Vet.Med.J.,Giza. Vol.42,No.1.(1994):77-81

EVALUATION OF SPECIFIC *FASCIOLA GIGANTICA* ANTIGENS FOR THE DIAGNOSIS OF FASCIOLIASIS IN EXPERIMENTALLY AND NATURALLY INFECTED SHEEP BY ELISA

By

MOUSA, W.M.
Dep. of Parasitology, Fac. Vet. Med., Cairo Unviversity.

(Received 19/1/1994)

SUMMARY

The value of peak II and E/S *Fasciola* antigens for diagnosis of experimental and natural sheep fascioliasis were studied by ELISA. The antibody levels of experimentally infected sheep with *F. gigantica* appeared at 2 weeks postinfection, gradually increased and the highest concentration of antibodies were found 9-13 weeks postinfection with both antigens. Also, both antigens gave 100% sensitivity for diagnosis of naturally infected sheep with fascioliasis. On the other hand, peak II antigen gave 100% specificity, while E/S antigen gave 94.29% specificity.

INTRODUCTION

Fascioliasis has an important impact on general health, weight gain and on the production (Hope Cawdery et al., 1977). In Egypt, fascioliasis caused by *F. gigantica* are wide spread and elevate mortality rate of sheep (Ezzat, 1949).

The diagnosis of fascioliasis among sheep is not confirmatory unless accompanied by the appearance of *F. gigantica* eggs in the faeces which occur about three months postinfection (Soulsby, 1968). Therefore, serological techniques are needed to increase sensitivity for detection of the disease in its early stage.

The present work was planned to evaluate two purified *F. gigantica* antigens for diagnosis of experimental and natural sheep fascioliasis by ELISA.

MATERIAL AND METHODS

Fasciola gigantica antigen

a) Preparation of crude worm antigen

The antigen was prepared using the method described by Hillyer and Santiago de Weil (1977). The adult *F. gigantica* worms were obtained from condemned bovine liver in Cairo abattoir. The worms were repeatedly washed in sterile PBS, homogenized, centrifuged at 20,000 rpm for one hour at 4°C. The protein content of the supernatant was measured by the method of Lowry et al. (1951).

b) Preparation of purified worm antigen (Peak II antigen)

The peak II antigen was obtained by gel filtration according to Mansour et al. (1983). Sephadex G-200 was suspended in 0.01 M PBS, pH 7.4 and allowed to swell for 3 hours in room temperature. The swollen Sephadex G-200 was packed in a glass column. The crude worm antigen was allowed to pass through the packed column. The protein fractions were collected in an ISCO fraction collector and the protein absorbance at 280 nm monitored by a gilford-250 spectrophotometer. The worm antigen was fractionated into 3 peaks. Peak II from several runs was pooled, lyophilised and reconstituted to 1/10 of the initial volume. The pro-

tein content was measured by the method of Lowry et al. (1951).

c) Preparation of Excretory/Secretory (E/S) antigen

The E/S antigen was prepared accoding to Hillyer and Galanes (1988). The living adult worm was washed repeatedly and then incubated in PBS (one worm/5ml) for 3 hours at 37°C. The supernatants were collected, centrifuged and concentrated using Pμ 10 membrane filters. The protein content was determined.

Serum sampels

a) Sera of experimentally infected sheep with *F.gigantica*

Six sheep (2-3 months old) were divided into twogroups. The first group included 4 sheep experimentally infected with 250 encysted metacercariae orally. The second group included 2 parasite free sheep as control. The serum samples from both infected and control sheep were collected during a period of 13 weeks after infection (viz.; 0, 1, 2, 3, 4, 5, 7, 9, 11 and 13 weeks) for evaluation and appearance of specific antibodies. Meanwhile, the faecal samples were collected from infected sheep at regular intervals to detect the first time when *F.gigantica* eggs appear in faeces. Also, the faeces of control sheep were examined routinely throughout t he time of the experiment.

b) Sera of naturally infected sheep with *F.gigantica*

Serum samples were obtained from slaughtered sheep in Cairo abattoir. parasitological and meat inspection were carefully performed to determine the presence of different parasites. Forty five serum samples were obtained from infected sheep with *F. gigantica* beside 35 serum samples from parasite free sheep as control.

Serodiagnostic technique

Enzyme linked immunosorbent assay

The test was carried out according to Zimmerm et al. (1982) with some modifications. The op mal reaction condition as regards sensitizing an gen concentration, antibody and conjugate dil tions were chosen for use with micro-ELISA af preliminary checker boad titration. In the prese study, the optimum conditions were 10 μg/m coating buffer antigen concentration, 1:50 seru dilution, 1:250 alkaline phosphatase labelled ra bit anti-sheep IgG as conjugate and 1 mg p nitrophenyl phosphatase dissolved in 1 ml su strate buffer as substrate. All incubation ste were carried out at 37°C in a moist chamber. Th positively threshold value was determined as mea of negative sera +3 standard deviations (mean 3SD).

RESULTS

The analysis of the obtained ELISA data as illu trated in Fig (1) showed significant antibody le els by two weeks postinfection with both antigen The antibody levels increased gradually till weeks postinfection and nearly remain in consta levels from 9 weeks till 13 weeks postinfection the end of experiment with both antigens (after th appearance of *Fasciola* eggs in the faeces, weeks postinfection).

On the other hand, the reaction of 45 naturally i fected sera and 35 control sera with E/S and pe II antigens was studid by ELISA. The positiv threshold value was also shown. It was found th all infected sera had an optical density higher th the corresponding threshold value indicating 10 (45/45) sensitivity with both antigens. In contra all control non-infected serum samples had an o tical density lower than the corresponding thre old value indicating negative result with 10 (35/35) specificity with peak II antigen, while serum smapels only gave negative results with E antigen indicating 94.29% (33/35) specific (Table 1).

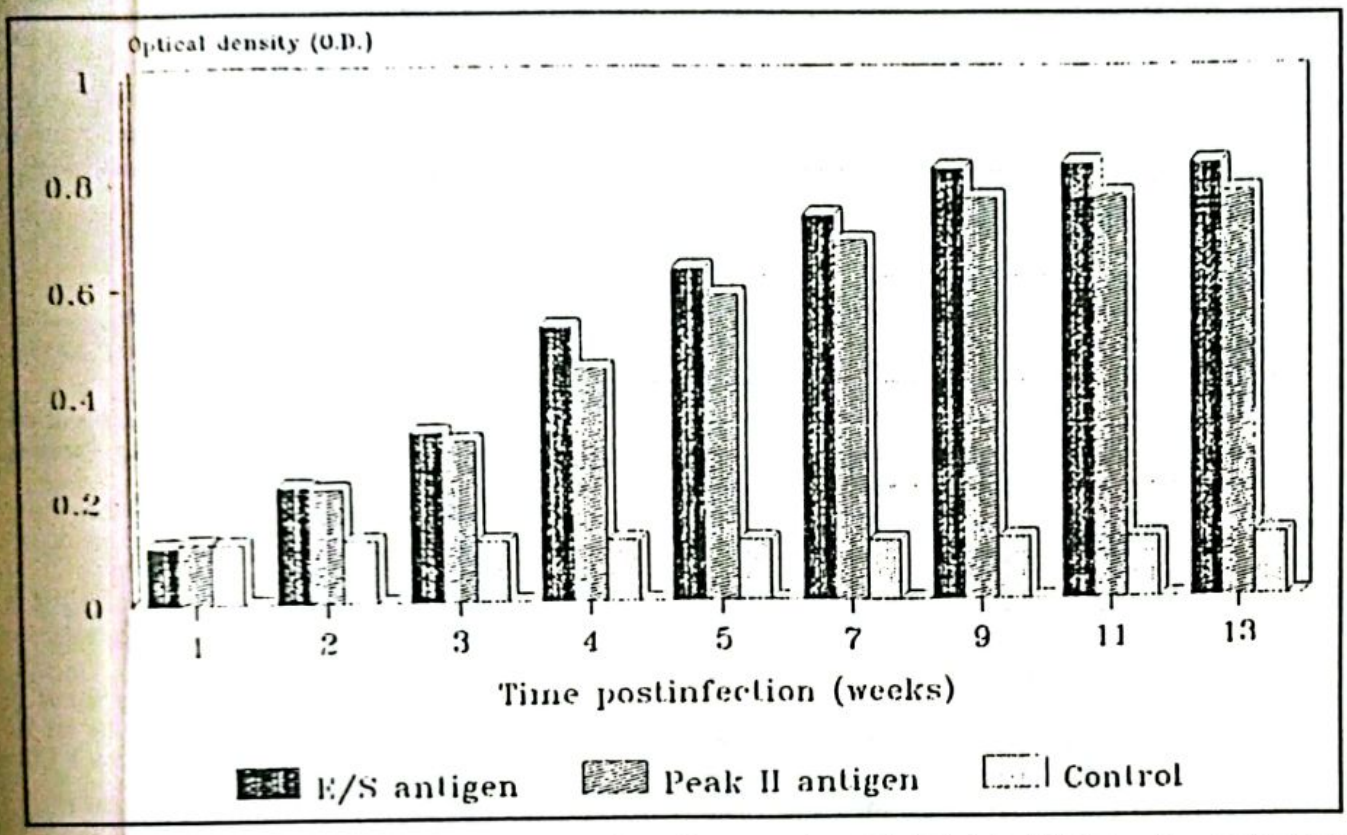

Fig. 1: Reactivity of two fasciolal antigens with sera from sheep experimentally infected with *F. gigantica* against IgG antibodies by ELISA

Table 1: The percentage of sensitivity and specificity of ELISA with E/S and peak II *Fasciola* antigens against IgG for diagnosis of naturally infected sheep.

	No. of collected + ve sera		No. of collected - ve sera	
	E/S Ag	Peak II Ag	E/S Ag	Peak II Ag
No. of tested cases	45	45	35	35
No. of positive cases	45	45	2	0
No. of negative cases	0	0	33	35
Sensitivity %	100	100	-	-
Specificity%	-	-	94.29	100

DISCUSSION

In the present study, the value of E/S and peak II antigens for diagnosing sheep fascioliasis were discussed by ELISA. The present study, revealed that the antibody levels of experimentally infected sheep with *F. gigantica* appeared at two weeks postinfection. The antibodies were gradually increased and reached the highest concentration 9-13 weeks postinfection. These data were in agreement with that of Babyanskas (1967); Kendall et al. (1978); Zimmerman et al. (1982) and Mousa (1992) who succeeded to detect the antibodies at early stage of fascioliasis (2-3 weeks postinfection. They used slide agglutination test, double immuno-diffusion, ELISA and EITB, respectively to detect the level of antibodies. These data disagreed with Farrel et al. (1981) who found that, the antibodies were detected in calves at late as 9-10 weeks postinfection by ELISA. This variation might be related to the quality of the used antigens where they used dead and lypholized *Fasciola* worm antigens.

The present study revealed that the sensitivity and specificity of peak II for diagnosis of natural sheep fascioliasis were 100% by ELISA. These data were in agreement with Hillyer and Santiago de Weil (1977); Mansour et al. (1983), who reported that the peak II was highly sensitive and specific for diagnosing human fascioliasis by immuno-electrophoresis technique.

The present investigation also found that the sensitivity and specificity of E/S antigen were 100% and 94.29% respectively. These data were in agreement with Hillyer and Galanes (1988) and Mousa (1992), who reported that the E/S antigen was reactive with all sera from fascioliasis by ELISA. These data disagreed with Babyanskas (1962) and mahmoud (1984), who found that the sensitivity of slide agglutination test for diagnosis of fascioliasis was 87.5% and 89.6% respectively. Such variation might be due to the difference of the test and antigen used.

From the previous discussion we can say that the peak II and E/S antigens are more helpful to determine the antibody levels at 2 weeks postinfection wih *F.gigantica* experimentally infected shee; On the other hand, peak II antigen is more helpf; for diagnosis of naturally infected sheep.

ACKNOWLEDGEMENT

The author would like to express his thanks to Prof. D M.K. Kamal for his help to use the chemical and instrume tal facilities of NAMRU-3 lab., Section immunopathology

REFERENCES

Babyanskas, M.A. (1962): Immunological reactions for d agnosis of fasioliasis in farm animals, Prof. Conf. Al Union, Soc. helminth Moscow 11: 15-16.

Babyanskas, M.A. (1967): Immunological diagnosis of be vine fascioliasis. Proc. 18th World Vet. Cong. Parasit 1: 131-134.

Ezzat, M.A. (1949): Helminth parasites of Egyptian shee Bull. Vet. Path. Labs., No., 250, Ministry of Agric Egypt.

Farrel, C.J., Shen, D.T., Wescott, R.B. and Lang, B.J (1981): An ELISA for diagnosis of *F. hepatica* infecti in cattle. Am. J. Vet. Res. 42 (2): 237-240.

Hillyer, G.V. and galanes, M.S. (1988): Identification of 1 KDa *F. hepatica* immune diagnostic antigen by the e zyme linked immuno-electransfer blot technique. J. Cli ic. Microbiol. 26 (10): 2048-2053.

Hillyer, G.V. and Santiago de Weil, N. (1977): Partial purif cation of *F. hepatica* antigen for immuno-diagnosis fascioliasis. J. Parasitol. 63 (3): 430-433.

Hope Cawdery, M.J., Strickland, K.L., Konway, A. an Crowe, P.J. (1977): Production effects of liver fluke cattle. Brit. Vet. J. 133: 145-158.

Kendall, s.B., Sinclair, I.J., Everet, G. and Parfitt, J.W (1978): Resistance to *F.hepatica* in cattle. I. parasitolog cal and serological observations. J. Comp. Path. 88: 11 122.

Lowry, O.H., Rosenbrough, N.J., Farr, A.I. and randall, R. (1951): Protein measurement with Folin-phenol reage J. Biol. Chem. 193, 265-275.

Mahmoud, A.A. (1984): Some studies on fascioliasis as an mal problem. Ph.D., Thesis, Vet. Med. Alexandria Uni viersity.

Mansour, N.S., Yousef, F.G., Mickhail, E.M. and Bactor, I (1983): Use of a partially purified *F. gigantica* wor antigen in the serological diagnosis of human fasciolias in Egypt. Am. J. Trop. Med. Hyg. 32 (3): 550-554.

Mousa, W.M. (1992): Studies on the cross-reactivity amon some helminths of Veterinary and medical importanc Ph.D., Thesis, Fac. Vet. Med., Cairo Univ., Egypt.

Soulsby, E.J.L. (1968): Helminths, arthropda and protoz of domesticated animals, 6th Ed. Baillier, Tindall an Cassel, London.

Zimmerman, G.L., Jen, L.W., Cerro, J.E., Farnsworth, K.L. and Wescott, R.B. (1982): Diagnosis of *F. hepatica* infection in sheep by an Enzyme linked immuno-sorbent assay. Am. J. Vet. Res. 43 (12): 2097-2100.