymes could be due to necrosis and lysis of the hyperbilirubinemia erythrocytes or during Babesia infection [31, 32].

The obtained results showed significant decrease in cholesterol, Triglyceride and glucose in cattle suffering from babesiosis theileriosis (Table, 7). Calve and adult infected with Theileria species had a lower levels of glucose, triglyceride and cholesterol [20, 25, 60], which might be attributed to partial anorexia and metabolic disturbances of the diseased animals [41]. It concluded that diagnosis of both babesiosis and theileriosis could be easily reached as babesiosis associated with red brown to coffee urine and microcytic hypochromic anaemia, while theileriosis is associated with enlargement of the superficial lymph nodes, corneal opacity and macrocytic hypochromic anemia.

Table 5 Proteins profile and Liver enzymes of healthy and diseased cattle (n=5)

Group	Total proteins (g/ml)	Albumin (g/ml)	Globulin (g/ml)	A/G Ratio
Control	7.64±0.51	3.91 ± 0.22	3.71±0.16	1.05±0.17
Babesiosis	5.82±0.24**	2.83±0.18**	$2.99\pm0.18^*$	$0.95\pm0.09^*$
Thelariosis	5.58±0.33**	$2.58\pm0.41^*$	$3.00\pm0.325^*$	0.86 ± 0.08

P < 0.05 and P < 0.01

Table 6 Liver function of healthy and diseased cattle (n=5)

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Group	ALP (U/L)	AST (U/L)	Alk.ph. (I.U/ml)	T. bilirubin (mg/dl)
Control	61.32±3.14	34.94±2.13	46.32±1.93	0.37 ± 0.07
Babesiosis	76.13±4.81*	42.42±2.31*	56.08±3.87*	$0.60\pm0.04*$
Thelariosis	73.71±3.54*	41.49±1.15*	59.95±3.91*	0.58±0.03*

 $^{^*}$ P < 0.05 and ** P < 0.01

Table 7 Lipogram and	glucose of healthy and diseased cattle (n=5

Group	Cholesterol (mg/dl)	Triacylglycerid (mg/dl)	Glucose (mg/dl)
Control	119.84±1.42	63.12 ± 2.67	65.08±3.04
Babesiosis	104.34±0194**	$47.39 \pm 1.92^{**}$	48.53±1.92**
thelariosis	$107.40 \pm 1.09^{**}$	$51.45 \pm 2.81^*$	50.17±1.83**

^{*}P < 0.05 and **P < 0.01

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استبیان دموی کیمائی للابقار المصابه بالبابیزیوزسس والثالاریوسس طارق حسن علام 1 ، وسامی شوقی محمد السد نصر 2 قسم الکیمیاء 1 والطفیلیات 2 معهد بحوث صحة الحیوان 2 الدقی 2 فرم الزقازیق

الملخص العربي

استهدفت هذه الدراسة محاولة تشخيص البابيزيا والثيليريا من خلال الاعراض الظاهرية، صورة الدم، صورة البروتين (البروتين الكلي، الزلال، الجلوبيولين)، ووظائف الكبد في الابقار المصابه (عدد 75 بقرة) في أماكن متفرقه بمحافظة الشرقية للمساعدة في العلاج المبكر. تم أخذ عينتين دم من وريد الاذن من كل الحيوانات على EDTA وعمل فحص طفيلي لشرائح الدم المصبوغه بصبغه جيمسا. اضافة الى قياس البروتين الكلي، الزلال، الجلوبيولين، وظائف الكبد في مصل الدم. تم تحديد 15 بقرة منها عشر بقرات مصابه بطفيليات الدم تتراوح أعمارها من 5-7 سنوات قسمت إلى ثلاث مجموعات متساوية كلاً منها تضم خمس بقرات المجموعة الأولى أبقار تتمتع بصحة جيدة (مجموعة ضابطة)، المجموعة الثانية أبقار مصابة بالبابيزيا (كانت تعانى من اعراض مرضية تتمثل في فقدان الشهية وارتفاع درجة الحرارة واحتقان بالأغشية المخاطية للعين وشوهد بول مدمم في بعض الأبقار مع وجود أعداد كبيرة من القراد ملتصقة على جسم الأبقار المصابة)، والمجموعة الثالثة أبقار مصابة بالثيليري (كانت تعانى من اعراض مرضية تتمثل في ارتفاع درجة الحرارة وافرازات انفية وعتامه بالعين وكحة واسهال واحتقان الجفون وحالات اخرى كانت تعاتى من شحوب وبياض الاغشية المخاطية للعين كما لوحظ في بعض الحالات تورم في الغدد الليمفاوية السطحية). افادت الفحوصات الاكلينيكيه بوجود تغيير في درجات الحرارة، عدد مرات التنفس، وحركة الكرش في كل الحيوانات. اشارت النتائج إلى ان الاصابة بالبابيزيا والثيليريا في الابقار ادت الى حدوث نقص معنوي في عدد كرات الدم الحمراء, تركيز الهيموجلوبين، حجم خلايا الدم المرصوصة و فقر دمز بالبابيزيا احدثت فقر دم المصحوب بالكريات الصغيرة قليله الهيموجلوبين. بينما الاصابة بالثيليريا احدثت فقر دم المصحوب بالكريات الكبيرة قليله الهيموجلوبين. كما وجد ان الاصابة بالبابيزيا والثيليريا تسببت في حدوث نقص معنوى في العدد الكلي لكرات الدم البيضاء، الخلايا المتعادلة والخلايا الحمضية بينما حدثت زيادة معنوية في الخلايا الليمفاويه، الخلايا القاعدية، والخلايا الملتهمة الكبيرة. اوضحت النتائج ان الابقار المصابة بالبابيزيا والثيليريا اظهرت نقص معنوى ملحوظ في قيم كل من البروتين الكلي, الزلال, الجلوبيولين الكلي ,الجلوكوز والكوليستيرول. زيادة معنوية في انزيمات الكبد (الأسبرتيت امينوترانس فيراز الالنين امينوترانس فيراز والفوسفاتيز القاعدي). نستخلص من هذه الدراسة أن تحديد التغيرات في صورة الدم بجانب الاعراض المرضيه في الابقار قد يكون وسيلة مساعدة ومفيدة لتشخيص والتفريق بين الاصابه بطفيليات الدم (البابيزيا والثيليريا).

(مجلة بنها للعلوم الطبية البيطرية: عدد 22 (2)، ديسمبر 2011: 118-126)