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STUDIES ON NATURALLY INFECTED SHEEP WITH HAEMONCHUS CONTORTUS WITH SPECIAL REFERENCE TO BLOOD AND SERUM HAEMATOBIOCHEMICAL CHANGES

(With 5 Tables and 2 Plates)

By

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دراسات علي العدوي الطبيعية لدودة المعدة (هيمونكس كونتورتس) في الأغنام مع الإشارة إلي التغيرات الدموية والبيوكيميائية في مصل الدم

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أجريت هذه الدراسة علي الأغنام التي تم ذبحها بمجزر مدينة المنصورة الرئيسي حيث تم الكشف عليها قبل الذبح لاكتشاف أي إصابات طفيلية أو أي علامات مرضية كذلك أيضاً تم فحص هذه الأغنام لطفيليات الدم. وبعد الذبح تعرضت للفحص لاكتشاف أي إصابات طفيلية. وقد اعتبرت الحيوانات الخالية من الإصابات الطفيلية كلية هي المجموعة الضابطة بينما الحيوانات التي ثبت وجود طفيليات في معداتها هي المجموعة المصابة والمعنية بالدراسة. تم أخذ عينات دم من جميع الحيوانات في المجموعة الضابطة والمجموعة المصابة علي مادة مانعة للتجلط وأخري بدون لعمل القياسات الدموية والبيوكيميائية. كذلك تم جمع الديدان الموجودة في معدات الحيوانات المصابة وتصنيفها وعد ديدان الهيمونكس لمعرفة نسبة الإصابة. وسجلت نتائج هذه الدراسة أن نسبة انتشار المرض بين الأغنام كانت ٤٤,١٢% وكانت نسبة الإصابة في الأغنام الصغيرة في السن أعلي من الأغنام الكبيرة وأن معدل المصابة في الشتاء عالية (٦١,٩٠%) بينما كانت في فصل الصيف منخفضة (٢٠%). وقد أثبت الفحص المعملي لعينات الدم والسيرم التغيرات التالية: بالنسبة لصورة الدم فقد ثبت وجود نقص معنوي عالي في العدد الكلي لكرات الدم الحمراء وحجم الخلايا المصمت ونقص معنوي عالي جداً في مستوي هيموجلوبين الدم. كذلك ثبت أن هناك نقص معنوي في متوسط حجم الكرات الحمراء (MCV) بينما كان هناك نقص معنوي عالي في كل من متوسط وزن الهيموجلوبين في الكرات الحمراء ومتوسط تركيز الهيموجلوبين في الكرات الحمراء (MCH & MCHC) علي الترتيب في المجموعة المصابة عنها في المجموعة الضابطة. وبالنسبة للعدد الكلي لكرات الدم البيضاء فقد أوضحت الدراسة وجود زيادة معنوية عالية جداً

في المجموعة المصابة بديدان الهيمونكس كذلك وجدت زيادة معنوية عالية جداً في نسبة الخلايا الحامضية (Eosinophils) وخلايا المونوسيت (Monocytes) وعلي الجانب الأخر وجد نقص معنوي عالي في نسبة خلايا الليمفوسيت (Lymphocytes). وبالنسبة للتغيرات في مصل الدم فقد ثبت بالفحص المعملي وجود نقص معنوي عالي ونقص معنوي عالي جداً في مستوي كل من البروتين الكلي والألبيومين علي الترتيب وفي المقابل تلاحظ وجود زيادة معنوية ومعنوية عالية في مستوي كل من بولينا الدم والكرياتينين وإنزيمات الكبد (S.AST & S.ALT) علي الترتيب. كذلك أوضحت الدراسة وجود نقص معنوي في مستوي كل من الكالسيوم والفوسفور والماغنسيوم ونقص معنوي عالي جداً في مستوي الحديد والنحاس في مصل الدم.

SUMMARY

The present study was conducted on sheep which were slaughtered in the main El-Mansaura abattoir, where they were exposed to antimortem examination for detecting any parasitological or signs of disease. Also the sheep were subjected to postmortem examination for detecting any parasitological infections. The sheep which were completely free from any parasitological infections were considered as control group. The sheep which proved to be infected with abomasal nematodes served as infected or parasitized group. Blood samples were taken from all animals (control and parasitized animals) with or without anticoagulant for haematological and biochemical investigations. After postmortem examination, the stomach worms were collected, identified as *H. contortus* and counted to estimate percentage of the infection. The prevalence of *H. contortus* was 44.12% among examined sheep, young animals were non susceptible to infection than adults and seasonal dynamics of *H. contortus* in sheep revealed that infection rate reached its peak during winter season (61.9%) while the lowest rate was in summer (20%). Laboratory investigations in the present study proved that; presence of high significant decrease in TRBCs count, PCV and very high significant decrease in Hb concentration. Also, the study showed significant decrease in MCV value while the decrease was highly significant for MCH and MCHC values in parasitized group. TWBCs count showed very high significant increase. On the other side, the lymphocytes showed high significant decrease. Serum biochemical analysis illustrated high significant and very high decrease in total protein and albumin respectively, on the contrary it was observed high and very high significant increase B. urea, creatinine and liver enzymes (S.AST & S.ALT) levels respectively. The level of Calcium, Phosphorus and Magnesium decreased significantly, while the significant decrease

was very high in the level of Iron and Copper in parasitized group when compared with control one.

Key words: *Haemonchus contortus, blood, biochemical changes, patho. gy.*

INTRODUCTION

Sheep are among the first domestic animals kept by man for the production of meat, milk, skin and wool. They are managed under all types of animal husbandry from intensive and sophisticated to the most extensive form of nomadic grazing (El-Maghawry *et al.*, 2000).

One of the most destructive enemies for animal wealth and biggest hinderness for successful production is parasitic infection. In Egypt, these enemies represent the most important causes of direct and indirect losses among sheep breeds (Ali *et al.*, 1994).

The stomach worm *Haemonchus contortus* has a worldwide distribution in both temperate and tropical zones (Urquhart *et al.*, 1996 and Abdel-Nabi, 2000).

Changes in the climatic factors throughout the year cause a significant variation in the dynamics of *Haemonchus contortus* population. Moreover, seasonal occurrence of Haemonchosis was recognized as a main feature in many parts of the world (Urquhart *et al.*, 1996).

The parasite is a blood sucking dewelling the abomasum of ruminants especially sheep and goats and to a lesser extent cattle, the third infective larvae of *H. contortus* enter the host and exsheath in the rumen and then migrate to the abomasum to complete their life cycle. During the presence of the parasite in the host tissue, it causes several pathological and haematobiochemical changes leading to clear anaemia and decreased weight gain (Darzi *et al.*, 2004; Sarkar *et al.*, 2005 and Beriajaya and Copeman, 2006).

So our study aimed to focus light on the prevalence and seasonal dynamics of *H. contortus* in addition to clearing the parasitological, haematobiochemical and histopathological alterations resulted from infection to help us in putting strategic treatment for controlling this parasite.

MATERIALS and METHODS

MATERIAL:

(I) Animals:

Sheep which were used for this study were selected those slaughtered in El-Mansoura abattoir during the period from December

2006 to November 2007. On the bases of clinical signs (unthriftiness, pica, pale mucous membrane and semisolid to watery diarrhea). They were subjected to complete clinical examination, for detecting any parasitological infections or signs of disease, including the general appearance, body temperature, skin lesions and mucous membranes.

Blood samples were obtained from the ear vein, stained with Gimsa stain for other identification of any blood parasites. Rectal faecal samples were collected in clean plastic bags and examined by standard parasitological technique for detection of parasitic infection, including direct smear method and concentration floatation technique (Georgi and Georgi, 1990).

After slaughtering, the sheep were subjected to postmortem examination for detecting any parasitological infection.

The sheep which were completely free from any parasitological infection were considered as control group, while the sheep which proved to be infected with gastric parasites were subjected to be the material of this study (parasitized group). Thus, a total number of 136 abomasa were selected. The technique for collection and identification of nematodes was done according to Urquhart *et al.* (1996).

The collected nematodes were fixed with hot solution of 5% glycerin in 70% alcohol. The small worms were cleared in alcohol glycerin mixture, while the large worms were cleared in lactophenol, then they were mounted in glycerin gelatin.

(II) Samples:

Blood samples:

Two blood samples were collected from each animal via jugular vein puncture before slaughter.

The first samples were whole blood collected in vacutainer tubes containing EDTA as anticoagulant and were used for haematological studies according to Jain (2000). The second samples were collected in centrifuge tubes and allowed to clot at 37°C and then nonhaemolysed blood serum was separated and used for measuring total protein (Doumas, 1975), albumin (Doumas *et al.*, 1971), Total globulin was calculated by substracting of albumin from total proteins, serum Aspartate aminotransferase (AST) and serum Alanine aminotransferase (ALT), (Reitman and Frankel, 1957), blood urea nitrogen (BUN) (Tabacco *et al.*, 1979), serum creatinine (Husdan and Rapoport, 1968), calcium (Gindler, 1972), inorganic phosphorus (El-Merzabani *et al.*, 1977) magnesium (Bouon, 1962), iron and copper levels were estimated by atomic absorption spectrophotometer (Allian and Mouros, 1979).

Histopathological samples:

The specimens for histopathological studies were fixed in 10% neutral formalin, dehydrated, cleared and embedded in paraffin wax. Paraffin sections obtained at 4-5mm and routinely stained with Haematoxylin and Eosin (Bancroft *et al.*, 1996).

(III) Statistical analysis:

All data in the present study were subjected to statistical analysis according to Snedecor and Cochran (1981).

RESULTS

Parasitological results:

Results obtained in our study concerning parasitological prevalence of *H. contortus* infecting sheep in El-Mansoura city are shown in Tables (1 and 2) while morphological features of male and female are shown in Plate (I A, B, C and D).

Table 1: The prevalence of *H. contortus* infecting sheep in El-Mansoura city.

Animal	No. examined	No. infected	% of infection
Lambs	59	28	47.45%
Ewes	77	32	41.55%
Total	136	60	44.12%

Table 2: Seasonal dynamics of *H. contortus* infection in sheep in El-Mansoura city.

Season	No. examined	No. infected	% of infection
Winter	42	26	61.90%
Spring	30	16	53.33%
Summer	35	7	20%
Autumn	29	11	37.93%

Haemonchus contortus female (Plate I B) varies in length between 18-30mm, the vulva is located at the posterior fourth of the body and covered by linguiform process or thumb-like flap (vf) (Plate I D).

In Plate (I D) intrauterine eggs are oval somewhat yellowish in colour and measure 70 to 85µm in length by 41 to 44µm in wide and are in early stages of cleavage.

The male (Plate I A) measures 10 to 20mm in length. Its capulatory bursa (cb, arrow) has elongated lateral lobes supported by slender long rays. The small dorsal lobe is a symmetrically situated and supported by Y-shaped dorsal rays (Plate I C). The spicules are 450 to 500µm long, each with a terminal barb; the gubernaculum is navicular.

PLATE I

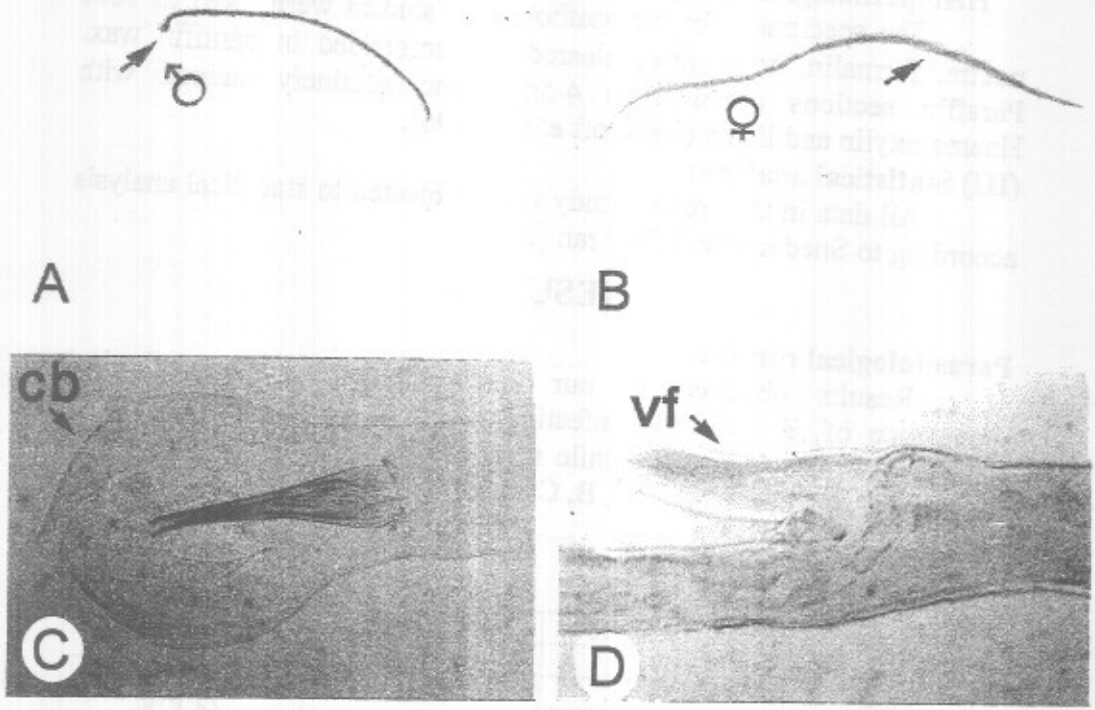

Plate I: (A) Male *Haemonchus contortus*
(B) Female *Haemonchus contortus*
(C) Posterior end of male *Haemonchus contortus*
(D) Posterior end of female *Haemonchus contortus* and intrauterine eggs

Haematological results:

Are shown in Table (3).

Table 3: Mean values of haemo and leucogram parameters of free parasitic and parasitized sheep with *H. contortus*

Parameter \ Group	*Free parasitic sheep control group (G_1)*	*Parasitized sheep with H. contortus (G_2)*
Total RBCs ($x10^6$/ mm^3)	9.75 ± 0.50	6.57 ± 0.58**
Hb (g/dl)	10.88 ± 0.54	7.41 ± 0.64***
PCV (%)	36.11 ± 1.06	30.31 ± 1.27**
MCV (FL)	38.43 ± 1.35	33.22 ± 1.44*
MCH (pg)	11.17 ± 0.57	8.31 ± 0.53**
MCHC (%)	27.93 ± 0.64	23.75 ± 0.59**
Total WBCs ($x10^3$/ mm^3)	9.95 ± 0.47	12.87 ± 0.46***
Neutrophils (%)	41.40 ± 0.39	42.10 ± 0.44 N.S
Eosenophils (%)	5.30 ± 0.27	7.20 ± 0.21***
Basophils (%)	0.20 ± 0.03	0.25 ± 0.04 N.S
Lymphocytes (%)	49.80 ± 0.92	45.05 ± 0.86**
Monocytes (%)	3.30 ± 0.33	5.40 ± 0.35***

*: Significant at ($p < 0.05$)
**: Highly significant at ($p < 0.01$)
***: very highly significant at ($P < 0.001$)
N.S: Non significant

Biochemical results:

Are illustrated in Tables (4 & 5).

Table 4: Mean values of liver and kidney function of free parasit. and parasitized sheep with *H. contortus*

Parameter \ Group	*Free parasitic sheep control group (G_1)*	*Parasitized sheep with H. contortus (G_2)*
Total protein (g/dL)	7.10 ± 0.23	6.01 ± 0.17**
Albumin (g/dL)	3.79 ± 0.12	2.67 ± 0.18***
Total globulin (g/dL)	3.31 ± 0.15	3.34 ± 0.19N.S
AST (Iu/ I)	15.65 ± 1.07	22.81 ± 1.39**
ALT (IU/ I)	28.71 ± 2.00	39.87 ± 2.04**
B. urea (mg/ dL)	22.80 ± 1.10	26.61 ± 1.06*
Creatinine (mg/dl)	0.68 ± 0.08	1.07 ± 0.13*

*: Significant at ($p < 0.05$)
**: Highly significant at ($p < 0.01$)
***: very highly significant at ($P< 0.001$)
N.S: Non significant

Table 5: Mean values of some serum biochemical parameters of free parasitic and parasitized sheep with *H. contortus*

Parameter \ Group	*Free parasitic sheep control group (G_1)*	*Parasitized sheep with H. contortus (G_2)*
Calcium (mg/dL)	10.57 ± 0.66	8.51 ± 0.59*
Phosphorus (mg/dL)	5.87 ± 0.51	4.22 ± 0.44*
Magnesium (mg/dL)	3.89 ± 0.43	2.66 ± 0.35*
Iron (µg/dL)	124.81 ± 3.27	80.47 ± 4.00***
Copper (µg/dL)	142.10 ± 3.88	93.98 ± 5.02***

*: Significant at ($p < 0.05$)
***: Very highly significant at ($P< 0.001$).

Histopathological results:

(1) Macroscopical finding:

The fundic and pyloric mucosa showed either focal or diffuse peticheal haemorrhages allover the whole mucosa. Deep red congested area mixed with erosions and ulcers of different sizes covered with hemorrhages and clotted blood were noticed on the fundic mucosa.

(2) Microscopical finding:

Various pathological changes were observed, the lesions produced in the abomasal wall were characterized by mononuclear cell infiltration with hyperactivity of goblet cells. Plate (II A and B) illustrates the following:

PLATE II

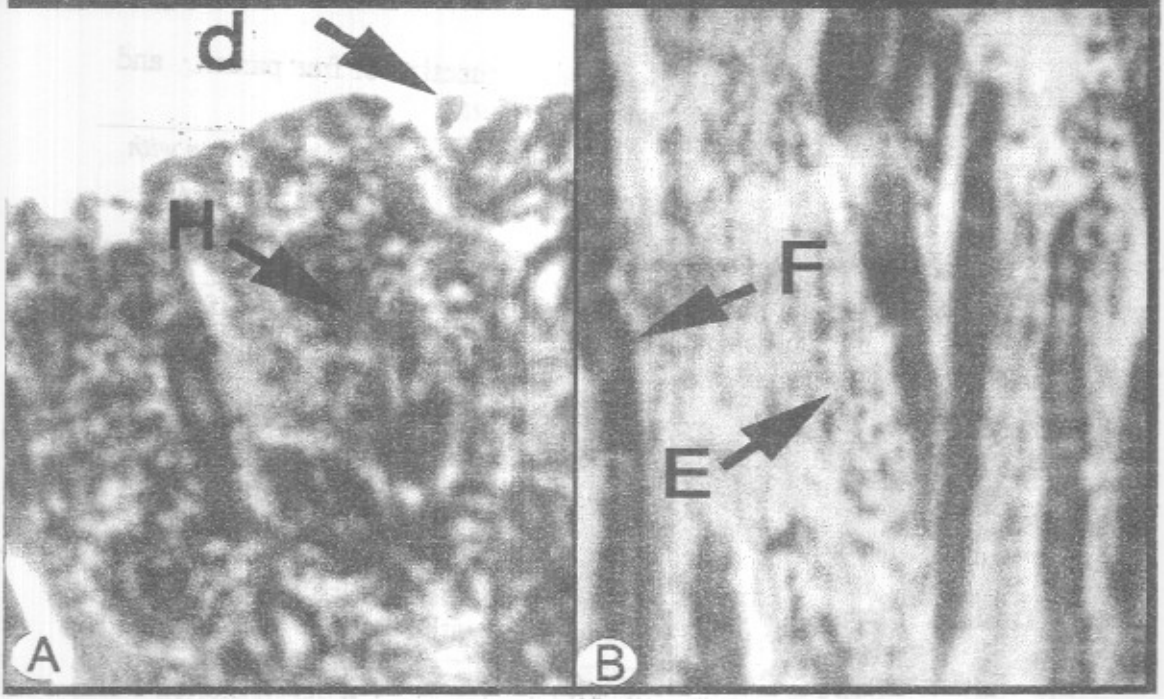

Plate II: (A) Showing focal haemorrhagic spots (H) with leukocytic infiltration in between the glands and desquamated (d) epithelial lining of the fundic mucosa (H & E x 400).

(B) Showing Streaks of fibrous connective tissue (F) and numerous mononuclear cells infiltration (E) with degenerative and necrotic changes of the abomasum (fundic region) owing to infection with *Haemonchus contortus* (H & E x 800)

DISCUSSION

We must offer veterinary care for sheep to overcome the problems and diseases to which they are exposed, specially in the field of endoparasites due to the great losses occurring. The gastrointestinal parasites are very common in sheep due to their grazing and watering habits (Godbole *et al.*, 1988).

Haemonchus contortus is one of predominant gastrointestinal parasites of sheep. It is distinguished by its blood sucking ability, due to a prominent buccal tooth presumed to function as lancet for slitting blood vessels (Noble *et al.*, 1989), in addition to the ability of the parasites to secrete cathepsin- L- like cystein proteases that can facilitate the parasite to suck host blood (Rhoads and Fetterer, 1995).

The present work revealed that out of 136 examined abomasa samples, 60 (44.12%) were infected with *H. contortus*. The recorded results agree with those reported by (Abdel-Aal and Khalaf-Allah, 2000) in their study on the incidence of *H. contortus* in Qalyoubia was 63.70%, while in Giza it was 38.7% (Hassona, 1979).

It has been found from this study that, the infection rate was higher in lambs (47.45%) than in ewes (41.55%), the lower percentage in ewes than lambs may be related to increased resistance to infection or reinfection with age or may be to development of immunity as a result of the intake of small numbers of larvae during early life. Young animals as lambs did not develop a suitable degree of immunity sufficient to compete infection (Stewart and Gordon, 1958). Also, Maqsood *et al.* (1996) reported that adult animals are generally less susceptible to helminth infection than young animals due to acquired immunity. In lambs immunity to *H. contortus* is delayed up to at least 4 months of age. This immune response, to a contaminated pasture, render the worm burden reach very high levels within a short period of time and contamination of the environment could also be proportionally higher.

Concerning seasonal dynamics of *H. contortus* infection, the present results showed that, the highest prevalence occurred in winter (61. 90%) and Spring (53.33%) seasons and the lowest was in summer (20%). The recorded results, were nearly similar to those reported by El-Akabawy (1987) and Abdel-Aal and Kalaf-Allah (2000), who mentioned that *H. contortus* had a peculiar seasonal dynamics with an increase in its prevalence during winter, this may be explained by the fact that most rainfall in Egypt is limited to winter season where sufficient amount of moisture is required for the development of larval stage. The lowest rate of infection was noticed in summer which may be due to inhibition of the development of infective larvae in pasture (El-Akabawy, 1987).

Moreover, *H. contortus* is a warm climate parasite which affect sheep during the four seasons, the parasitic stage of *Haemonchus* can thrive at temperature above 18°C (El- Azazy, 1995).

The data of haemogram parameters that presented in the present study revealed significant decrease ($p < 0.05$) in the mean value of MCV, high significant decrease ($P < 0.01$) in total RBCs count, PCV, MCH, MCHC and very highly significant decrease ($P < 0.001$) in Hb levels in parasitized sheep if compared with parasitic free ones (control), indicating a case of microcytic hypochromic anaemia. These findings could be attributed to chronic blood loss specially by *Haemonchus contortus* spp. and impairment of absorption, utilization and assimilation

of amino acids and some elements essential for erythropoiesis (William *et al.*, 1983 and Cales, 1986).

The observed microcytic hypochromic anaemia was confirmed by the decreased serum iron and copper levels in infected group. The results in our study were supported by those obtained by El-Maghawry *et al.* (2000).

It is evident from the present study that there was very highly significant increase ($P < 0.001$) in total WBCs count, Eosinophils and monocytes mean values in parasitized group when compared with the control group. In contrary marked reduction ($P < 0.01$) in lymphocytes was observed.

These results were similar to those recorded by Mousa *et al.* (1998) and Hamdy and El-Sayed (2004). The significant alterations in leucogram parameters could be explained by the fact that parasitism has eosinophil chemotasis properties and produced toxins and histamine due to antigen antibody reaction leading to eosinophilia, in the same respect monocytosis followed the induced chronic inflammation (Tizard, 1987).

The present data indicated a high and very high significant decrease ($P< 0.01$ and $P < 0.001$) in total proteins and albumin levels respectively in infested group below the control level. On the other hand, a significant and high significant increase ($P< 0.05$ and $P< 0.01$) in B. urea, creatinine, serum AST and serum ALT levels respectively above the control level was observed. The hypoproteinaemia and hypoalbuminaemia could be referred to the state of anorexia and inability of the liver to synthesis protein (El- Masry and El-Maghawry, 2000). The results in proteinogram in the present study coincide with those previously recorded by El-Sayed. (1993) and Bricarello *et al.* (2002).

The marked increase in liver and kidney functions in parasitized sheep in the present study were closely fitted with the results of El-Dessouky (2003) who attributed these changes to the primary or secondary liver and kidney involvement by metabolic products of parasites.

In the present investigation the mean values of calcium, phosphorus and magnesium significantly decreased ($P< 0.05$) below the control level, while very high significant decrease ($P< 0.001$) in the mean values of iron and copper were observed below the control group.

These present results partially agreed with El-Maghawry *et al.* (1998) in camels and completely with Hamdy and El-Sayed (2004) and Bashandy *et al.* (2006). They attributed such changes to the nematode

infection which may induce desquamation of epithelium, villous atrophy, mucosal flattening and accumulation of mucous debris. Accordingly, this lead to male absorption and impaired assimilation of nutrients.

It can be concluded from the present study that natural infection of sheep with *H. contortus* still constitutes a serious problem on health condition of these animals, lowering their productivity. It is therefore recommended that the farmers must be trained about the trends of parasitic infection, the role of pastures in the spread of infection. Veterinarians also depending on haematobiochemical and pathological changes could select suitable anthelimintic for deworming of sheep periodically, consequently improving general economy of sheep industry.

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