PARASITOLOGICAL, HAEMATOLOGICAL AND BIOCHEMICAL STUDIES ON NATURALLY INFECTED GOAT WITH THEILERIA SPECIES IN EGYPT

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SUMMARY

The prevalence of theileria infection was investigated in 65 goats with suspected symptoms of theilerioses from a private farm in Giza Governorate. In addition, a total of 51 blood samples were collected from apparently healthy goats from private farms and during slaughtering at El-Bassatine abattoir. The incidence of theileria infection in the farm was 86.1% while, the incidence in the apparently healthy goats was 43.1%. Ring form of theileria species was detected in infected erythrocytes. Macroschizonts and microshizonts were detected in the lymphocytes with different shapes of chromatin materials. Haematological studies revealed significant decline in haemoglobin concentration, packed cell volume and red blood cell count. Total leucocyte count showed significant leucopenia in diseased animals. Examination of serum revealed significant rises in levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea, creatinine and cholesterol. Concentration of serum total protein, albumin and globulin showed significant decrease in theileria infected animals as compared with the healthy control goats. Levels of serum iron, inorganic phosphorus, magnesium and calcium showed also significant decline as compared with healthy goats. No changes were observed in serum sodium and triglycerides levels of goats with theilerioses.

Therefore, it is important to put in consideration the great economical losses caused by theilerioses in such important small ruminants.

INTRODUCTION.

Theilerioses, a tick-borne hemoprotozoan disease,

is one of the most economically important diseases of wild and domestic ruminants transmitted trans-stadially by Ixodid ticks.

There were two named species of theileria infecting goats as well as sheep, theileria lestoquardi (T. hirci) and Theileria ovis (Schnittger et al., 2003). Theileria ovis had been considered to be non pathogenic and the infection was known as benign theilerioses, while T. lestoquardi was associated with high morbidity and mortality rates, and the infection was known as malignant theilerioses (Mary et al., 1994). The clinical manifestations in goats which suffered from malignant theilerioses included fever, anemia, markedly swollen lymph nodes and lacrimation. In addition, infected animals may suffer from congestion of conjunctivae and necrosis of eye balls which may lead to blindness (Aslani and Mohri, 2002).

During the course of the disease, there were a marked decrease in milk production, emaciation and recumbancy which may lead to the death of infected animal. Also, in benign theilerioses, transient fever and mild lymph node swelling were recorded (Mary et al., 1994). In Egypt, Nagaty (1947) found *T. lestoquardi* in blood of sheep and goats in Kena Governorate. Altay (2005) recorded that *T. lestoquardi* and *Theileria* ovis may cause malignant theilerioses of sheep and goat in Africa, Asia and Southern Europe. Mazyad and Khalaf (2002) recorded that the percentage of infection of goats with Theileria ovis in North Sinani was

7.5% and double infection with theileria and babesia was found to be 25% of the total examined animals. Tageldin et al. (2005) in the Sultanate of Oman found that the percentage of infection of goats infected with theileria species was 2.4% while Lnci et al. (2003) in Turkey recorded that the infection rate of theileria species in goats was 8%.

Although goats are considered one of the important sources of animal protein in Egypt, especially among bedouins, scarce literature, are available about goat theilerioses. The average population of goats in Egypt is about 1.5 millions (General Organization for Veterinary Services, 2007).

As many bedouins depend in their life on such small ruminants as source of meat, milk and hairs, the present study was conducted to throw some light on the prevalence of theileria infection in these economically important animals, as well as, to achieve better understanding of the changes in haematological and biochemical constituents of blood as a result of theileria infection, which will help to better understanding of the pathogenesis of tropical theilerioses in goats.

MATERIALS AND METHODS

1. Animals:

a. A total of sixty five goats from a farm in Giza Governorate of both sexes with suspected symptoms of theilerioses were bled and examined clinically for theileria infection. The clinical symp-

toms include fever (>39°C), loss of weight, anaemia and ocular manifestations such as conjunctivits and corneal opacity.

b. A total of fifty-one apparently healthy goats form another private farms and from slaughtered goats at El-Bassatine abattoir were also examined.

2. Samples:

Two blood samples were collected from each animal and divided into two portions. The first one was collected on an anti-coagulant (EDTA) for parasitological and haematological examinations. Thin blood films were prepared, fixed with methanol, stained with Giemsa stain and examined microscopically for the presence of any blood parasite. The second portion was left to clot 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.

3. Laboratory processing of samples:

After parasitological examination, 35 samples were proved to be highly positive for theileria infection, were subjected to haematological and serum biochemical analysis. While, 15 microscopically negative blood samples were subjected to the same examinations and were considered as a control negative.

The haematological examinations of tested blood samples included hemoglobin concentration (Hb) according to Crosby et al. (1954), Packed cell volume (PCV), by the micro-haematocrite method of Schalm (1986) and the total erythrocytes (RBCs) and leucocytes (WBCs) counts according to Thompson (1980).

The biochemical examination of serum samples included determination of the levels of serum total protein by Biuret method as described by Weichselbaum (1946), serum albumin level according to Dumas et al. (1971), serum creatinine as reported by Henry (1974), serum urea according to Patton and Crouch (1977), serum cholesterol according to Richmond (1973), high density lipoprotein cholesterol was carried out by using the method of Burstain et al. (1970), low density lipoprotein cholesterol using the method of Steinberg (1981) and triglycerides level as described by Glindler and King (1972). Serum inorganic phosphorus and serum magnesium level were determined according to Kilchling and Freiburg (1951) and Glindler (1971), respectively. 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 aminotransferase (AST) and alanine aminotransferase (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).

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RESULTS AND DISCUSSION

I. Parasitological examination:

Blood film examination revealed the presence of theileria infection in goats which suffered from evident clinical symptoms as rise in temperature (>39°), anorexia, loss of weight and anaemia. As well, clear ocular manifestations, such as bilateral congestion of the conjunctival mucous membrane, lacrimation, mild bulging appearance of the eye with milky infiltration and corneal opacity ending by complete blindness were observed (Fig., 1). Out of 65 clinically suspected farm goats, 56 animals were highly infected with theileria with an incidence rate of 86.1% and out of 51 from apparently healthy goats 22 animals were found to be positive with an incidence of 43.1%.

In the present study, the parasite was found to occur within the infected erythrocytes, ring form with transparent central part and nucleus located at the margin was found in most cases and measured 1.2µm (Fig., 2). The schizonts were detected in the lymphocytes with two forms macroschizonts and microschizonts. Macroschizont measured 7.5 X 2.5 µm (Fig., 3-a), while, microschizont measured 8.75 X 3.75 µm (Fig., 3-b). Most of the chromatin appeared as granules, which represented nuclear material without cytoplasm that might be indicative to severe infection with theile-

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ria species.

These results were with agreement with those of Aslani et al. (2002) and Salama and Magdy (2007) who reported that ocular manifestations in ... buffaloes were characterized by milky infiltration and opacity of the cornea. In the present study, the rate of infection was 86.1% in clinically suspected goats and 43.1% in apparently healthy animals, these percentages were higher than those of Mazyad and Khalaf (2002) who recorded that the percentage of infection with Theileria ovis alone in North Sinani was 7.5% and double infection with theileria and Babesia ovis was 25%. Also, these results were higher than that recorded by Tageldin et al. (2005) who found that the infection rate in Sultanate of Oman was 2.4%. On the other hand, this percentage was lesser than that of Luo-Jianxun et al. (1997) who found that the percentage of infection with theileria species in goats was 86.6% in China, Variation of the percentage of infection in goats could be due to difference of locality and/or the environment of the applied breeding system including tick population (Hala and Eman, 2006). These results may prove that theileria species infection among goats is wide. spread in Egypt. The differentiation between pathogenic and non-pathogenic species of theileria was not feasible. However, Schnittger et al. (2004) in North Africa indicated that, malignant theilerioses occurred in this region of the control of the control

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II. Haemtological and biochemical examinations:

Haematological and biochemical analysis of serum was commonly used to monitor health status and disease diagnosis in different animals, in these studies realistic values of different biochemical changes in goats suffering from theilerioses were analyzed.

The result obtained in table (1) revealed significant decrease in the haemoglobin, packed cell volume, total numbers of erythrocytes and leucocytes in goats infected with theileria, compared with clinically healthy non infected goats. Hala and Eman (2006) reported similar haematological picture in sheep infected with theileria. This decrease could be probably attributed to destruction of the parasitized erythrocytes by macrophage in the spleen, lymph nodes and other organs of the reticuloendothelial system (Omer et al., 2003). Leukopenia could be resulted from destruction of lympocytes by schizogeny in lymphoid organs and infiltration of these cells into various organs resulting in decreased count in the peripheral circulation as reported earlier by Al-Kushali et al. (1981).

Table (2) showed a significant increase in the cholesterol, HDL, LDL and triglycerides level compared with the same level in healthy goats. While, in table (3), a significant increase in AST, ALT and alkaline phosphatase values was obtained in sera of theileria infected goat. These re-

sults agree with those obtained by Yadav and Sharma (1986); Omer et al. (2003) and Hala and Eman (2006) and causes hepatic tissue damage which includes coagulative necrosis, distortion of hepatic cords and heavy infiltration of lymphocytes in the peripheral areas that indicates severe damage in the hepatobiliary system due to hypoxia resulting from anaemia and jaundice as has been reported by Sandhu (1996).

Analysis of the obtained data in table (3) indicated decline in mean values of total protein, albumin and globulin in sera of infected goats with theileria, compared with non-infected ones. The low total serum protein in infected goat with theilerioses may be due to hypoalbuminaemia and hypoglobulinaemia which arises from liver dysfunction. Similar results and explanations were obtained by Sandhu et al. (1998) and Hala and Eman (2006). Omer et al. (2003) reported significant decrease in serum total protein in crossbred calves experimentally infected with Theileria annulata.

The results in table (4) showed significant increase in serum urea and creatinine levels in infected goats with theileria as compared with apparently healthy ones. These findings are probably due to kidney damage and coincided with those of Yin et al. (2003) who noticed focal diffuse coagulative necrosis and severe damage in the collecting tubules; haemorrhages and lymphocytic aggregations in the interstitial spaces.

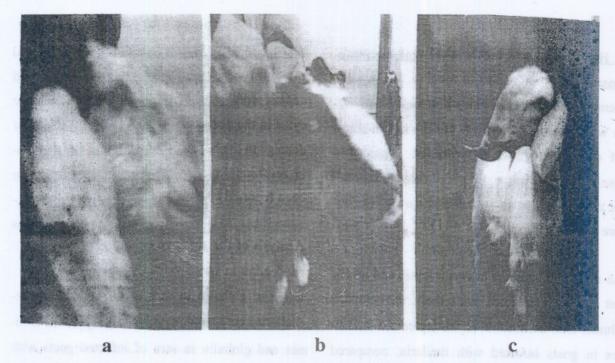


Fig. (1): "a, b, c" Ocular manifestations in goats showing milky infiltration and corneal opacity.

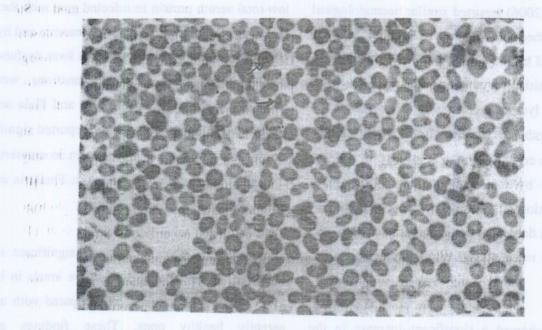


Fig. (2): Blood smear of a goat showing ring form of theileria species within infected erythrocytes.

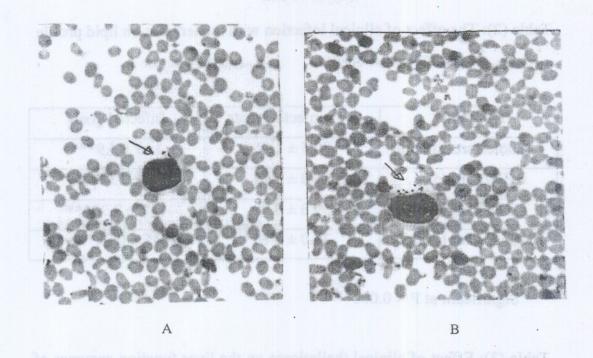


Fig. (3): Blood smears of a goat showing schizonts in the lymphocytes.

- a- Macroschizonts.
- b- Microschizonts.

Table (1): The effect of clinical theilerioses on some haematological parameters in goats.

Parameter	Non infected goats	Infected goats
Hb (gm/dl)	11.5 ± 0.34	7.4 ± 0.19***
PCV%	35.6 ± 0.45	19.0 ± 0.35***
RBCs X 10 ⁶ /μl	7.4 ± 0.44	4.8 ± 0.31***
WBCs X 10 ³ /μl	8.1 ± 0.63	3.9 ± 0.11***

^{***} Significant at P < 0.001

Table (2): The effect of clinical infection with theilerioses on lipid profile parameters in goats.

Parameter	Non infected goats	Infected goats
T. Cholesterol (gm/dl)	80.5 ± 1.05	145 ± 4.9***
HDL (mg/dl)	15.0 ± 0.94	25 ± 1.09***
LDL (mg/dl)	59.0 ± 0.98	111 ± 2.30***
Triglycerides (mg/dl)	30.0 ± 0.86	45 ± 0.94***

*** Significant at P < 0.001

Table (3): Effect of clinical theilerioses on the liver function enzymes of goats.

Parameter	Non infected goats	Infected goats
ALT (u/l)	27.4 ± 0.95	59.5 ± 1.09***
AST (u/l)	60.0 ± 1.33	165.0 ± 2.11***
ALP (u/l)	113.0 ± 2.15	194.0 ± 2.50***
. T. protein (gm/dl)	7.4 ± 0.71	4.3 ± 0.31***
Albumin (gm/dl)	3.1 ± 0.11	2.0 ± 0.09***
Globulin (gm/dl)	4.3 ± 0.31	2.3 ± 0.13***

*** Significant at P < 0.001

Table (4): The effect of clinical infection with theilerioses on some kidney function of goats.

Parameter	Non infected goats	Infected goats
Urea (mg/dl)	18.5 ± 0.25	27.2 ± 0.32***
Creatinine (mg/dl)	1.2,± 0.05	2.1 ± 0.09***
Uric acid (mg/dl)	7.6 ± 0.51	7.9 ± 0.68

*** Significant at P < 0.001

Table (5): The effect of clinical infection with theilerioses on some mineral profile parameters of goats.

Parameter	Non infected goats	Infected goats
Calcium (mg/dl)	9.5 ± 0.51	7.8 ± 0.19***
Inorganic Phosphorus (mg/dl)	5.2 ± 0.20	3.9 ± 0.25***
Sodium (mEq/l)	148 ± 1.22	146.0 ± 0.23
Potassium (mEq/l)	4.6 ± 0.05	$4.5 \pm 0.01***$
'Magnesium (mg/dl)	2.5 ± 0.03	1.6 ± 0.10***
Iron (µg/dl)	124 ± 1.23	65.0 ± 1.22***

^{*} Significant at P < 0.01

Table (5) showed decreased serum calcium level in goats infected with theileria. This could be attributed to hypoalbuminaemia and hypomagnesamia (Omer et al., 2003). As well the decline in serum inorganic phosphorus and magnesium levels could be due to renal tubular defects (Sandhu et al., 1998, Omer et al., 2003 and Hala and Eman, 2006). The significant decrease in iron lev-

el in the serum could be attributed to the inability of the damaged liver to synthesize transferrin (Kumar and Malik, 1999 and Omer et al., 2003). While the decreased potassium concentration in the serum of the infected goat may be associated with the inappetence and hypomagenesemia. Similar results were observed by Omer et al. (2003) and Hala and Eman (2006).

^{***} Significant at P < 0.001

It was obvious from this study that theilerioses constitutes a real problem among goats in Egypt. Therefore, due to the high rate of infection with theileria among goats, and to the great need of goat as an important source of animal protein, control measures of goats theilerioses should be undertaken and further studies for differentiation between theileria species infecting goat must be carried out.

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دراسات طفیلیة، هیماتولوجیة وبیوكیمیائیة على الماعز المصابة بطفیل الثیاریا في مصر در اسات طفیلیه در محمود محمد عرفه* ، در ابراهیم جوده حافظ**

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الملخص العربي

في خلال هذه الدراسة تم عمل أستبيان عن نسبة الأصابة بالثياريا في عدد ٦٥ ماعز من مزارع خاصة بمحافظة الجيزة والتي تظهر عليها أعراض الأصابة بالمرض، وبالأضافة الي ذلك فقد تم جمع عدد ٥١ عينة من ماعز سليمة ظاهرياً من بعض المزارع وأثناء الذبح بمجزر البساتين بالقاهرة. وكانت نسبة الأصابة بالثياريا في الماعز التي يظهر عليها الأعراض بالمزارع ٢٠١٨% أما في الحيوانات السليمة ظاهريا والتي تم تتبعها في بعض المزارع ومجزر الساتين فكانت نسبة الأصابة ٢,٦١%، وعند الفحص ظهرت أشكال الثيليريا المستديرة داخل كرات الدم الحمراء للحيوانات المصابة، وأيضاً تم الكشف عن وجود أطوار مختلفة لطفيل الثيليريا في كرات الدم البيضاء (الليمفوسيت)، وقد أظهر فحص الدم أنخفاض ملحوظ في نسبة الهيموجلوبين والهيماتوكريت وكرات الدم الحمراء في الحيوانات المصابة أما بالنسبة لكرات الدم البيضاء فكانت الليكوبينيا الظاهرة هي السمة الغالبة في هذه الحيوانات. وعند فحص السيرم في الحيو إنات المصابة لوحظ زيادة كبيرة في مستويات الالانين أمينوتر انسفيرين و الأسبرتيت أمينو تر انسفيريز والفوسفاتين القلوى والبولينا والكرياتينين والكولستيريول، أما بالنسبة لمستويات البروتين الكلى والألبيومين والجلوبيولين فقط ظهر إنخفاض واضح في هذه الحيوانات وكذلك عند فحص مستويات الحديد والفسفور الغير عضوي والكالسيوم والماغنسيوم في الحيوانات المصابة لوحظ أنخفاض ملحوظ في مستوياتها وذلك عند مقارنة جميع هذه النتائج بمثيلاتها من الحيوانات الضابطة والغير مصابة ولم يلاحظ تغير واضح في مستويات الصوديوم ودهينات الدم الثلاثية في الماعز المصابة بهذا المرض.

ولهذا يجب الأهتمام بالإصابة بطفيل الثيليريا في الماعز مع الأخذ في الأعتبار الخسائر الفادحة التي يمكن أن تسبيها الأصابة في هذه المجترات الصغيرة.