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**PARASITOLOGICAL AND BIOCHEMICAL STUDIES  
ON CATTLE THEILERIOSIS AT DAKAHLIA  
GOVERNORATE WITH SPECIAL REFERENCE  
TO ITS CONTROL**  
(With 6 Tables and 2 Figures)

By

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**دراسات طفيلية وبيوكيميائية علي مرض ثيلريا الأبقار في محافظة الدقهلية  
مع الإشارة إلي طرق المقاومة**

**نبيلة محمود المصري ، صبري عبده الدسوقي ، شعبان عبد ربه أبو الخير**

تم إجراء هذه الدراسة علي عدد ١٥٠ بقرة تتراوح أعمارها من ٦ شهور - أكثر من ٥ سنوات تنتمي إلي بعض المزارع الخاصة في المناطق المختلفة لمحافظة الدقهلية في الفترة من سبتمبر ٢٠٠٤ إلي نهاية أغسطس ٢٠٠٥. وقد أظهر الفحص الإكلينيكي (الظاهري) الاشتباه في عدد ٤٦ حيوان عليها أعراض الإصابة بطفيل الثيلريا وبإجراء الفحص الطفيلي لهذه الحيوانات معمليا تأكد إصابة عدد ٤٤ حيوان بطفيل الثيلريا أنيولاتا (٢٩,٣٣%) بالنسبة للعدد الكلي حيث وجد ارتفاع في درجة الحرارة (٤٠-٤١م°)، تضخم في بعض الغدد الليمفاوية، فقدان الشهية، احتقان الأغشية المخاطية، زيادة في معدلات التنفس وفي الحالات المتقدمة من المرض ظهرت أعراض الأنيميا والضعف العام بدرجات متفاوتة مع تواجد القراد علي جسم الحيوان. وقد أظهرت الدراسة أن أعلى نسبة إصابة كانت في فصل الخريف (٤٧,٦١%) يليها فصل الصيف (٣٩,٤٧%) ثم الربيع والشتاء بنسبة (٢٠%)، (٥,٧١%) علي الترتيب. أوضحت الدراسة أن الحيوانات عند عمر ٦ شهور إلي سنة كانت نسبة الإصابة (٣٧,٥٠%) يلها العمر من ١-٣ سنوات (٣٢,٥%) فالعمر من ٣-٥ سنوات (٢٧,٧٧%) وفوق خمس سنوات (٥,٨٨%). وبالنسبة للتغيرات في صورة ومصل الدم للحيوانات المصابة بالثيلريا أنيولاتا عند مقارنتها بحيوانات المجموعة الضابطة كانت علي النحو التالي: أظهرت الفحوص الدموية وجود أنيميا تمثلت في وجود نقص معنوي عال في كل من العد الكلي لكرات الدم الحمراء وتركيز الهيموجلوبين وحجم الخلايا المضغوطة وأيضا لخلايا الليمفوسيت ومعنوي لخلايا المونوسيت وفي الجانب الآخر وجدت زيادة معنوية عالية في الخلايا المتعادلة والحمضية ومعنوي في الخلايا القاعدية وفيما يخص

الفحوص البيوكيميائية لمصل الدم للحيوانات المصابة وجد نقص معنوي عال في مستوي كل من الألبومين وسكر الدم وعلي النقيض وجدت زيادة معنوية عالية في مستوي كل من الجلوبيولين الكلي والألفا جلوبيولين، والجاما جلوبيولين، أنزيمات الترانس أميناز، الحديد، النحاس ومعنوية في المستوي الكلي للأصباغ المرارية، بولينا الدم، الكرياتينين، السيلينيوم، المنجنيز، الزنك عند مقارنتها بالمجموعة الضابطة. وقد تم عزل الحيوانات المصابة حيث عولجت بعقار بيوتالكس وتيراميسين طويل المفعول وأنتوبكس كمقوي عام. وقد تم رش الحظائر والحيوانات للقضاء علي القراد بعقار البوتكس مما أدى إلي تمام الشفاء وعودة الحيوانات لحالتها الطبيعية واختفاء الأعراض وتحسن ملحوظ في صورة الدم والقياسات البيوكيميائية وعودتها إلي معدلاتها الطبيعية.

## SUMMARY

This study was carried out on 150 cow, their ages ranged from 6 months - over 5 years belonged to some private farms in different localities at El-Dakahlia governorate in the period from September 2004 to August 2005. The clinical examination showed 46 suspected cases which have the signs of theileria infection, laboratory parasitological examination confirmed that infection of 44 animals infected with *Theileria annulata* (29.33%) to total animal number where high temperature (40°-41°C), enlargement of some superficial lymph nodes, anorexia, congestion of mucus membranes, increased respiratory rates were noticed. In advanced cases of the disease signs of anaemia, general weakness were present with different degrees, in addition to presence of ticks on animal body. The study revealed that the higher rate of infection was in Autumn season (47.61%) followed by Summer season (39.47%), Spring and Winter (20%) & (5.71%) respectively. The study cleared that animals at the age of 6 months to one year have the higher rate of infection (37.50%) followed by the age of 1-3 years (32.50%), 3-5 years (27.77%) and finally over 5 years (5.88%). Concerning the changes in the blood and serum of *Theileria annulate* infected animals when compared with control group were as follow: Blood picture investigations proved presence of anaemia represented by high significant decrease for total erythrocytic count, haemoglobin concentration and packed cell volume and lymphocyte cells, also significant decrease for monocyte cells. On contrary presence of high significant increase in neutrophil and oesinophil cells and significant in basophil cells. Serum biochemical investigation of infected animals, showed that high significant decrease in Albumin and glucose level, while on the other side there was high significant increase in the level of both total globulin, alfa-globulin, gamma-globulin, transaminase enzymes, iron and copper, while the level of total bilirubin, blood urea, creatinine, selenium, manganese and

zinc showed significant increase. All infected animals were isolated and treated with Butalex®, Terramycin L.A and Antoplex as general haematonic. Animals and their houses were sprayed for ticks using Butox, who lead to complete recovery for diseased animals and marked improvement in haematological and biochemical parameters towards its normal levels.

**Key words :** *Theileria annulata*, prevalence, serum, biochemical changes.

## INTRODUCTION

Tropical theileriosis is a tick born disease of cattle caused by *Theileria annulata*, transmitted by hard tick (*Hyalomma anatolicum*) (Shkap *et al.*, 2003 and Akats *et al.*, 2004). The disease is a major constraint to livestock improvement programmes in many parts of the middle east and Asia, where about 200 million cattle are said to be at risk of infection (Bakheit and Latif, 2002). The disease is clinically manifested by marked fever, swelling of superficial lymph nodes, inappetence, tachycardia, dyspnoea and weakness (Omer *et al.*, 2003). *Theileria annulata* infection rate, reach its maximum in both autumn and summer seasons (El-Metenawy, 2000). Studies of Forsyth *et al.*, (1999) had presented a new view on the destructive effect of the parasite on the host organs and tissues. As the number of cattle in Dakahlia governorate reaches 172099 head (GOVS, 2005). So the present study aimed to facus light on disease situation at Dakahlia governorate, its clinical signs, seasonal prevalence, age susceptibility, the changes of some blood biochemical components associated with *Theileria annulta* infection in addition to treatment trails of diseased animals.

## MATERIALS and METHODS

### I- Animals:

The present study was carried out on 150 cows aged from 6 months to over 5 years old collected from different private farms in different localities at Dakahlia governorate during the period extending from the beginning of September 2004 to end of August 2005. The animals were clinically examined for signs of health and disease, special care to body temperature, nasal discharge, enlargement of superficial lymph nodes, corneal opacity and wasting according to the method described by Radostits *et al.*, (2000), the animal divided into two groups the first consisted of 104 apparently healthy animals (free from blood parasite after blood examination), the second consisted of 46 animals suffered from signs of the disease blood examination of these animals.

Revealed that, 44 animals was infected with *Theileria spp.* The adult ticks were collected manually from the animal and was examined and identified according to Bovattour *et al.*, (1996).

## **II- Samples:**

1- Thin blood smears were individually prepared from superficial ear vein, fixed and stained with Giemsa stain for detection of blood parasites microscopically according to Coles, (1986).

2- Aspirated smears were also taken from superficial enlarged lymph nodes and examined according to Marquardt *et al.*, (2000).

3- Two blood samples were collected from jugular vein via venoject system, from each examined animal, in a dry sterile centrifuge tubes. The first blood samples was collected with anticoagulant (sodium salt of EDTA) and used for the evaluation of haemo and leucograme parameters according to the method adopted by Jain, (2000). The second blood sample was collected without anticoagulant for preparation of serum needed for biochemical investigations, where both of serum total proteins, serum glucose, serum aspartate aminotransferase (S.AST), serum alanine aminotransferase (S.ALT), serum total bilirbin, serum blood urea and serum creatinine were estimated using commercial diagnostic test kits supplied by BioMerieux France. Serum protein electrophoresis was separated according to their respective electrical charges at pH 8.8 on a cellulose plate using both electrophoretic and electroendosmotic force present in the system according to Alper, (1974). The serum iron, copper, selenium, manganese and zinc were estimated by atomic absorption spectrophotometer (PYE, Unicum, SP-90, England) according to Khan *et al.*, (1995).

## **III- Treatment trails and control:**

Butox® (intervet) was used for eradication of ticks vectors on animals and in the surrounding areas with concentration of 25 PPM with spray manner according to manufacture recommendation. Theileria infected cows were treated with Butalex® (schering plough animal health), a single injection of 2.5 mg/kg (1ml Butalex per 20 kg.b.w) was used via i/m route in the muscle of the neck. Supportive treatment was done (Terramycin L-A (phizer) as antibiotic for secondary infection at a dose of 200mg (1mg/10kg.b.w), repeated after 3day, beside Antoplex® (Labortrios Tornel, Mexico) as haematinic was used at a dose of 10 ml twice a week, via i/m route.

## **IV- Statistical analysis:**

All data were subjected to statistical analysis according to Snedcor and Cochram, (1982).

## RESULTS

The present study was carried out to focus light on disease situation at Dakahlia governorate, its clinical signs, seasonal prevalence, age susceptibility, changes of some blood biochemical components in addition to treatment trials.

### I- Clinical findings:

Close observation concerning the clinical survey conducted on 150 cows revealed presence of lacrimation, nasal discharge, enlargement of superficial lymph nodes, inappetance, tachcardia, weakness and corneal opacity on 44 animals. Gradual improvement in the general health condition of the diseased cows was noticed during treatment course, complete recovery was obtained after relapse of 15 day manifested by disappearance of clinical signs of the disease.

### II- Laboratory findings:

Parasitological examination of blood smears revealed that there is no presence of Theileria infection after treatment. The results of the study dealing with parasitological and blood biochemical parameters before and after treatment were illustrated in Tables (1-6) and Figs. (1 and 2).

**Table 1:** The prevalence of *T. annulata* infection among cattles in Dakahlia Governorate:

Examined animals	No.	No. of infected	Infection %
Apparently healthy	104	0	0%
Suspected diseased	46	44	95.65
Total	150	44	29.33

**Table 2:** Seasonal prevalence of *Theileria annulata* infection among cattle in Dakahlia Governorate:

Season	No. of examined animals	No. of infected	% of infection
Summer	38	15	39.47
Autumn	42	20	47.61
Winter	35	2	5.71
Spring	35	7	20
Total	150	44	29.33

**Table 3:** The prevalence of *Theileria annulata* infection among cattle of different age:

Age group	No. of examined	No. of infected	Infection %
6M→1 year	40	15	37.50
1→3 years	40	13	32.50
3→5 years	36	10	27.77
Over 5 years	34	2	5.88

**Table 4:** Haemo and leucogram of cattle infected with *Theileria annulata* before and 15 day after treatment.

Parameters	Item	Control healthy group (g <sub>1</sub> )	Diseased group (g <sub>2</sub> )	
			Before treatment	After treatment
RBCs (x10 <sup>6</sup> /μl)		8.71±0.33	6.91±0.38**	8.47±0.31 <sup>N.S</sup>
Hb (g/dl)		11.37±0.44	8.57±0.49**	11.13±0.37 <sup>N.S</sup>
PCV (%)		34.63±1.30	28.40±1.46**	33.87±1.44 <sup>N.S</sup>
Total WBCs (x10 <sup>3</sup> /μl)		9.66±1.73	17.89±1.41**	10.98±0.97 <sup>N.S</sup>
Neutrophils (%)		31.45±1.85	44.24±2.95**	31.50±2.47 <sup>N.S</sup>
Eosinophils (%)		5.55±0.37	8.47±0.45**	5.81±0.36 <sup>N.S</sup>
Basophils (%)		0.75±0.10	1.34±0.17*	0.77±0.12 <sup>N.S</sup>
Lymphocytes (%)		56.75±2.09	41.75±2.98**	57.43±1.33 <sup>N.S</sup>
Monocytes (%)		5.50±0.29	4.20±0.37*	4.49±0.38 <sup>N.S</sup>

\*: Significant at (P < 0.05) \*\*: Highly significant at (P < 0.01) N.S: Non significant.

**Table 5:** Biochemical changes in serum of cattle infected with *Theilerice annulalta* before and 15 day after treatment.

Parameters	Item	Control healthy group (g)	Diseased group (g <sub>2</sub> )	
			Before treatment	After treatment
Total proteins (g/dl)		7.66±0.16	8.17±0.25 <sup>N.S</sup>	7.89±0.22 <sup>N.S</sup>
Albumin (g/dl)		4.13±0.19	2.87±0.29**	4.07±0.22 <sup>N.S</sup>
Total globulin (g/dl)		3.35±0.28	5.30±0.36**	3.82±0.30 <sup>N.S</sup>
α-globulin (g/dl)		1.123±0.064	1.501±0.068**	1.280±0.061 <sup>N.S</sup>
β-globulin (g/dl)		0.567±0.071	0.979±0.077**	0.578±0.067 <sup>N.S</sup>
δ-globulin (g/dl)		1.840±0.102	2.820±0.136***	1.962±0.103 <sup>N.S</sup>
Glucose (mg/dl)		55.16±5.31	31.74±5.43**	58.91±4.78 <sup>N.S</sup>
AST (IU/L)		56.43±2.05	68.86±2.16**	56.98±1.69 <sup>N.S</sup>
ALT (IU/L)		17.51±1.41	25.89±1.57**	18.23±1.32 <sup>N.S</sup>
Total bilirubin (mg/dl)		0.945±0.061	1.190±0.055*	0.961±0.052 <sup>N.S</sup>
Blood urea (mg/dl)		27.43±1.41	33.71±1.94*	28.61±1.73 <sup>N.S</sup>
Greatinine (mg/dl)		1.60±0.12	2.11±0.17*	1.66±0.14 <sup>N.S</sup>

\*: Significant at (P < 0.05)

\*\*\*: Very highly significant at (P < 0.001)

\*\* : Highly significant at (P < 0.01)

N.S: Non significant.

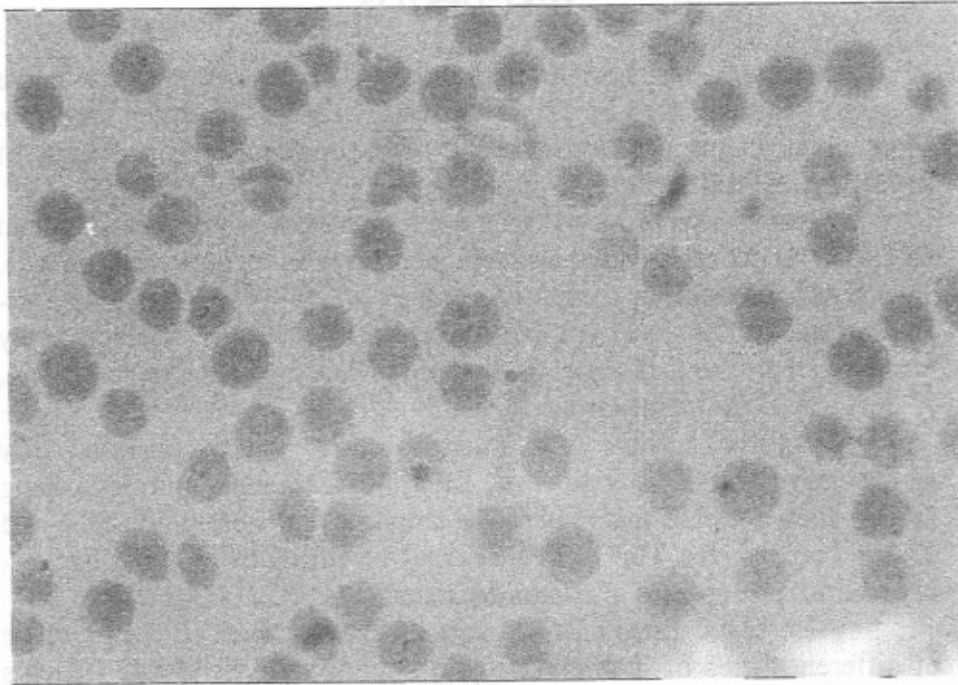
**Table 6:** Mean values of serum microelements in cattle infected with *Theileria annulalta* before and 15 day after treatment.

Parameters	Item	Control healthy group (g)	Diseased group (g <sub>2</sub> )	
			Before treatment	After treatment
Iron (μg/dl)		227.81±3.50	246.37±3.45**	229.11±2.72 <sup>N.S</sup>
Copper (μg/dl)		113.26±2.69	129.78±2.96**	114.51±1.91 <sup>N.S</sup>
Selenium (μg/dl)		3.295±0.274	4.395±0.235*	3.191±0.243 <sup>N.S</sup>
Manganese (μg/dl)		51.18±2.59	59.43±2.20*	52.76±2.27 <sup>N.S</sup>
Zinc (μg/dl)		121.52±4.15	136.55±4.49*	123.87±3.78 <sup>N.S</sup>

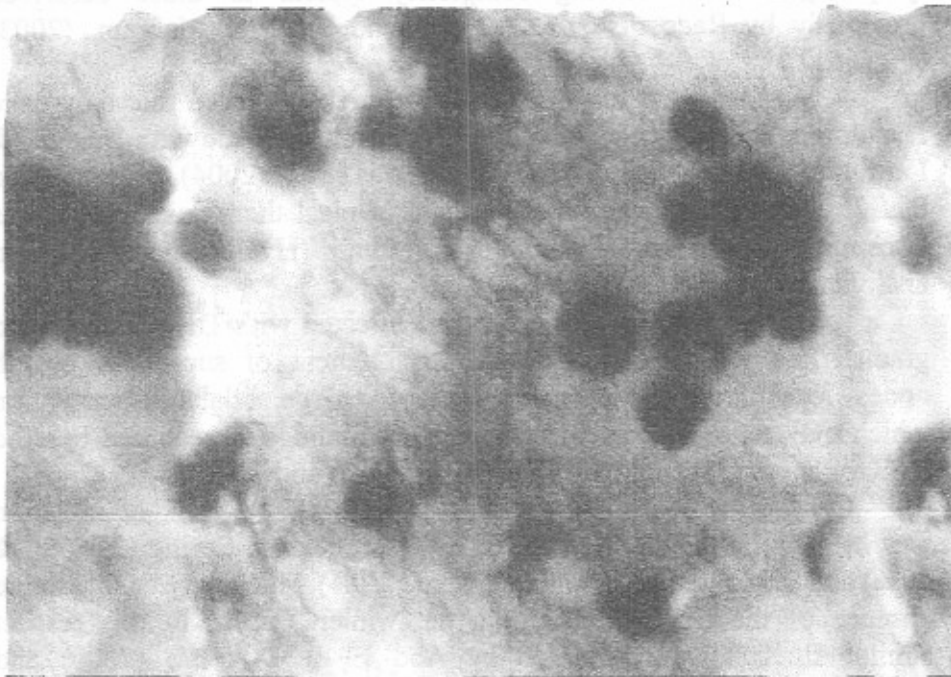
\*: Significant at (P < 0.05)

\*\* : Highly significant at (P < 0.01)

N.S: Non significant.



**Fig. 1:** Blood film showing theileria trophozoites (X →10 x 100)



**Fig. 2:** Koch's blue bodies stained by Gimsa stain (X →10 x 100)

## DISCUSSION

Mediterranean coast fever or tropical theileriosis is a tick borne disease of cattle caused by *Theileria annulata* infection, extending from Morocco to China and consider one of the major health problems of livestock (Devendra, 1995).

Examination of blood films and lymph node smears revealed that 44 (29.33%) out of 150 animals were infected with theileriosis (Table 1). This result was similar to that of Loria *et al.*, (1999) in Italy, Abd El-Raof *et al.*, (2000) in Egypt and Inoue *et al.*, (2001) in Vietnam, they recorded that the incidence of theileriosis among cattle were (29.4%, 33.66% and 27.5%) respectively.

However, higher prevalence rates were reported by El-Metenawy, (2000), Inoue *et al.*, (2001) in Cambodia and Song Sang (2003), they detected that theileriosis among cattle were (76.5, 50.4 and 67.8%) respectively. This differences may be attributed to the difference, in localities and species of examined animals.

Also blood films and lymph node smears revealed the presence of different forms of intra-erythrocytic trophozoites. Schizonts (Koch's blue bodies) were observed in the lymph nodes and the circulating lymphocytes. This finding completely typical to those described previously by Radostits *et al.*, (2000) and Saleh & Mahram, (2003) (Figs. 1, 2).

The identification of the collected ticks revealed that they were *Hyalomma anatolicum anatolicum*. This result coincide with Bakheit & Latif, (2002), Shkap *et al.*, (2003) and Aktas *et al.*, (2004), they recorded that *T. annulata*, the causative agent of tropical theileriosis in cattle is transmitted by ticks of genus *Hyalomma* (*Hyalomma anatolicum anatolicum*).

Clinical examination of animals infected with *Theileria* revealed gradual rise in body temperature, enlargement of superficial lymph nodes, loss of weight, anorexia, nasal discharge, bilateral lacrimation which may reach to corneal opacity. This findings as recorded by Abd El-Raof *et al.*, (2000), Saleh & Mahran, (2003) and Omer *et al.*, (2003).

Concerning seasonal prevalence (Table 2), the peak of infection was in Autumn (47.61%) followed by Summer and Spring (39.47% & 20%) and the lowest rate was in winter (5.71%) which may be due to the absence of the tick vector during the winter season. In this respect similar finding were recorded by Abd El-Raof *et al.*, (2000) and



El-Metenawy, (2000) with some variation in the ratio which may be attributed to the number of animals examined in each season.

The present data in Table (3) revealed that calves with age of 6 months to 1 year were highly susceptible to theileriosis (37.50%) which is nearly equal to that from 1-3 years (32.50%), they decreased at the age from 3-5 years (27.77%) to reach its lowest infection rate at animals over 5 years (5.88%) which may be attributed to age resistance or to previous infection and presence of a sort of immunity. Around this point Abd El-Raof *et al.*, (2000) reported 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%), while Song & Sang, (2003) found that *T. sergenti*, infection among the over 3 years old group of cattle was (75%) had a higher prevalence than that among the less than 3 years old group (61.8%). This variation may be attributed to the species of the examined animals.

Concerning the changes in haemogram parameters in *Theileria annulata* infected cows, (Table 4) showed that there was a noticeable microcytic hypochromic anaemia as a result of high significant decrease ( $P < 0.01$ ) in the levels of total red blood cells count (tRBCs), haemoglobin content (Hb) and packed cell volume (PCV%) when compared with control group.

This result come in accordance with those of Omer *et al.*, (2002) and Emam and Al-Kabbany, (2004). Such changes could be due to the destructive effect of the parasites on erthrocytes and failure of bone marrow to produce enough erthrocytes response to the toxic material to compensate such losses (Radostits *et al.*, 2002).

Dealing with the changes in leucogram parameters in the present study (Table 4) revealed that there was a high significant leucocytosis ( $P < 0.01$ ) as a result of high significant increase ( $P < 0.01$ ) of neutrophils, eosinophils and basophils, also Table (4) clarified a high significant and significant decrease ( $P < 0.01$  &  $P < 0.05$ ) in lymphocytes and monocytes in comparison with control group respectively.

Guglielmone *et al.*, (1996), Omran and El-Kholany, (2003) explained such alterations by the fact that the stress exerted on the animals lead to release of corticoids into circulations so both of lymphocyte and monocyte number became reduced, at the same time neutrophils were attracted to injury site to participate in the developing inflammation.

Table (5) indicated a high significant decrease ( $P < 0.01$ ) in albumin level, on contrary globulin level showed high significant

increase ( $P < 0.01$ ), also Table (5) revealed high significant increase ( $P < 0.01$ ) in  $\alpha$ ,  $\beta$  globulins and very high significant ( $P < 0.001$ ) in  $\delta$ -globulin fractions. The electrophoretic pattern of blood serum proteins obtained in this study coincides with the protein electrophoretogram previously reported for buffaloes (Abd El-Raof *et al.*, 2000 and For neonatal calves Thomas, 2000 a, b).

El-Sawalhy, (1999) attributed the marked reduction in albumin level to the harmful effect of the parasite on the liver function and consequently reduction of albumin synthesis.

The increased values of  $\alpha$ -globulin in the current work agreed with those finding of Saleh and Mahran, (2003) in Friesian calves.

Concerning the mean values of  $\beta$ -globulin Table (5) showed a high significant increase ( $P < 0.01$ ) than control group, Abd El-Raof *et al.*, (2000) found increase in  $\beta$ -globulin fractions in buffaloes infected with *Theileria annulata*, while Singh *et al.*, (2001) found reduction in this fraction during the course of experimental *T. annulata* infection in crossbred calve. The present study showed marked increase in the value of  $\delta$ -globulin fractions Table (5), this could be attributed to initial uncontrolled proliferation and neoplastic transformation of  $\beta$ -lymphocytes which are responsible for humoral immunity and production of immunoglobulins, Moreau *et al.*, (1999).

Blood serum glucose values in cows infected with *Theileria annulata* indicated a high significant decrease ( $P < 0.01$ ) when compared with control group Table (5). The obtained value in this study was supported by those reported by Attia, (2001) who attributed hypoglycaemia to anorexia and depraved metabolic process of diseased animals.

The mean values of blood serum AST and ALT enzymes in this study revealed high significant elevation ( $P < 0.01$ ) and significant increase ( $P < 0.05$ ) for both of total bilirubin, creatinine and B.urea respectively Table (5). Similar results were obtained by Ceci *et al.*, (1997) who referred such increase in liver enzymes and total bilirubin to the degenerative change of the liver, while the increase in serum creatinine and B. urea values referred to the failure of excretion in addition to lysis of erythro cytes during *Theileria* infection. Pathological studies of Abou El-Hassan, (1997) and Singh, (1998) showed several and different macroscopic and microscopic lesions in liver and kidney of *Theileria* infected calves, resulting in hepatic and renal damage due to excessive production of cytokines.

Regarding trace elements change for cows infected with theileria annulate Table (6) showed a high significant increase ( $P < 0.01$ ) of serum iron and copper values. Goldberg *et al.*, (1991) attributed such change due to the degenerative process of red blood cells caused by hemoglobinase enzyme of the parasite which is responsible for release of copper and iron. The significant increase ( $P < 0.05$ ) of serum selenium, manganese and zinc values. Table (6) in the present work agreed with those illustrated by Ashmawy *et al.*, (1994) who said that such increase could be due to the damage of glutathione dependent enzymes and superoxide dismutase proceeding liberation of them.

#### **Chemotherapeutic trials:**

The corrective therapy in the present work was based on:

- (1) Eradication of ticks on the diseased animals and surroundings using Butox® at dose of 25 PPM.
- (2) Treatment of diseased animals with specific antitheilerial drug Butalex® at a dose of 1ml/20k.g.b.w via i/m injection in addition to Antoplex® as haematinic at a dose of 10 ml twice a week via i/m route.

Furthermore the haematological and biochemical values showed marked improvement towards their normal levels in the diseased treated animals as shown in Table (4-6). In this respect these results were agreeable with those of Omran and El-Kholany, (2003).

In conclusion, the present study proved that ticks are very dangerous in animal house and must be eradicated, specially during summer and Autumn seasons, also cattle theileriasis, based on studied indices was accompanied by blood picture changes, impairment of liver and kidney function and changes in the levels of microelements. Application of specific antiprotozoal drugs with supportive treatment restored the normal haematological values and liver and kidney functions in addition to serum microelements.

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