Clinical, haematological and biochemical study to cattle naturally infected with *Theileria annulata* in north of Basrah province

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Abstract

A total of 447 cows , 114 cows were naturally infected with *Theileria annulata* clinically normal cows were served as control. The clinical result indicated that the cows exhibited sings of fever, enlargement of superficial lymph nodes , respiratory signs with corneal opacity, soft yellowish diarrhea with subcutaneous accumulation of fluid. The blood examination showed the high level of parasitemia varied between 13-53% and observed all parasite stages; erythrocyte stage 69.43% the lymphocytic stage 25.43% while both stage (erythrocytes and lymphocytic)5.26%. The hematological parameter showed the Hypochromic macrocytic anemia with significant decreases in Hb(6.95±0.59) g/l , PCV(29.06±4.56)%, MCHC(24.71±4.55)%, RBCC(4689482±638753) 10/ mm , WBCC (5013.2±711.1)10/ mm , neutrophils(19±3.7)% while recorded a significant increases in the lymphocytes(69.74± 4.9)% and MCV(61.09±7.55)fl . The biochemical parameter recorded a significant increases in total serum bilirobin(1.19±0.40) and active serum enzyme ALT(19.77±2.94) and AST(67.98± 16.36)with recorded a significant decreases in the total serum protein(4.27±0.40)and albumin(1.56±0.36).

Introduction

Tropical theileriosis is a tick-borne disease caused by *Theileria annulata* and transmitted by ticks from genius *Hyalomma* spp (1). Tropical theileriosis is one of the most prevalent and economically important fatal diseases of cattle in Iraq (2).In their mammalian hosts, they have complex life cycle. Infection is initiated by transmutation of microschizont infected the cell in the lymph nodes draining the site of inoculation of sporozoites by ticks, the sporozoit transformation into schizont in the lymphocytes , the schizont undergoes further differentiation to merozoites, which are released upon lysis of the infected cells. This is followed by the development of piroplasms in erythrocytes and the parasite becomes infective for the vector (3, 4) .

Materials and Methods

Field of study area and animals.

The area of the study includes two location in the Basrah province these locations are Al-Hartha and Shat-Al- Arab. A total number of (447) cows in deferent ages , sexes and breeds were examined randomly belonging to different localities in Shat AL-Arab and AL Hartha during the period from 15 September 2008 to 30 Jun 2009. The hematological and biochemical studies performed in 100 animals divided into 25 animals representive control group and 75 representive infected group.

Clinical Examination

Clinical examination was performed on all animals .This test include measure the body temperature, auscultation, percussion and palpation of superficial lymph nods .The signs in clinical cases of bovine theileriosis were observed and recorded.

Samples Collection

Two types of samples blood and lymph were collected from animals under study; Blood sample was collected from ear vein, and used for marking blood film. Another a blood sample was collected by vein puncture of the jugular vein 5 ml EDTA tube and 5 ml without EDTA tube. The blood without EDTA was allowed to coagulant and spirited

the serum by using centrifuge (3000 rpm). Both sets of samples were stored at -20°C . The EDTA samples were used in (Hb, PSV, TRBCC and TWCC investigation) and decanted serum used for biochemical tests .Lymph samples were collected from the enlargement superficial lymph node specially prescapular lymph node by puncture and diatheses the lymph to marking lymph film according the procedure of (5).

Haematological Examination

Haematological examination was done according to (6), including red blood cells count (RBCC) and white blood cells count (WBCC) , Hemoglobin Concentration (Hb), Packed Cell Volume (PCV), Differential White Blood Cells Count (DWBCC) , Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC).

Biochemical parameter

Biochemical serum analysis include Serum total protein Serum, total Bilirubin, Serum Aspartate aminotransferase (AST), Serum Alanine aminotransferase(ALT), Serum Total Albumin were estimated spectrophotometry by using commercial chemical kits supplied by (SPECTRUM).

Statistical analysis

In order to determine the statistical significances among different variables SPSS program (Statistical program for social sciences) version 11.Students t-test was used for comparison between two groupes (control and diseased cattle). Chi-Square test was used to comparison between the epidemiological result. All hematological and biochemical test values were expressed as mean and stander error of mean and $P<0.05$ was considered as statistically significant.

Results

Clinical Signs

Clinical examination to animals subjected to the study showed clinical signs varied from the rise of body temperature up to 40.5°C, the enlargement of lymph nodes specially the superficial prescapular lymph node (fig.1), increasing to respiratory rate, increasing to heart rate ,nasal and ocular discharge some time associated with corneal opacity (fig.2), soft yellowish diarrhea, pale-yellowish mucus membranes, straw yellowish urine and the same time showed emaciate, recompenses(fig. 3) and subcutaneous accumulation to fluid table (1).

Table(1): clinical sings and percentage of infection .

Clinical sings	No. of animals	Percentage of infection %
Fever	96	84.42
Enlargement of prescapular lymph node	92	80.70
Pale –yellowish of mucosal membrane	73	64.03
Respiratory signs	68	59.64
Eye congestion and lacrimation	87	76.31
Corneal opacity	18	15.78
Emaciation	23	20.17
Straw yellowish urine	78	68.42
Odem	21	18.42
Neurological signs	0	0
Recompancy	33	28.94
Ticks infestation	101	88.59
Yellowish soft diarrhea	31	27.19

Morphology and Laboratory Examination.

Through laboratory examination of blood and lymph smears by using stained thick and thin blood films the study showed high level of parasitemia in infected animals varied from 13-53 % and the RBC can be infected by 6 parasites in maximum limit (fig.4). Observe all forms of *Theileria annulata* piroplasms including cocci, rod, comma, signet-ring, and pear-shaped forms with abnormalities in erythrocyte structure. While in the WBCs. observe the schizont stage koch' s blue body (fig. 5). Moreover the examination showed 25.43% of blood cells infected by schizont lymphocytic stage, 69.29% infected by erythrocytes stage and 5.26%showed the erythrocytes and lymphocytic form(table 2).

Table(2): percentage of infected blood cells with different stages of parasite.

Phase of parasites	No. of animals	Percentage of infection
Lymphocytic stage (Koch s blue body)	29	25.43
Erythrocytes stage	79	69.29
Erythrocytes and Lymphocytic stage	6	5.26

Fig(1) Enlargement to prescapuler lymph nod

Fig(2) corneal opacity

Fig(3) Calve recompensed position

Fig.(4) shows the erythrocyte stage of *Theileria annulata* infection 1000x

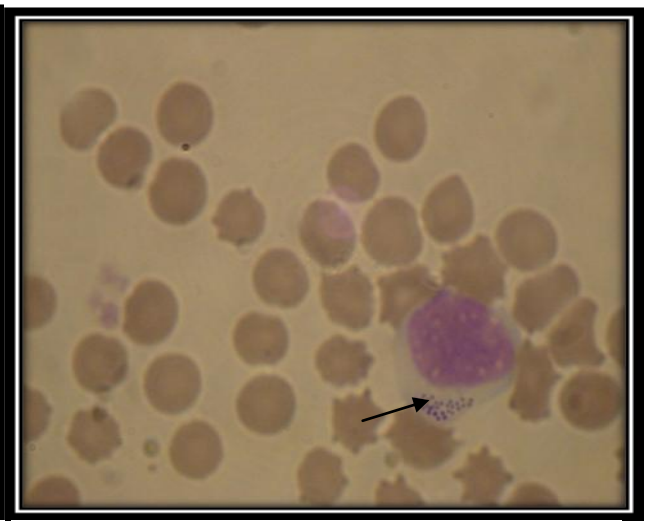

Fig.(5) shows the lymphocytic stage (Koch s blue body) 1000x

Hematological Parameter

The study result showed the animals infected by bovine theileriosis complained of macrocytic hypochromic anemia and as a result in table (3). the hematological analysis showed. statistically a significant decreases was observed ; mean hemoglobin amount(Hb) ,pocket cell volume(PCV), Mean corpuscular hemoglobin concentration(MHCH),red blood cells count (RBCC),white blood cells count (WBCC) and neutrophils (P<0.001),while statistically a significant increases was founded at lymphocyte count(P<0.001) and Corpuscular volume(MCV). Farther more, other parameters were showed statistically an important changer.

Table(3): effected of bovine theileriosis on some hematological parameter

Hematological parameters	Control animals	Infected animals
Hb (g/l)	10.93±1.03	6.95±0.59***
PCV (%)	34.81±1.40	29.06±4.56***
MCV (fl)	50.09±4.08	61.09±7.55***
MCH (pg)	15.85±0.26	15.27±0.255NS
MCHC (%)	32.32±2.67	24.71±4.55***
RBCC (10/mm)	6997510±671104	4689484±638753***
WBCC (10/mm)	8537.4±519.1	5013.2±711.1***
Lymphocytes(%)	60.2±4.58	69.74±4.9***
Neutrophils (%)	30.2±3.76	19±3.7***
Monocytes (%)	4.5±1.	2.76±2.65NS
Eosinophils (%)	3.9±1.5	2.7±2.3NS
Basophils (%)	0000	0.12±o.3NS
No. of animals	25	75
# = mean± std. Deviation	NS= Non significant	***=P<0.001

Biochemical Parameter

As a result in the table(4) the biochemical analysis were showed statistically significant increases in serum total bilirobin and active serum enzyme ALT,AST while observe statistically significant decreases in the serum total protein and albumin.

Table (4) The effected of some biochemical parameters by Bovine theileriosis.

Biochemical parameters	Control animals	Infected animals
Bilirubin	0.2959±0.28	1.19±0.40***
Total protein	6.29±0.68	4.27±0.74***
Albumin	3.70±0.51	1.56±0.36**
ALT	15.78±4.22	19.77±2.94***
AST	35.47±5.41	67.98±16.36***
No. of animals	25	75
# = mean± std. Deviation	NS= Non significant	***=P<0.001

Discussion

Clinical Signs

In the present study the animals which infected by *Theileria annulata* clinically were showed fever, enlargement of superficial lymph node ,anorexia respiratory signs, pale-yellowish of mucous membrane and eye congestion and lacrimation. All these signs were agree with results of most studies like (5 ,7 , 8). While the other signs as cornel opacity, yellowish soft diarrhea, emaciation and recompensey agreed with results of (7, 9 , 10).The increases of body temperature occur due to the liberation of endogenous progeny because cellular lysis and high level of parasitemia lead to the stimulation of thermoregulatory center in the hypothalamus (11).The anorexia could be attributed to present fever (12). The enlargement of superficial lymph node could be explained by lymphoid hyperplasia in early stage of disease that occurs due to increases of proliferation of microschizonts inside the lymphocyte caused inflammatory reaction in the infected lymph node(13). Paleness of mucous membranes exhibited the development of anemia and reduction of hemoglobin concentration and the total erythrocytes count was due to the distraction and the removed of the infected erythrocytes by reticulo- endothelial system (14).The cornel opacity was explained by (7 , 10) as a result of white blood cells infiltration. Diarrhea seen in 27.19% of infected cattle was explained as a result to inflammatory reaction and ulceration to abomasal and gastro intestinal tract (15).The other clinical signs as straw yellowish urine explained by the increases of the total bilirobin level in the blood and body lead to stimulating the body to extracting the bilirobin with body extraction and secretion(16). Respiratory signs occur due to the accumulation of edematous fluid inside the lung and thoracic cavity (17).

Hematology

The present study was showed a high level to parasitemia which varied between 13-53% and the erythrocyte infected by six parasites in maximum level with abnormalities in erythrocytes shape moreover observe all parasite shape with domination to ring stage and recorded 69.29% erythrocytes form 25.43% lymphocytic form and 5.26% showed both stage. All these results were agreement with (15 , 5 , 18, 19, 8). Infection erythrocytes by more than one parasite was explained by the occurrence of asexul reproduction inside

erythrocyte. As well as the dominated of ring stage was explained by the increases of these stage in *Theileria annulata* infection (19). Moreover the abnormality erythrocytes shape are mainly due to toxic action to parasite in the erythrocyte cell, erythrocyte oxidation , and immune-mediated process(14 , 18).The present study was showed a severe to mild change in the hematological parameters that occurs due to the infection with bovine theileriosis. The macrocytis hypocromic anemia was observed in infected cattle these result was agreement with (20). The animals infected with bovine theileriosis were showed significant decreases in hemoglobin concentration Hb and total RBCs count these results were agreement with most hematological studies as (15,21, 22, 7, 23). Moreover observe a significant decreases in PCV and MHCH and significant increases in MCV with unimportant statically change in MCH. these result were in agreement with (5, 9, 23). While disagreement with (7).All these changes occur as a result of anemia which occurs due to toxic metabolites of *Theileria* spp. which have harmful effect on bone marrow as they interfere with the process of erythropossis. Persistent loss of blood caused by permanent blood sucking ticks which play a role as well (24, 25).Boulter and Hall (26) mentioned that tumor necrotic factor- (NTF-) has been implicated in pathogenesis of anemia in bovine theileriosis by suppressing haemopiotic progenitors. The other importing cause to make the anemia in bovine theileriosis infection was the hemolytic anemia caused by an immune–mediated hemolytic which is indicated by the presence haemagglutinin (22 , 27) as while as the modern research explains the mechanism of anemia that occurs due to the activity of antioxidant enzymes such as superoxide dismutase (SOD) was effective by parasites and results increased fragility of RBCs and thus ,acceleration of erythrocytes clearance by Phyagocytic cells(28,29 30, 31).In the present study the leucogram was showed a significant decreases in total WBCC and neutrophils while the basophiles, eosinophils and monocytes don't showed importing statisticully change these results were agree with (14, 7) Furthermore, lymphocytes was showed a significant increases and these agree with (15, 5 , 21, 7). Such change in leucogram might be attributed to the persistent harmful effects of toxic metabolites of *Theileria* in haemopiotic organs especially bone marrow and their interference with process of leuocogenesis (24). Relative increases in the number of lymphocytes reflect compensatory mechanism as target cells in response to invasion with *Theileria* (13).In the present study significant increases in ALT and AST were observed in infected cattle when compared with control group. These results agree with those of (32,5 , 7,16). AST and ALT are involved in amino acid and carbohydrate metabolism. These enzymes are present in high concentration in the muscles and liver. The elevation of these enzymes in the blood are indicator of organ necrosis(33). *Theileria annulata* infection that caused hepatic tissue damage that includes coagulative necrosis, distraction of hepatic cords and heavy infiltration of lymphocyts in periportal area, indicating severe damage to hepatobiliary system due to hypoxia resulting from anemia and jaundice(32, 18).The present study was recorded a significant increases in the total serum bilirubin in the *T. annulata* infected cattle. these result may be related to the hepatic dysfunction and the, presumably, hemolytic anemia(7). Similar result has been obtained by others (15, 5, 14, 34 ,16). The biochemical analysis in the present study was recorded significant decreased in serum total protein ,albumin concentration. These results were in agreement with(14, 34, 7, 16). The hypoproteinaemia and hypoalboumineamia is possibly due to the harmful effect of toxic metabolites of *Theileria* and due to liver failure.

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دراسة سريريه ، دموية وكيموحيوية للأبقار المصابة طبيعيا بالطفيلي *Theileria annulata* في شمال محافظة البصرة

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الخلاصة

شملت الدراسة الحالية 447 بقرة حيث أظهرت 144 بقرة علامات سريريه تمثلت باألحمى , تضخم العقد أللمفاوية السطحية, عتمه العين متصاحبة علامات تنفسية و إسهال مع تجمع السوائل تحت الجلد في بعض الحالات .الدراسة الدموية أظهرت مستوى عالي من الطفلنيمية في الدم تتراوح بين 15−53% مع مشاهدة جميع أطوار و إشكال الطفيلي آذ كانت نسبه الطور الدموي 69.43% بينما نسبة الطور اللمفاوي 25.43% وكانت نسبة الإصابة بكلا الطورين 5.26%. أظهرت الدراسة الدموية أن الحيوانات المصاب بطفيلي الثيليريا الحلقي تعاني من فقر الدم نوع كبير الحجم قليل الصباغ مع قله معنوية في كل من قيم خضاب الدم Hb (6.95 ± 0.59) غم / لتر وحجم الخلايا المرصوص % PCV (29.06±4.56) وعدد خلايا الدم الحمر RBCC(4689482±638753) 10/ ملم و عدد خلايا الدم البيض WBCC(5013.2±711.1) 10/ ملم و عدد العدلات في الدم (19±3.7) % كما سجلت زيادة معنوية في العد التفريقي للخلايا اللمفاوية (69.74± 4.9) % وMCV (61.09±7.55) . بينما أظهرت الدراسة الكيموحيوية زيادة معنوية في كمية البلروبين الكلي (1.19±0.40) و إنزيمALT (19.77±2.94) AST و(16.3 ±67.98) في الدم كما أظهرت نقصان معنوي في كل من مستوى بروتين(4.27±0.40)الدم الكلي والألبومين(1.56±0.36).