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STUDIES ON THE RECENT METHODS OF DIAGNOSIS OF CLINICAL AND SUB CLINICAL FASCIOLIASIS IN SHEEP

(With 5 Tables and 3 Figures)

By

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دراسات عن الطرق الحديثة لتشخيص الحالات الإكلينيكية والتحت إكلينيكية للإصابة بالديدان الكبدية في الأغنام

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التشخيص المعملي للإصابة بالديدان الكبدية يشكل صعوبة، خاصة في بداية الإصابة وحتى المزمنة منها، لأنه يعتمد على الكشف عن بويضات هذا الطفيل في البراز. لذلك استهدف البحث الحالي إلقاء الضوء على استخدام الطرق الحديثة (اختبار الاليزا) في التشخيص المبكـــر للإصابة بالديدان الكبدية في الأغنام، وكذلك مقـــارنة نتائج الفحص الباراسيتـــولوجي لبراز الحيوانات ونتائج اختبار الاليزا بها. كما استهدفت أيضا دراسة الأعراض الإكلينيكية والتغيرات البيوكيميائية في الأغنام المصابة بالديدان الكبدية. تم أجراء الدراسة على ١٤٥ رأسا من الأغنام المصرية البلدية من كلا الجنسين تراوحت أعمارها بين ٣ إلى ٧ أعوام , وقد اختيرت مجموعات هذه الحيوانات من قرى مختلفة بمحافظة أسيوط. كان تسع وثلاثون (٣٩) من هذه الحيوانات سليمة من الناحية الاكلينيكيه وفي حاله صحية جيدة ، أثبتت الفحوصات المعملية خلوها من الطفيليات الداخلية و الخارجية واستخدمت هذه الحيوانات كمجموعه ضابطة. باقي الحيوانات (١٠٦) كانت تعاني من أعراض الضعف العام والهزال وشحوب الأغشية المخاطية والنقص الواضح في الإنتاج والخصوبة، هذا بالإضافة إلى أن هذه الحيوانات كانت تعانى من درجات متباينة من الإسهال الذى تراوح قوامة من الطرى ألي المائى في بعض منها، كما شوهد معاناة بعض من هذه الحيوانات من استسقاء بالمنطقة تحت الفك وبعضها ظهرت علية درجات متفاوتة من سقوط الصوف. تم اخذ عينات براز من هذه الحيوانات لفحصها مجهريا كما تم اخذ عينات دم منها لاستخدام أمصالها في إجراء اختبار الاليزا و الاختبارات البيوكيميائية عليها وذلك لتشخيص

الفاشيولا ومعرفة التغيرات البيوكيميائية المصاحبة للإصابة بها. وقد أظهرت نتائج الفحص الباراسيتولوجى للبراز وجود بويضات الفاشيولا في عدد ٢٢ حيوان [٢٠,٧٥% من إجمالي الحيوانات المريضة (١٠٦) و١٥,١٧% من إجمالي الحيوانات الكلية المستخدمة في هذة الدراسة (١٤٥)] بينما وجدت بويضات لطفيليات أخرى في عدد ٣٥ حيوان. وبأجراء اختبار الأليزا على أمصال الدم للحيوانات في المجموعات المختلفة، أظهر الفحص السيرولوجى إصابة عدد ٧٤ حيوانا بديدان الفاشيولا (كانت موجبة لاختبار الأليزا ، ٦٩,٨% من إجمالي الحيوانات المريضة والتي كانت تعاني من أعراض اكلينيكية و ٥١,٠٣% من إجمالي الحيوانات الكلية). وصلت درجة حساسية الاختبار إلى ٩٠,٩١% ودرجة تخصص الاختبار إلى ٩٤,٨٧% كما أثبتت النتائج عدم وجود تداخلات في نتيجة الاختبار مع الطفيليات الأخرى مما يوضح دقة اختبار الأليزا في اكتشاف الحالات المصابة وخاصة حالات الإصابة المبكرة والتي لاتظهر عليها أعراض اكلينيكية واضحة ويصعب وجود بويضات للطفيل في عينات البراز المأخوذة منها. أوضحت النتائج البيوكيميائية عدم وجود اختلاف معنوى في مستوى البروتين الكلى لمصل الدم في جميع المجموعات وكان هناك نقص معنوى واضح في مستوى الألبيومين في المجموعات اللآتى عانين من الاصابه بالديدان الكبدية والإصابة بديدان أخرى غير الديدان الكبدية. بينما وجدت زيادة معنوية في مستوى الجلوبيولين في المجموعتين الثانية والثالثة مصحوبا بنقص معنوي جدا في نسبة الألبيومين إلى الجلوبيولين في نفس المجموعات. أظهرت النتائج أيضا عدم وجود أي اختلاف معنوي في مستوى الصفراء الكلى والمباشر لمصل دم الحيوانات في جميع المجموعات التي شملتها الدراسة ، بينما كانت هناك زيادة معنوية عالية في نشاط إنزيمات الأسبرتيت أمينوترانسفيريز والألانين أمينوترانسفيريز وإنزيم الجاما جلوتميل ترانسفيريز في المجموعتين الثانية والثالثة واللآتي عانين من الاصابه بالديدان الكبدية.

SUMMARY

Parasitological diagnosis of fascioliasis and the detection of *Fasciola* egg in feces alone is generally inadequate. This study aimed to use the enzyme linked immunosorbent assay (ELISA) in the diagnosis and early detection of fascioliasis in sheep, also comparing results of ELISA test and that of parasitological examination of feces for the detection of *Fasciola* egg and studying the clinical signs and the biochemical changes associated with sheep fascioliasis. 145 Egyptian Balady sheep of both sexes, 3-7 years old were investigated in this study. Clinical and laboratory investigation proved that 39 animals of the examined cases were clinically healthy, showing sings of soundness, good body condition score and free from any internal or external parasites. These animals were kept as control. The rest (106 animals) of the examined sheep were found suffering from variable degrees of emaciation, debility and paleness of the mucous membranes. Frequent to continuous attack of diarrhea was also observed. Some cases showed edema in the

submandibular space (bottle jaw) in addition to severe reduction in both productive and reproductive state. ELISA test revealed high incidence of fascoliasis among ill-health sheep [74 positive cases of the 106 ill-health animal (69.8 %)]. *Fasciola* spp. eggs were detected only in 22 of the 74 fecal samples of the infested sheep (29.73 %). The application of ELISA test for the detection of antibodies against *Fasciola* in sheep infested with helminthes parasites other than *Fasciola species* revealed negative results, which proved that ELISA is considered a good and specific test for the routine and earliest diagnosis of liver fluke infestation in sheep livestock. The probability by which the infested animal is identified by the ELISA test as positive in this work was 90.91 % (sensitivity of ELISA). On the other hand the probability by which a non-diseased animal is identified by the ELISA test as negative reached 94.87 % (specificity of the ELISA). By the using of ELISA test the false positive cases reached 9.1 % and the false negative cases reached 5.3 %. Biochemical investigations of blood serum revealed that the average values of the total protein in the infested animals were not changed, however the level of albumin in the blood serum of sheep infested with *Fasciola* and those infested with internal parasites other than *Fasciola* was highly reduced. On the other hand high significant elevation of globulin was found in blood of sheep infested with *Fasciola*. Concerning the blood serum levels of total and direct bilirubin, there were insignificant changes in their mean values in the different groups. There were highly significant increases in the serum activity of γ-glutamyl transferase enzyme (γ-GT), aspartate aminotransferase (AST) and allanine aminotransferase (ALT) in sheep infested only with *Fasciola* and those infested with *Fasciola* and other helminthes parasites.

Key words: *Fusciola, ELISA, sheep fasccioliasi.*

INTRODUCTION

Fascioliasis is one of the major causes of economic losses resulting from mortality and morbidity in sheep (Njau *et al.*, 1989). In Egypt liver flukes are the most helminthes parasites among animals. The direct impacts of *Fasciola* are liver lesions, reduction in feed utilization efficiency, deprivation of animals from digested nutrients and reduced feed intake through loss of appetite and discomfort leading to reduced feeding time. *Fasciola* infestation causes the condemnation of the affected liver or the part, which had undergone cirrhosis, and

calcification rendering it unfit for human consumption (Ayoub, 1983). El-Bahy (1997) mentioned that the pathological changes that occurred during the migration of liver flukes was economically and immunologically dangerous as it decrease body resistance and increasing their susceptibility to infection by other disease as well as marked decreasing in animal productivity. Also fascioliasis has an adverse effect on conception and establishment of fetus (Caudery, 1976) in addition to reduction of the fertility (Rommanik, 1977 and Arthur, 1977).

Fascioliasis in animals is of special interest during the last years in Nile delta region of Egypt. This is mainly due to the increase in percentage of human infestation (Hassan *et al.*, 1995). Human is infested accidentally by ingestion of raw aquatic vegetables or water contamination with metacercaria (Han *et al.*, 1996). Human fascioliasis is becoming an increasingly important clinical and epidemiological problem in Egypt (Hammounda *et al.*, 1995). A case of fascioliasis in person dealing with sheep was reported by Jones and Smith (1963). It was diagnosed by a strongly positive complement fixation test for *Fasciola* without detecting ova in his feces. The authors suggested that the patient acquired the infection from his hand while eating after handling sheep.

Parasitological examinations for the detection of eggs in feces are often negative in humans even after infections are patent. Thus, serologic diagnosis is an attractive alternative to help define infection as an adjunct to clinical findings. Serodiagnosis is also useful in epidemiological studies for human and animals infections. Antibody detection assays are both sensitive and early predictors of infection. For example ELISA test using. *F. hepatica* excretion secretion (*F.h.* ES) antigens become positive within 2 weeks of animal infection.

Clinically the disease in sheep is manifested by dullness, weakness, lack of appetite, pallor of mucus membranes and conjunctivae and pain if pressure is exerted over the area of the liver. In acute fascioliasis death occurs quickly, usually less than 48 hours from the appearance of the disease and may be accompanied by the passage of blood stained discharge from nostrils and anus. Furthermore outbreaks are most common and severe in young sheep and are relatively of short duration (Bhaskara and Madhubala, 1998). Sub acute fascioliasis is also described in sheep, it results from the ingestion of large number of metacercaria over a longer period of the time, and the major signs in infested animals are weight loss and pallor of mucous membranes. The

submandibular edema could be seen in only few cases, but many animals will resent palpation over the region of the liver (Urquhart *et al.*, 1996). Chronic Fascioliasis occurs when small numbers of metacercaoriae usually 200 - 500 are ingested over long period. Infested sheep are characterized by the classical clinical signs of liver fluke infestation, which are loss of weight, developed submandibular edema (bottle jaw) and pallor of the mucosa over a period of weeks. Shedding of wool may occur. The course of the disease is long as 2-3 months in those, which die, however many can survive but are emaciated for a long period (Radostits *et al.*, 1994; and Bowman, 1999). Anemia could not detected in sheep in migratory phase (Sinclair, 1962), however a single dose of 5000 larvae of *Fasciola* could produce severe anemia (Ross *et al.*, 1967).

Thabet (1995) and Nwiyi and Chaudrai (1996) found that the total level of protein in serum of female and male sheep suffering from *F. gigantica* was decreased. The blood serum level of albumin in two breeds of sheep experimentally infested with *F.gigantica* was declined 7 - 10 weeks post infection (Waweru *et al.*, 1999). On the other hand Thabet (1995) reported that the mean value of blood serum globulin in male and female sheep suffered from *F. gigantica* was elevated.

Significant increase in blood serum level of total bilirubin in sheep infested with *Fasciola gigantica* was recorded by El-Samani *et al.* (1985). Aspartate aminotransferase (AST) and allanine aminotransferase (ALT) activities were directly increased in blood in response to obstructive disease of liver (Fascioliasis) (Alan, 1994 and Margri Sirios, 1995).

This study aimed to: throw a light on the uses of the recent methods as ELISA test in diagnosis and early detection of fascioliasis in sheep, comparing results of ELISA test and that of parasitological examination of feces for the detection of *Fasciola* egg and studying the clinical signs and the biochemical changes associating sheep fascioliasis.

MATERIALS and METHODS

1- Animals:

A total number of 145 Egyptian Balady sheep of both sexes, 3-7 years old were investigated in this study. The animals were belonged to individual private farms in some villages at Assiut Governorate, Egypt. Careful routine clinical examinations of these studied cases were carried out as methods described by Rosenberger (1990) and Radostits *et al.* (1994). Laboratory investigations including blood analysis, liver function

tests and fecal examination were carried out on samples from each animal of the studied cases.

II- Samples and adopted methods:

A) Blood samples:

8-10 ml whole blood samples without anticoagulants were collected from each animal of both control and diseased groups. These samples were used for obtaining blood serum for the determination of total protein (T. ptn. g/l), albumin, g/l; total bilirubin (umol/l); direct bilirubin (umol/l); γ-glutamyletransferase (γ-GT,U/l); aspartate aminotransferase (AST, U/l) and allanine aminotransferase (ALT, U/l) by the using of test kits supplied by Sclavo/Italy (Annino, 1960 and Colombo *et al.*, 1974) and Biomerieux/France (Reitman and Frankel, 1957). Also part of the blood serum was used for the detection of the antibodies against *Fasciola* flukes by ELISA test.

B) Fecal samples:

These were directly collected from the rectum of each animal of the examined cases. The samples were transferred in labeled, clean and dry plastic bags. Examination of fecal samples were carried out at the same day of its collection according to Coles (1986)

Enzyme linked immunosorbent assay test (ELISA), was used for the detection of the antibodies against *Fasciola* in serum of sheep according to the method described by Santigo and Hillyer (1988) with the uses of excretory secretory (ES) antigen [Live intact adult *Fasciola* worms were obtained from infested liver at local abattoir of Assiut city and washed 3-4 times at room temperature during the first hour with 0.01M phosphate buffer saline (PBS) pH, 7.2 to remove all traces of blood and bile. The worms were then incubated (20 worms/100 ml PBS) at 37C° for 3 hours. The supernatant was then centrifuged at 20,000 rpm for one hour to remove particulate materials. Prepared ES antigen was stored at –20C° until further uses].

111-Statistical Analysis:

Statistical Analysis of the obtained data was done by means of software computer program (Spsswin, 1995).

RESULTS

Clinical and laboratory investigation proved that 39 animals of the examined cases were healthy, showing sings of soundness, good body condition score and free from any internal or external parasites,

these animals were kept as control. 106 animals of the examined sheep were suffering from variable degrees of emaciation, debility and paleness of the mucous membranes. Frequent to continuous attack of diarrhea was also observed. Some cases showed edema in the submandibular space in addition to severe reduction in the productive and reproductive state of the animals. According to the results of clinical examination and laboratory findings of parasitological analysis of feces and findings of ELISA test the studied cases were classified into five groups (Tables, 1, 2, & 3). Clinical signs observed on examined sheep of the studied groups are illustrated in Table 1 and Figures 1, 2 & 3.

Results of parasitological examinations of feces for the detection of parasitic ova as well as those of parasitological examinations and ELISA test for the diagnosis of fascioliasis are shown in Tables 2 & 3.

Blood serum levels of total protein, albumin, globulin, A/G ratio, total bilirubin, direct bilirubin, Γ-glutamyl transferase (Γ-GT), aspartate aminotransferase (AST) and allanine aminotransferase (ALT) in healthy and diseased groups of sheep are shown in Table 5.

DISCUSSION

Clinical findings:

In the present study animals infested with *Fasciola* showed paleness of the mucous membranes, roughness of the coat, emaciation, dullness, depression and various degrees of diarrhea. There was a severe reduction in the reproductive and productive performance of the infested sheep (Figures, 1, 2 & 3). The body condition score was not markedly affected in some cases however in others was poor to very poor. Depletion of subcutaneous and tail fat was observed in several surviving infested sheep (Fig. 2). Shedding of the wool, partial to complete alopecia, loss of weight and presence of submandibular edema (bottle jaw) were observed on several cases of infested animals (Fig. 1). In mild fascioliasis there were a small number of migrating flukes not sufficient to cause appreciable damage to liver parenchyma and consequently did not affect the weight of the animals. The same picture was described previously by Bulgin *et al.* (1984). In moderate and sever fascioliasis there were a severe destruction in the liver parenchyma causing the above mentioned signs and lead to poor body gain (Radostits *et al.*, 1994 and Nwiyi and Chaudrai, 1996).

In an area where liver flukes occur, every case of chronic ill health in sheep must be considered as a possible case of fascioliasis

(Radostits *et al.*, 1994). In a flock where fluke is present it is necessary to determine whether or not they could be the sole or main contributing factor to the ill health or death of the animals. The infestation may be the main cause of disease or may occur due to another debilitating disease such as nutritional deficiencies of copper or cobalt, other internal parasites or John's disease. In addition to ill-health and the other clinical signs of fascioliasis observed on infested sheep, chronic fascioliasis may follow and can be confirmed by the detection of large numbers of characteristic operculated egg of *Fasciola* in the feces, and by hepatic lesions characteristic of the disease in the liver at necropsy.

Laboratory finding:

Flukes eggs in the feces are not suspended satisfactory by all flotation solutions, consequently sedimentation tests are more accurate and it has been suggested that treatment may cause improvement if any egg are not present (Happich and Boray, 1969). Parasitological diagnosis of fascioliasis and detection of *Fasciola* egg in feces alone is generally inadequate because the incubation period presenting clinical finding is from a few days to 2-3 months, yet the flukes require a period of at least 3-4 months to attain sexual maturity (Chen and Mott, 1990). Thus infested animals have clinical finding long before eggs could be found in the feces, making parasitological finding as an early diagnosis tool impossible. A number of serological technique (Ghauvin *et al.*, 1995) has been developed as a promising alternative for diagnosis. Serological tests have shown promise, that ELISA is the best test for routine diagnosis of fascioliasis in sheep and cattle (Radostits *et al.*, 1994). A rise in antibody could be detected by 2 weeks after infection but was not sufficiently high to be diagnostic until 6-8 weeks (Zimmermann *et al.*, 1982 and Radostits *et al.*, 1994).

In the present study parasitological examination revealed that *Fasciola* species eggs were detected in fecal samples of only 22 cases of 74 positive infested sheep by ELISA test (29.73 %) which may be attributed to either early infestation with the parasite (Radostits *et al.*, 1994) or inefficient fecal analysis tests caused by inherent problem in the fecal sedimentation procedures (Dixon and Wescott, 1987). In the last decade, excretory secretory antigens have been widely used to detect antibodies in parasitic infection with varied success (Santiago and Hillyer, 1986 & 1988). The authors used adult worm homogenates and found that sheep and calves recognized major antigenic polypeptides (immunodiagnosis) by 8 weeks of infestation.

ELISA test in this study gave proper and promising results where high incidences of fascoliasis among ill-health sheep were detected (69.8%). Furthermore the probability by which the infested animal is identified by this test as positive in this work was 90.91% (sensitivity of ELISA). On the other hand the probability by which a non-diseased animal is identified by the ELISA test as negative reached 94.87% (specificity of the ELISA). The false positive cases reached 9.1% and the false negative cases among the examined cases reached 5.3%. Cornelissen *et al.* (1992) found that the specificity of ELISA to detected antibodies against *Fasciola* species was 98 % and 95 % by using somatic and excretory antigens respectively. The results of ELISA test in serum samples of the control group (Group 1) revealed that there were 2 clinically healthy sheep having no detectable *Fasciola* egg in feces but positive by ELISA (false +ve, Table 4). These two cases were treated with anti *Fasciola* drugs 4 weeks before sampling and this lead to absence of the *Fasciola* egg in feces however; the antibodies against *Fasciola* were present in low titer in serum that gave false positive result. Dumenigo *et al.* (1999) suggested that presence of antibodies might indicate previous exposure to the parasite rather than the existence of the current infection and absence of egg in feces of animals 2 weeks after chemotherapy. On the other hand there were 2 animal voiding eggs in their feces but negative for ELISA (False -ve, Table 4). The anti *Fasciola* antibodies response of hosts are relatively intense when parasites are migrating through the liver parenchyma and falls when they establish in the bile duct, such phenomena probably explains the lower anti *Fasciola* antibodies in hosts voiding fluke eggs and subsequent to the fasciolicidal drugs (Njau *et al.*, 1989). The microscopic examination of fecal samples for the presence of *Fasciola* egg is often difficult because they are not found during the prepatent period, on the other hand, in some cases it is also difficult during the patent period because of the intermittent excretion of parasitic eggs (Sandeman and Howell, 1981; Njau *et al.* 1989 and Dumenigo *et al.*, 1999). Eggs of *Fasciola* do not appear in several conditions of chronic fascioliasis and these is due to sterility of adult worm, so the excretion of *Fasciola* eggs among these animals was lowering (Kendall, 1975)

Hillyer *et al.* (1992) in a study on human fascioliasis recorded that 19 0f 20 (95 %) individual who were positive parasitologicaly were also positive by ELISA test. Hillyer *et al.* (1996) found that all sheep,

which are positive parasitologically, were also positive serologically which mean that ELISA test sensitivity reached 100 %.

ELISA test for diagnosis of fascioliasis revealed negative results in sheep infested with helminthes parasites other than *Fasciola* (Group IV) and also with those suffering ill health not associated to parasitic infestation (Group V). These findings revealed that there were no cross reaction between *Fasciola* and other helminthes parasites, also support the results of ELISA test and declared that ELISA is considered an important specific and sensitive test for diagnosis and screening of fascioliasis in sheep livestock. In this respect Zimmerman *et al.* (1982) noticed that nematode infection did not appear to interfere with interpretation of ELISA results in sheep infested with *Fasciola*.

Regarding the biochemical investigations the average values of serum total protein were not changed in the examined groups. There were a high significant reduction ($p < 0.01$) in the blood serum levels of albumin (hypoalbuminemia) in sheep infested only with *Fasciola*, (group 11) sheep infested with *Fasciola* and other helminthes parasites (group 111) and those sheep infested with internal parasites other than *Fasciola* species (group IV). On the other hand high significant elevation ($p < 0.01$) were found in blood, serum globulin of sheep infested with *Fasciola* and sheep infested with *Fasciola* and other helminthes parasites respectively. It was evident from these results that, although sheep infested with *Fasciola* were suffering from anorexia and their livers were not efficiently synthesizing protein, the elevated globulins substituted the reduced albumins and thus total proteins were not changed. Drastic alterations in plasma protein values were observed usually with liver diseases, decrease in albumin levels may by attributed to inhibition of its synthesis, its rapid breakdown and its losses (Coles, 1986). Hypergloulinemia reflects the reactivity of the immune body defense in response to invasion of the bile duct tissue with *Fasciola* and it means in the same time that the injury process of the body defense is still active (Robert and keith, 1986). The obtained results in this concept come in accordance with the findings of Kadhim (1976), Moragg (1991) and Waweru *et al.* (1999).

The insignificant changes in the values of blood serum levels of total and direct bilirubin in the different groups agreed with Hjerpe *et al.* (1971) who recorded that the concentration of total serum bilirubin did not depart appreciably from normal during the first 6 weeks of

experimentally infested sheep. Similar results were recorded by Sykes *et al.* (1980) and El-Samani *et al.* (1985).

The highly significant increases ($P < 0.01$) in the serum activity of gamma-glutamyl transferase enzyme (γ-GT) in sheep infested with *Fasciola* gave an indication for the importance of the use of this enzyme in diagnosis of fascioliasis. Sykes *et al.* (1980) suggested that γ-GT was considered the most suitable test in the field for the detection of fascioliasis. A direct proportion relationship was recorded between the number of fluke burden in liver and γ-GT level in serum (Wyckoff and Bradley, 1985 and Wiedosari and Copeman, 1990).

Regarding the activities of serum transaminases the high significant elevation ($P < 0.01$) in the activity of AST and ALT in sheep infested with *Fasciola* could be attributed to the necrotic degeneration of the liver, periductable fibrosis and blockage of the bile ducts during the fluke migration through the liver (Urquhart *et al.*, 1996 and Waweru *et al.*, 1999). Furthermore the authors added that higher serum aminotransferase level might be used as an index of liver damage caused by fascioliasis. In this respect AST levels have been used in the past but gamaglutamyl transferase is nowadays more relatively sensitive indications of liver cell damage associating fascioliasis (Hawkins, 1984). In this work although ALT levels was significantly elevated when compared with that of the control group, it could not be of a significant diagnostic value for fluke infestation in general.

Finally it could be concluded that in big herds one can judge the sub clinical individuals of fascioliasis by the using of ELISA test without big error. Further work comparing serological results and necropsy examination of naturally infested animals must be done to determine whether these seronegative animals actually represented non-exposed sheep or individuals that failed to produce or maintain detectable antibody to *Fasciola hepatica*.

REFERENCES

Alan, H. and Ba, W. U. (1994): Diagnostic enzymology, in "Clinical Laboratory Medicine". Edited by Kenneth, D.M.C. Clatchey, Williams, Wilkins company: 239-240.

Annino, J.S. (1960): Clinical Chemistry Principle and procedure little, Brown and Co., Boston and Toronto: 202.

Arthur, G.H. (1977): Veterinary reproduction and obstetrics. Maemi Uian Publishing company, New York, 4th Ed.

Ayoub, A.A.M. (1983): The interpretation of the different tests used for the Estimation of parasitic status of *Fasciola gigantcia* in Charkia Governorate Thesis, M.V.Sci. Faculty of Vet. Medicine Dept. of parasitology, Cairo University.

Bhaskara Ro, P. and Madhubala, K. (1998): Out breaks of fascioliosis in sheep with pulmonary involvement. Indian Vet, 75: 183-184. 1MSD, AGVET.

Bowman, D., Dwigh, I.J. and Randy, C. (1999): Dwigh D. Bowman Georgis Parasitology for Veterinarians 7th ed. W.B. Saunders company, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo: 114-117.

Caudery, M.J. (1976): The effects of fascioliasis on infertility. British Vet. J., 2: 568-575

Chen, M.G. and Mott, (1990): Progress in assessment of morbidity due to *Fasciola hepatica* infection. Trop Dis Bull, 87.R1, R36.

Coles, E.H. (1986): A Text book of Veterinary Clinical Pathology. 4th Ed., W. B Sounders company philoidelphia, London, Toronto, 331-333.

Colombo, J.P., Peheim, E., Kyburz, S. and Hoffmann, J.P. (1974): Cli.Chim.Acta, 51: 217-119.

Cornelissen, J.B., Leeuw, W.A. and Heijden, P.J. (1992): Comparison of an indirect hemagglutination assay and an ELISA for diagnosing *Fasciola hepatica* in experimentally and naturally infected sheep. Vet. Q., 14: 152-156.

Dixon, R.C. and Wescott, R.B. (1987): A fast accurate fecal examination technique for diagnosis of *Fasciola hepatica.* Proc. Am. Assoc. Vet. Parasitol, Portland, OR: July 17 - 18: 28.

Dumenigo, B. E., Espino, A. M., Finlay, C.M. and Mezo, M. (1999): Kinetics Of antibody-based antigen detected in serum and feces of sheep experimentally infected with *Fasciola hepatica*. Vet. Parasit, 86: 23-31.

El-Bahy, M.M. (1997): Fascioliasis among animal, snail and human hosts in Kafr El-Sheikh Governorate with special reference to species infecting humans. Vet.Med.J.Giza, 45: 187-209.

El-Samani, F., Mahmoud, O.M., Fawi, E.T., Gameel, A.A. and Haroun, A.M. (1985): Serum enzyme activity and bilirubin concentration in sheep experimentally infected with *Fasciola hepatica*.J. Comp.Path., 95:499-503.

Ghauvin, L., Bouvet, G. and Boulard, Ch. (1995): Humeral and cellular immune responses to *Fasciola hepatica* experimental primary and secondary infection in sheep. Int.J.Parasitol. 25:1227-1241.

Hammounda, N.A., El-Mansoury, S.T., El-Azzouni, M.Z. and El-Gohari, Y. (1995): Therapeutic effect of tripendazole in patients with fascioliasis in Egypt. J. Egypt. Soci. Parasitol, 25:(1): 173-174.

Han, J.K., Han, D. and Choi, B.L. (1996): MR findings in human fascioliasis. Trop. Med.Inter. Health, 3: 367-372.

Happich, F.A., and Boray, J.C. (1969): Quantitative diagnosis of chronic fascioliasis.2.The estimation of daily total egg production of *Fasciola hepatica* and the number of adult flukes in sheep by fecal egg counts. Aus. Vet. J., 45:329-331.

Hassan, M.A., Nahed , E.M., Laila, A.M., Bessa, E.A. and Mohamed, H.A. (1995): Prevalence of *Fasciola* infection among school children in Sharkia Governorate. Egypt. J.Egypt.Soc. Parasitol., 25: (2) 543-549.

Hawkins, C.D. (1984): The use of hemoglobin, PCV and serum sorbitol dehydrogenase as indicator of the development of fascioliasis in sheep. Vet. Parasitol. 15: 125-133.

Hillyer, G.V., Solor De Galanes, M Buchon, P. and Bjorland, J. (1996): Herd evaluation by enzyme linked-immuonosorbent assay for the determination *Fasciola hepatica* infection in sheep and cattle from the Altiplano of bolivia, Vet. Parasitol., 61: 211-220.

Hillyer, G.V., Solor De Galanes, M and Battisti, G. (1992): Fasciola hepatica host responders and non-responders to parasite glutathione s transferase. Exp. Parasitol., 75: 176-186.

Hjerpe, C.A., Tennant, B.C., Crenshew, N.F. and Beker, N.F. (1971): Ovine fascioliasis in California. J.of the American Vet. Med. Association 159: 1266-1277.

Jones, I. and Smith, P. (1963): Liver fluke infection in man. Lancet, 85:1241.

Kadhim, J.K. (1976): Changes in serum protein values of sheep infected with *Fasciola gigentica* .Am. J.Vet. Res. 37.

Kendall, S.B. (1975): Some parasites of domestic animals in Aswan Governorate. Egypt. Trop. Animal. Health and prod, 6, 3: 128-130.

Margri Sirios, M. S. (1995): Clinical chemistry, in (Vet. Clinical Laboratory procedure). Mosbyst. Louis Baltimore, Berlin. Boston Carlsbad Chicago, Madrid, pages 107-111.

Moragg, K. (1991) : Veterinary laboratory Medicine Ist Ed .Black well Scientific publication, London Boston 68-73.

Njau, B.C., Kasali, O.B., Scholtens, R.G. and Alkalework, N. (1989): The Influence of watering practices on the transmission of *Fasciola* among sheep in the Ethiopian highlands. Vet. Res. Com., 13: 67-74.

Nwiyi, T.N. and Chaudrai, S.U.R. (1996): Observations on Prevalence hematological and Pathological changes in cattle, sheep and goats naturally infected with *Fasciola gigantica* in Arid Zone of Boron state, Nigeria, Pakistan Vet.J, 16(24): 172-175.

Radostits, O.M., Gay, C.C., Blood, D.C. and Hincheliff, K.W. (1994): Veterinary Medicine, 9th ed., W.B.saunders London, New York, Philadelphia, San Francisco, St. Louid, Sydney

Reitman, S. and Frankel, S. (1957): Am. J. Clin. Path, 28: 56.

Robert, J.D. and Keith, W.P. (1986): Veterinary Laboratory Medicine 2nd Ed., Laboratory Congress Publication: 105-109.

Rommanik, K. (1977): Search for the relation ship of chronic Fascioliasis in cattle and experimental Fascioliasis hepatica in rats to the reproduction processes in these animals. Veterinaries, 8: 3-49.

Rosenberger, G. (1990): Die Klinische Untersuchung des Rinds: 3.Auflage VonDirksen, G., Grunder H- D and ST ber Ms. 670-677: Verlag Paul parley. Berlin and Hamburg.

Ross J.G., Dow, C. and Todd, J.R. (1967): A study of *Fasciola hepatica* infection in sheep. The Veterinary Record, 80: 543-546.

Sandeman, R.M. and Howell, M.J. (1981): Response of sheep to challenge infection with *Fasciola hepatica.* Research in veterinary Science, 30:294-297.

Santiago, N. and Hillyer, G.V. (1986): Isolation of potential Serodiagnostic *Fasciola hepatica* antigens by electro elution form polyacrylamide gels. Am. J. Trop. Med. Hyg, 35: 1210-1217.

Santiago, N. and Hillyer, G.V. (1988): Antibody profile by EITB and ELISA of cattle and sheep infected with *F. hepatica* J. parasitol, 78:810-818.

Schalm, O.W., Jain, H.C. and Carrol, E.J. (1979): Veterinary Hematology. 3rd Ed. Lea and Febriger, Philadelphia. PP.29.

Sinclair, K.B. (1962): Observation of clinical pathology of ovine fascioliasis. Brit.Vet. J. 118: 37 –53.

Spsswin, (1995): Software packed for statistical (Under Window U.S.A., (1995).

Sykes, A.R., Coop, R..L. and Rushton, B. (1980): Chronic sub clinical fascioliasis in sheep effects on food intake, food utilization and blood constituents. Research in Veterinary Science, 28:63-70.

Thabet, M.H. (1995): Coagulogram following some liver diseases in sheep. M.V.Sci. Thesis, Faculty of Vet. Med. Assiut University.

Urquhart, G.M., Armour, J., Duncon, J.L., Dunn, A.M. and Jennies, F.W. (1996): veterinary parasitology. 2nd Black well science Ltd.

Waweru, J.G., Kanyari, P.W.N., Mwanji D.M., Negate, T.A. and Nasen, P.A. (1999): Comparison of serum biochemical changes in two breads of sheep (Red massoni and Dorper) experimentally infested with *Fasciola gigantica.* Onderstepoo journal of Vet, Research 66: 47-49.

Wiedosari, E. and Copeman, D.B. (1990): High resistance to experimental infection with *Fasciola gigantica* in Javaness thin tailed sheep. Veterinary Parasitology, 37:101-111.

Wyckoff, J.H. and Bradley, R.E. (1985): Diagnosis of *Fasciola hepatica* infection in beef calves by plasma enzyme analysis. American Journal of Veterinary Research, 46:1015-1016.

Zimmerman, G.L.; Jen, L.W.; Cerro, J.E.; Fornsworth, K.L. and Wescott, R. B. (1982): Diagnosis of *Fasciola hepatica* infections in sheep by an enzyme-linked immunosorbent assay. American Journal of Veterinary Research.43: 2097-2100.

Table, 1: Observed clinical signs on sheep of the studied groups.

Aspect of examinations	Group 1 No. = 39	Group 11 No. = 55	Group 111 No. = 19	Group 1V No. = 16	Group V No. = 16
Appetite	Good	Slight inappetance to complete loss of appetite, increase desire to drink water (19).	Mild to moderate loss of appetite.	Good appetite (9), partial loss of appetite (7)	Complete loss of appetite (3), Partial loss of appetite (13).
Mucous membrane (m. M.)	Rosy red No lesion No discharge	Pale (33); Pale mucous membrane of eye with nasal discharge (4).	Pale in all animals	Pale, (10) Pale m. m. eye with nasal discharge (2)	Pale m. m. of eye with nasal discharge (5)
Body condition score	Good to very good	Emaciation, thin and poor (37).	Emaciation (12), Emaciation with dehydration (3).	Emaciation (6)	Emaciation (4)
Gait	Normal posture and gait	Laying down for long periods followed by difficult to get up standing (15), Complete recumbancy (4)	Laying down for long periods followed by difficult standing (3) recumbancy (3)	Tendency to laying down difficult standing (13)	Recumbancy (2)

Group I: Healthy control sheep. Group II : Sheep infested only with *Fasciola* spp. (positive either by fecal examination or ELIZA)
Group III : Sheep infested with *Fasciola* spp. and other internal parasite.
Group IV: Sheep infested with internal parasite other than *Fasciola* spp.
Group V: Sheep showing clinical signs of fascioliasis however fecal examination and ELIZA test revealed – ve results of infestation.

Table, 1:Continuation,

Aspect of examinations	Group 1	Group 11	Group 111	Group 1V	Group V
Character of feces	Normal pellets	Diarrhea with alternative intervals of constipation (12), greenish watery diarrhea with offensive odor (9), indigestible food particles (14).	Different forms of diarrhea and blood in feces (5) Indigestible food particles (14)	Soft mucoide feces (3), Soft pasty (2), presence of adult worms in feces (2)	Soft feces (3), greenish watery diarrhea (4), presence of adult worm in feces (2) (Nematode worm)
Mental state	Active, sound	Dullness and depression (12)	Dullness and depression (16)	Dullness & depression in all animals	Dullness & depression in all animals
Coat	Good appearance	Easley detached wool (28), partial alopecia in different part (5)	Easley detached wool (5), partial alopecia (12)	Easley detached wool (4)	Partial loss of wool (5), mange mites (3)
Productive and reproductive state	Fertility and reproductive efficiency were high with high milk yield and meat production	Infertility (19)	Infertility (19)	Infertility (10)	Infertility (3)
edema	-------	Submandibular edema (13)	-------	-------	Submandibular edema (2), Subcutaneous edema (2)

Table, 2: Results of parasitological examinations of feces for the detection of parasitic ova.

Group 1 (No. = 39)	Group 11 (No. = 55)	Group 111 (No. = 19)	Group 1V (No. = 16)	Group V (No. = 16)
Fecal samples were free from any egg of internal parasites	Positive for *Fasciola* spp. egg (20), The remaining was positive by ELIZA test. Media	Feces containing Faciola spp egg and other eggs of internal parasites (2) -*Fasciola* spp. egg + oocyst of *Eimeria* spp +*Trichostrongylus* spp egg (1)m -*Faciola* spp egg + *paramphistomum* spp egg (1) Positive for *Fasciola* by ELIZA test and feces contain eggs for other parasite (17 -*Neoascaris vitulorum* (2) -*Trichuris* egg (1) -*Trichuris* + *Eimeria oocyst* (2) -*Trichostrongylus* spp egg containing variable number of cells (6) -*Trichostrongylus* spp egg + oocyst (2) -*Dictyocallus* spp.egg contain larva (2) -*Eimera oocyst* (2)	-Egg of Nematodirus Spp. Contain 2-8 distinctive large cell (2) -Eimeria oocyst (6) -Tricostrongyle spp. egg + oocyst (2) -Trichuris ovis egg (1) -Paramphistomum spp egg (2) -Moniezia spp. Egg variable in shape (2) -Dictycalus spp. egg contain larva (2)	Feces free from any egg of internal parasite.

Table, 3: Results of parasitological examinations and ELISA test for the diagnosis of fascioliasis.

Animal group	No. of examined Cases	Positive cases by fecal examination		Positive cases by ELISA	
		Number	%	Number	%
Group 1	39	00	00	2	5.13
Group 11	55	20	36.6	53	96.36
Group 111	19	2	10.35	19	100
Group 1V	16	00	00	00	00
Group V	16	00	00	00	00
Total	145	22	15.17	74	51.03

Table, 4: Sensitivity and specificity of ELISA test.

Test result	True positive*	True negative**	Total
Test +ve	20	2	22
Test -ve	2	37	39
Total	22	39	61

* : Animals were ill health and *Fasciola* eggs were detected in feces.

**: Animals were healthy; *Fasciola* eggs were not detected in feces and ELISA test for diagnosis of *Fasciola* gave negative results.

$$\text{Sensitivity} = \frac{20}{20+2} = 90.91\ \% \qquad \text{Specificity} = \frac{37}{37+2} = 94.87\ \%$$

$$\text{False positive} = \frac{2}{20+2} = 9.1\ \% \qquad \text{False negative} = \frac{2}{2+37} = 5.13\ \%$$

Sensitivity: Probability by which a diseased (infested) animal is identified by the test as positive.
Specificity: Probability by which a non-diseased animal is identified by the test as negative.
False positive: Probability by which an animal is identified as test positive, while in fact it is non diseased.
False negative: Probability by which an animal is identified as test negative, while in fact it is diseased.

Table, 5: Blood serum levels of total protein, albumin, globulin, A/G ratio, total bilirubin, direct bilirubin, Γ-glutamyl transferase (Γ-GT), aspartate aminotransferase (AST) and allanine aminotransferase (ALT) in healthy and diseased groups of sheep.

Animal group		Total protein g/l	Albumin g/l	Globulin g/l	A/G Ratio	Total bilirubin µmol/l	Direct bilirubin µmol/l	Γ-GT U/l	AST U/l	ALT U/l
Group 1	x±sd	77.3 ± 14.1	28.3±6.3	48.5±13.6	0.61±0.2	8.7±4.3	2.1±2	31.2±8	68.9±19.5	16.9±7.7
	range	61.7- 122	17.9-43.1	30.2-98.3	0.24-1.1	2.1-16.8	0.0-8.7	15.5-50	30-100	7.5-34
Group 11	x±SD	74 ± 22.7	19.5±4.9	54.6±21.9	044±031	8.9±6.4	2.2±1.7	59.9±25	108.2±45	24.7±10.6
	range	37-130.2	11.5-35.6	12.5-107.3	014-2.08	2.1-36.1	0.2-10.1	26-158	45-230	9-55
Group 111	x±SD	73±17.5	19.6±3.9	53.4±18.63	0.43±0.23	10.8±6.3	1.4±1.2	40.5±8	102±28.3	25.3±8
	range	47.494.9	13.7-27.4	28.3-80.5	0.18-0.87	2.4-22	0.3-4.8	23-54	78-185	7.5-36
Group 1V	x±SD	72.7±19.1	22.5±7.1	48.3±19.4	0.54±0.26	7.9±3.9	1.6±1.5	37.9±16	69.8±11	20.5±9.3
	range	43-108	11.1-34	21-90	0.2-1.04	2.6-16.8	0.0-5.0	19-66	54-90	5-32.5
Group V	x±SD	74±20.3	23.5±7.3	50.5±15.3	048±0.15	9.4±5.13	2.1±1.7	32.1±10	77±22	19.8±10.4
	range	46.5-127	14.5-37.3	31.7-89.7	0.31-0.8	2.4-18	0.3-6.2	13-52	45-120	6-38

Figure, 1: Ram infested with *Fasciola* spp. showing bottle jaw and paleness of the mucous membrane. (ELISA test positive, parasitological examination of feces for the detection of *Fasciola* spp. ova: positive).

Figure, 2: Ewe from sheep herds infested with *Fasciola* spp. showing weakness, emaciation and depletion of the subcutaneous fat (ELISA test positive, parasitological examination of feces for the detection of the ova of *Fasciola* Spp.: negative).

Figure, 3: Pale mucous membrane of weak, emaciated sheep in herd infested with *Fasciola* spp., (ELISA test positive, parasitological examination of feces for the detection of *Fasciola* spp. ova: positive).