

HAEMATOLOGICAL AND BIOCHEMICAL CHANGES IN BLOOD OF SHEEP SUFFERING FROM THEILERIA INFECTION

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SUMMARY

The prevalence of Theileria infection was investigated in 40 sheep from a private farm in Gharbia governorate with suspected symptoms of theileriosis. In addition, a total of 96 blood samples were collected from apparently healthy sheep during slaughtering at El-Bassatine and El-Warak abattoirs. The incidence of Theileria infection in the farm was 87.5%, while, the incidence in the apparently healthy sheep of abattoirs was 18.75%. Ring and rod forms of Theileria species were detected within infected erythrocytes. Macroschizonts and microschizonts were detected in the lymphocytes with different shapes of chromatin materials. Haematological studies revealed significant progressive declines in haemoglobin concentration, packed cell volume and red blood cell count. The total leucocyte count showed significant leucopenia in diseased animals. Examination of serum revealed significant rises in levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphates, urea, creatinine and cholesterol. The concentrations of serum total protein, albumin and globulin showed significant decrease in Theileria infected animals as compared with the healthy control sheep. Levels of serum iron, inorganic phosphorus, magnesium and calcium showed also significant declines as compared with healthy sheep. No changes were observed in serum sodium and triglycerides levels of sheep with theileriosis.

INTRODUCTION

Theileria species are economically important, intra-cellular protozoa, transmitted transstadially by Ixodid ticks, which infect wild and domestic ruminants. In the mammalian host, the parasite infects leukocytes of mono-nuclear lineage transform into schizonts, followed by the development of piroplasms in erythrocytes. In the arthropod vector, the different stages of *Theileria* species develop in gut epithelial cells and salivary glands till reaching the sporozoite which is the infecting stage to the mammals. The main symptoms of *Theileria* disease are anemia, pyrexia and swelling of superficial lymph nodes which used to demonstrate the disease. Clinically, in addition inappetence, tachycardia and weakness were recorded. There are different species of *Theileria* infecting sheep, including, *T. lestoquardi* (*T. hirci*), *T. ovis* and *T. separata* (Schnittger et al. 2003). The pathogenicity of *Theileria* is variable depending on strain virulence. *T. lestoquardi* is considered the highly pathogenic one (Uilenberg 1981). In Egypt, the widely distributed species is *Theileria ovis* which causes a mild disease manifested by fever, lymphadenopathy at site of the tick bite and slight anemia in sheep (Hooshmand-Rad and Hawa 1973). *T. lestoquardi* were found in blood of sheep and goat from Kena governorate (Nagaty 1947). Hafez et al, (1981) demonstrated that *Theileria hirci* (*T. lestoquardi*) was the most predominating species in sheep in Egypt. Altay (2005) recorded that *Theileria lestoquardi* and *Theileria ovis* cause malignant theileriosis of sheep and goat in Africa, Asia and southern Europe. Mazyed and Khalaf (2002) recorded that the percentage of infection of sheep with *Theileria*

ovis in north Sinai was 2.7% and double infection with *theileria* and *Babesia ovis* was found to be 24%. Also in Egypt **Harfoush (1998)**, found that the percentage of infection with *Theileria* species in sheep was 58.2%. The Schizont stage of the unidentified *Theileria* of sheep was demonstrated in liver, spleen, lung, kidney, lymph node and peripheral blood (**Yin et al., 2003**).

The available literature about sheep theileriosis in Egypt is limited, although their population is about 5 millions (**FAO 2005**). Sheep are important source of meat, milk and wool.

The objective of this study was to investigate the prevalence of *Theileria* infection in sheep and detection of different forms of their schizont stage. Most of the previous studies concerning biochemical parameters in *T. annulata* infection have been carried out on experimentally infected calves (**Singh et al., 2001 and Omer et al., 2003**). This investigation was undertaken to ascertain the changes in hematological and biochemical constituents of blood as a result of *Theileria* infection in sheep. These studies will help to achieve better understanding of the pathogenesis of tropical Theileriosis in sheep.

MATERIALS AND METHODS

A total of forty sheep from a farm in Gharbia governorate of both sexes with suspected symptoms of theileriosis were bled and examined for thei-

leria infection. The clinical symptoms include fever ($>39^{\circ}\text{C}$), enlarged lymph nodes and some cases of abortion. In addition, a total of ninety-six blood samples were collected from apparently healthy sheep during slaughtering at El-Bassatine and El-Warak abattoirs. The blood samples were collected and divided into two portions. The first portion of each blood sample was collected on an anticoagulant (EDTA) for parasitological and haematological examinations. Thin blood films were prepared, fixed in methanol, stained with Giemsa stain and examined microscopically for the presence of any blood parasite. The second portions of the blood samples were left to clot and then centrifuged at 3000 rpm for 15 minutes for obtaining clear serum sample. Serum samples were kept at -20°C until biochemical analysis was performed.

After parasitological examination, 33 positive samples proved to be highly positive for blood parasites were subjected to haematological and biochemical analysis. In addition, samples from 10 microscopically negative healthy adult sheep were subjected to the same examinations as controls.

The haematological examinations for the tested blood samples included hemoglobin concentration (Hb), packed cell volume (PCV), total erythrocytes count (RBCs) and leucocytes count (WBCs). Hemoglobin concentration (Hb) was measured according to Crosby et al. (1954), packed cell volume (PCV) was determined by the micro-haematocrite method of Schalm (1986). The total erythrocytes (RBCs) and leucocytes (WBCs) were counted according to Thompson (1980).

The biochemical examination for serum samples included determination of the levels of serum total protein, albumin, globulin level, urea, creatinine, serum total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, triglycerides, serum calcium, inorganic phosphorous, magnesium, sodium and potassium, serum aspartate aminotransaminase (AST), alanine aminotransaminase (ALT) and alkaline phosphatase (ALP).

Estimation of serum total protein was determined by Biuret method as described by **Weichselbaum (1946)**. Serum albumin level was determined according to **Dumas et al, (1971)**. The serum creatinine was determined according to **Henry (1974)**. The serum urea was determined according to **Patton and Crouch (1977)** the serum cholesterol was determined according to **Richmond (1973)**, High density lipoprotein cholesterol was carried out by using the method of **Burstain et al (1970)**, while low density lipoprotein cholesterol was determined according to **Steinberg (1981)**. The triglycerides level was estimated according to **Henry et al (1974)**. Serum calcium was determined as described by **Glindler and King, (1972)**. Serum inorganic phosphorous was determined according to **Kilchling and Freiburg (1951)**. Serum magnesium level was determined as described by **Glindler (1971)**. Serum sodium and potassium were estimated using the flame photometer (Corning photometer 410) according to **Dawborn et al. (1965)**. Serum iron level was estimated according to **Smith et al. (1981)**. Serum transaminases including aspartate aminotransaminase (AST) and alanine aminotransaminase (ALT) were determined according to **Reitman**

and Frankel (1957) while alkaline phosphatase was determined as described by Eastman and Bixter (1977).

The obtained data were statistically analyzed according to Petrie and Watson, (1999).

RESULTS AND DISCUSSION

Parasitological examination revealed the presence of *Theileria* infection in both sheep from the farm in El-Gharbia which were suffered from rise in temperature, enlargement of lymph nodes and abortion and the examined sheep from slaughter houses which were apparently healthy. These results agreed with those of Harfoush (1998) who found thileria infection in sheep exhibited symptoms such as the rise in temperature, pale or congested mucous membrane and sometimes anorexia. Out of 40 examined farm sheep, 35 animals were found positive for *Theileria* infection with an incidence of 87.5%, and out of 96 slaughtered sheep 18 animals were found positive with an incidence of 18.75%. This percentage was higher than that of Mazyad and Khalaf (2002) who recorded that the percentage of infection with *Theileria ovis* in north Sinai was 2.7% and double infection with *Theileria* and *Babesia ovis* was 24%. Also this percentage was less than the result of Harfoush (1998) who found that, the percentage of infection with *Theileria* species in sheep was 58.2%. The variation of the percentage of infection with *Theileria* in sheep could be due to the difference of locality. These results support that *Theileria* species infection in sheep is wide-

spread in Egypt. A differentiation between pathogenic and non-pathogenic species of *Theileria* by microscopic examination is not feasible. However reports from Sudan (Salih et al. 2003) and North Africa (Schnittger et al. 2004) indicated that, malignant Theileriosis occurred in these regions.

In the present study, the parasite was found to occur in different morphological forms within the infected erythrocytes. Ring form with transparent central part and nucleus located at the margin was found in most cases and measured 1.2 μm (Fig.1.a). Rod form with nuclei located at two ends was found in less frequency and measured 1.5x 0.5 μm (Fig.1.b). The schizonts were detected in the lymphocytes with two forms macroschizonts and microschizonts. Macroschizont measured 7.5 x 2.5 μm (Fig.2.a). Microschizont measured 8.75x3.75 μm (Fig.2b). There were different shapes of chromatin material. Most of chromatin appeared as granules, which represent nuclear material without cytoplasm. In addition several chromatin of less well-recognized appearance were observed as comma, oval-round and rod shapes (Fig.3.a&b). These different shapes might be indicative to severe infection with *Theileria* species. Yin et al, (2003) demonstrated that, *Theileria* schizonts were found in liver, spleen, lung, kidney, lymph node and peripheral blood. They also observed merozoites in microschizonts with comma shaped.

Haematological and biochemical analysis of serum is commonly used to monitor health status and disease diagnosis in different animals, in these studies realistic values of different biochemical changes were analyzed in

sheep suffering from theilerioses. The data obtained in table (1) revealed a decrease in haemoglobin, haematocrit, the total numbers of erythrocytes and leukocytes in sheep infected with *Theileria*, compared with healthy sheep. **Leemans et al (1999)** reported similar haematological picture in sheep infected with *T. lestoquardi*. The significant decrease in Hb concentration, PCV and RBCs, count were probably resulted from destruction of the parasitized erythrocytes by macrophages in the spleen, lymph nodes and other organs of the reticuloendothelial system (**Dhar and Gautam 1979, Sharma 1979, Sandhu et al 1998 and Omer et al 2003**). They also recorded a progressive decrease in these parameters in acute *Theileria annulata* infection in cattle. Leukopenia could have resulted from large scale destruction of lymphocytes by schizogony in lymphoid organs and infiltration of these cells into various organs resulting in decreased count in the peripheral circulation as reported earlier (**Sharma and Gautam, 1971 and Al-Kushali et al, 1981**).

The analysis of the obtained data in Table (2) showed a decline in the mean values of total protein, albumin and globulin in sera of infected sheep with *Theileria*, compared with the non infected ones. The low total serum protein in infected sheep with theilerioses may be due to hypoalbuminaemia and hypoglobulinaemia arising from liver dysfunction. Similar results were obtained by **Saudhu et al. (1998)**. **Singh et al. (2001)** and **Omer et al. (2003)** reported significant decrease in serum total protein in crossbred calves experimentally infected with *Theileria annulata*.

The results in Table (2) clarified that the serum urea and creatinine significantly increased in infected sheep with *Theileria* as compared with healthy ones. This is probably due to kidney damage. These findings agree with that of Sandhu (1996), Sandhu et al. (1998) and Yin et al. (2003). They noticed focal diffuse coagulative necrosis and severe damage in collecting tubules, haemorrhages and lymphocytic aggregations in interstitial spaces. But Kerr (1989) noticed a decline in serum creatinine which was not clinically significant.

The significant increase in AST, ALT and ALP observed in the serum of sheep infected with *Theileria* (Table, 2) indicates liver damage. These results agree with the results of Sandhu et al. (1998) and Omer et al. (2003). *Theileria annulata* infection causes hepatic tissue damage that include coagulative necrosis, distortion of hepatic cords and heavy infiltration of lymphocytes in the peripheral areas which indicate severe damage in the hepatobiliary system due to hypoxia resulting from anaemia and jaundice, (Sandhu, 1996).

Table (2) represents a significant increase in the cholesterol, HDL and LDL while triglyceride level showed no significant change, compared with the same level in healthy sheep. This may be due to liver damage. These results agree with the results of Yadav and sharma (1986) while Singh et al. (2001) recorded a significant decrease in serum cholesterol and HDL level in infected sheep with theileriosis. This phenomenon might partially

depend on the acceleration of erythropoiesis as reaction anemia caused by *Theileria sergenti* infection. On the other hand **Sindhu et al. (1998)** reported no change in serum cholesterol levels which may be due to that animals were young and fed on whole milk.

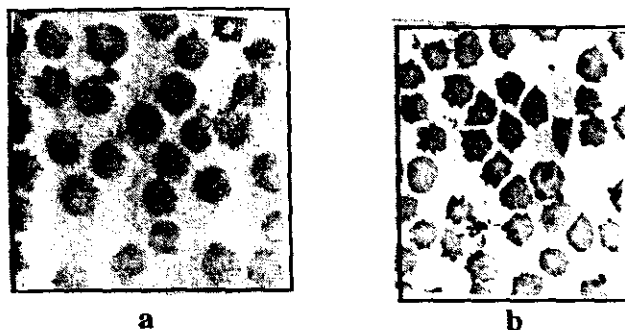


Fig. 1: Morphological forms of Theileria species within infected erythrocytes.

a- Ring form

b- Rod form

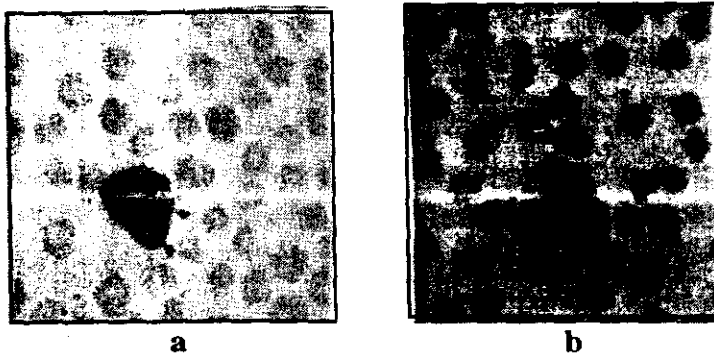


Fig. 2: Blood smears of sheep showing schizonts in the lymphocytes.

a- Macro-schizonts

b- Micro-schizonts

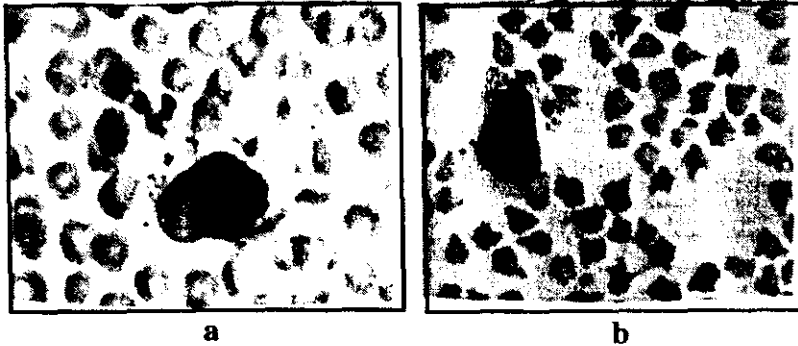


Fig.3: Schizonts in the lymphocytes showing different shapes of chromatin materials.

a- Ring and oval-round shapes.

b- Dot granules, rod and comma shapes.

Table (1): The effects of clinical theileriosis on some haematological parameters in sheep

Parameter	Non infected	Infected
Hb gm%	10.33±0.21	6.71±0.14***
PCV %	33.57±0.43	21.82±0.082***
RBCs x 10 ⁶ /ul	9.60±0.24	5.28±0.13***
WBCsx 10 ³ /ul	7.53±0.31	4.89±0.20***

***p<0.001

Table (2): Effect of clinical infection with theileriosis on some biochemical parameters of sheep

Parameter	Non infected	Infected
Total Protein gm/dl	6.89±0.16	5.71±0.13***
Albumin gm/dl	2.92±0.09	2.43±0.08***
Globulin gm/dl	3.96±0.13	3.29±0.11***
Creatinine mg/dl	1.11±0.02	1.44±0.04***
Urea mg/dl	13.95±0.32	20.64±0.68***
Cholestrol mg/dl	67.96±0.93	91.54±1.64***
Triglycerides mg/dl	26.6±0.69	28.44±1.01
HDL mg/dl	13.23±0.91	20.1±0.87***
LDL mg/dl	49.26±0.25	63.2±1.5***
ALT U/L	24.8±1.4	30.76±1.86*
AST U/L	58.55±1.48	174.39±6.39***
ALP U/L	108.44±4.94	152.5±4.82***

*p<0.01; ***p<0.001

Table (3): Effect of clinical infection with theileriosis on some elements in the serum of sheep

Parameter	Non infected	Infected
Calcium mg/dl	10.04±0.21	8.94±0.19***
Inorganic phosphorus mg /dl	5.43±0.12	4.73±0.19*
Sodium m Eq/L	152.2±0.73	151.44±0.91
Potassium m Eq/L	4.92±0.06	4.53±0.06***
Magnesium mg/dl	2.73±0.15	1.78±0.09***
Iron ug/dl	134.98±3.47	63.10±3.29***

*p<0.01; ***p<0.001

The decreased serum calcium level in sheep infected with *Theileria* (Table, 3) can be attributed to hypoalbuminaemia and hypomagnesaemia (**Burtis and Ashwood, 1996 and Omer et al. 2003**). **Dhar and Gautan (1977)** and **Sandhu et al (1998)** reported no change in calcium level in the sera of sheep experimentally infected with theileriosis which may attributed to the young age of the used animals in their studies and to the fact that they were fed on whole milk.

The decline in serum inorganic phosphorus and magnesium levels in the sheep with theileriosis (Table, 3) may be due to renal tubular defects, (**Agus, et al 1982, Sandhu, et al, 1998 and Omer et al, 2003**).

A significant decrease in iron level in the serum of sheep with *Theileria* (Table, 3) may be attributed to the inability of the damaged liver to synthesize transferrin (**Burtis and Ashwood, 1996, Kumar and Malik 1999, and Omer et al. 2003**). While, the decreased potassium concentration in the serum of the infected sheep may associated with the inappetence and hypomagnesaemia. Similar results were observed in the crossbred calves experimentally infected with *Theileria annulata* (**Yadav and Sharma, 1986 and Omer et al. 2003**).

It was obvious from this study that theileriosis is a real problem among

sheep in Egypt. Continuous surveillance of the prevalence of this disease and tick vector in different localities is needed which must be through a national program for control of this disease and other blood parasites. This program must including treatment and eradication of the vector. Quick and periodic treatment of sheep may improve the general health conditions of the animals and reduce the biochemical alteration that may happen due to infection.

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التغيرات الهيماتولوجية والبيوكيميائية فى الأغنام المصابة بالثيليريا

هالة حسين وهيب وإيمان محمد قش

معهد بحوث صحة الحيوان

تم فى هذه الدراسة الإستقصاء عن الإصابة بالثيليريا فى عدد ٤٠ أغانم من مزرعة بمحافظة الغربية تظهر عليها أعراض الإصابة بالمرض ، وبالإضافة إلى ذلك فقد تم جمع عدد ٩٦ عينة من أغانم سليمة ظاهرياً أثناء الذبح بمجزرى البساتين والوراق بالقاهرة والجيزة وقد ظهر أن نسبة الإصابة بالثيليريا فى الأغانم التى يظهر عليها الأعراض بالمزرعة ٨٧,٥٪ أما فى الحيوانات السليمة ظاهرياً والتى تم تتبعها فى المجرى فكانت نسبة الإصابة ١٨,٧٥٪ ، وعند الفحص ظهرت الأشكال المستديرة والعصوية من الثيليريا داخل كرات الدم الحمراء للحيوانات المصابة وأيضاً تم الكشف عن وجود أطوار مختلفة لطفيل الثيليريا فى الخلايا الليمفاوية (الليمفوسيت) ، وقد أظهر فحص الدم إنخفاض متدهور محسوس فى نسبة الهيموجلوبين والهيماتوكريت وكرات الدم الحمراء فى الحيوانات المصابة أما بالنسبة لكرات الدم البيضاء فكانت الليكوبينيا المحسوسة هى السمة الغالبة فى هذه الحيوانات . وعند فحص السيرم فى الحيوانات المصابة لوحظ زيادة محسوسة فى مستويات الالانين ترانسفيريز والإسبرتيت ترانسفيريز والفوسفاتيز القلوى والبولينا والكرياتينين والكستروى ، أما بالنسبة لمستويات البروتين الكلى والألبومين والجلوبيولين فقد ظهرت إنخفاضات محسوسة فى هذه الحيوانات وكذلك عند فحص مستويات الحديد والفوسفور الغير عضوى والكالسيوم والمغنسيوم فى الحيوانات المصابة لوحظ إنخفاضات محسوسة فى مستوياتها وذلك عند مقارنة جميع هذه النتائج بمثيلاتها من الحيوانات الظابطة والغير مصابة ولم يلاحظ تغير محسوس فى مستويات الصوديوم ودهنيات الدم الثلاثية فى الأغانم المصابة بهذا المرض .