

Short Paper**Cross reactivity between ES and somatic antigens of *Fasciola spp* in enzyme linked immunosorbent assay****Moazeni, M. * ; Gaur, S. N. S and Shahangian, A.**

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***Correspondence:** M. Moazeni, Department of Pathobiology, School of Veterinary Medicine, University of Shiraz, Shiraz, Iran. E-mail: moazeni@shirazu.ac.ir**Summary**

Excretory-secretory (ES) and somatic antigens of *Fasciola hepatica* and *Fasciola gigantica* were prepared from freshly collected flukes. Laboratory bred rabbits were immunized with antigens for preparation of antisera. ES antigens of both species showed strong positive reaction with antisera raised against ES and somatic antigens of parasite. Somatic antigens of both species also showed strong positive reaction with antisera raised against somatic and ES antigens of parasite. In homologous combination of antigens and antisera higher enzyme linked immunosorbent assay (ELISA) values was observed in comparison with heterologous combination, so it was concluded that ES and somatic antigens of *Fasciola spp* have strong cross reaction with each other but the antigenic materials of ES and somatic products of parasite are not completely the same.

Key words: *Fasciola*, ELISA, ES antigen, Somatic antigen**Introduction**

The estimated annual losses to livestock due to fascioliasis all over the world are more than \$2000 million dollars. It is also an emerging zoonoses and about 2.4 million people are presently affected with this infection in different parts of the world (Dalton, 1999).

Faecal egg detection (Coprological diagnosis) is the most reliable diagnostic method for infection with adult flukes but this method can not detect immature flukes migrating in the liver parenchyma in the early stages of infection (Itagaki *et al.*, 1989). The pathology of immature fascioliasis is manifested as early as 2 weeks after the infection, whereas parasitological diagnosis is impossible until about 10-14 weeks when eggs are detected in the faeces (Guobadia and Fagbemi, 1997). Enzyme linked immunosorbent assay (ELISA) allows for early detection of fascioliasis in animal herds and their owners so that

humans and livestock can be treated prior to the development of liver pathology, thus minimizing morbidity due to this disease (Hillyer *et al.*, 1996). Considering the importance of fascioliasis in human and domestic animals and the role of ELISA in the early detection of the disease, in present study the cross reactivity of ES and somatic antigens of the parasite were investigated.

Materials and Methods**Collection of worms**

Adult flukes including *Fasciola hepatica* and *Fasciola gigantica* were collected in fresh state from the bile ducts of naturally infected sheep from Shiraz slaughterhouse, Fars province, Iran. The flukes were washed several times in phosphate buffered saline (PBS).

Preparation of ES antigen

Freshly collected, living flukes after proper washing were incubated in 0.01 M

PBS, 7.2 pH at 37°C for 6 hrs (one worm in 2 ml PBS). The incubation fluid was centrifuged at 4°C for 35 min at 27000 g, the supernatant was stored at -20°C until used.

Preparation of somatic antigen

Freshly collected flukes were homogenized in PBS (1 worm in 3 ml). The homogenized fluke materials was sonicated for 15 min under chilled condition and the mixture was centrifuged at 4°C for 35 min at 27000 g. The supernatant was stored at -20°C until used.

Protein estimation of antigens

The protein concentration of antigens were determined by the procedure described by Lowry *et al.*, (1951).

Immunization of rabbits

Laboratory bred, rabbits were used for the preparation of antisera. The rabbits were divided into 5 groups comprising 3 animals in each group. Each rabbit received 5 injections of antigen at interval of 7 days starting from 0.5 ml to a maximum of 2.5 ml. The antigens were used at a protein concentration of 2 mg/ml. The first injection was given with Freund's complete adjuvant and the second with Freund's incomplete adjuvant. (an equal volume of antigen and adjuvant was used). All injections were done subcutaneously. Ten days after the last injection, the rabbits were bled intracardially, serum separated under sterile conditions and was stored at -20°C until used. The serum samples of 3 normal healthy rabbits were also collected simultaneously and stored at -20°C to be used as negative control.

Enzyme linked immunosorbent assay

The ELISA was performed in 96 wells, microtiter plates (Dynatech Lab, USA) as

described by Hillyer *et al.*, (1996), Martinez *et al.*, (1996), Mbuh and Fagbemi (1996), Fagbemi *et al.*, (1997) and Ferre *et al.*, (1997) with some modifications. Briefly, the wells were coated with 100 µl of antigens (20 µg/ml) diluted in carbonate bicarbonate buffer (pH = 9.6) and incubated at 37°C For 1 hr and overnight at 4°C. Plates were washed 4 times with 0.01 M phosphate buffered saline (pH = 7.2) containing 0.05% Tween 20 (PBS/T). The wells were blocked with 100 µl of 1% bovine serum albumin (ICN Farmaceutical Inc.) solution in PBS/T and incubated for 1 hr at 37°C followed by 4 washes with PBS/T. A pool of three immune rabbit sera at 1 : 400 dilution in PBS/T was added to wells (100 µl/well) and incubated for 1 hr at 37°C followed by four washes with PBS/T. A pool of three normal rabbit sera at 1 : 400 dilution was used as negative control. 100 µl of goat anti rabbit immunoglobulines conjugated horseradish peroxidase (Dako A/S Denmark) at a dilution of 1 : 2000 in PBS/T was added to each well and incubated for 1 hr at 37°C followed by four washes with PBS/T. 100 µl of orthophenylen diamine (OPD: Merck, Germany) as substrate was added per well and incubated for 30 min at room temperature in the dark. The reaction was stopped by adding 50 µl of 12.5% H₂SO₄ (Merck, Germany) per well. The optical densities (OD) were read at 490 nm using a microtiter plate reader (Dynatech laboratories Inc, USA).

Results

ES and somatic antigens of *F. hepatica* and *F. gigantica* were tested with their homologous and heterologous antisera. The ELISA values (optical densities) of positive antisera and also negative control are presented in Tables 1 and 2.

Table 1: ELISA values (optical densities) for homologous and heterologous combination of *F. hepatica* antigens and antisera

Antiserum	ES antigen	Somatic antigen	Control sera
Antigen			
ES antigen	0.79	0.54	0.02
Somatic antigen	0.82	0.94	0.03

Table 2: ELISA values (optical densities) for homologous and heterologous combination of *F. gigantica* antigens and antisera

Antigen	Antisera	ES antigen	Somatic antigen	Control sera
ES antigen		0.72	0.46	0.01
Somatic antigen		0.85	0.95	0.02

Discussion

In the course of this study efforts were made to prepare and compare the ES and somatic antigens of *F. hepatica* and *F. gigantica* in ELISA test. According to the results, ES antigen of *F. hepatica* showed strong reaction with antisera raised against somatic antigen of this parasite (27 times greater than negative control). Somatic antigen of *F. hepatica* also showed strong reaction with antisera raised against ES antigen of this parasite (27.3 times greater than negative control).

ES and somatic antigens of *F. gigantica* also showed strong reaction with their heterologous antisera (46 and 42.5 times greater than negative controls, respectively).

The results of present study showed that ES and somatic antigens of both species of *Fasciola* have cross reaction with each other and both antigens can be used for the detection of antibodies in the serum of immunized rabbit. These mean that some antigenic materials are common between ES and somatic antigens.

Santiago *et al.*, (1986) reported that crude *F. hepatica* ES products, when tested by ELISA, had a high reactivity with the sera from rabbit in acute fascioliasis. ELISA test using crude adult somatic antigen is advantageous for diagnosis of naturally occurring *Fasciola* infection in cattle (Itagaki *et al.*, 1989). Cornelissen *et al.*, (1992) used ELISA with somatic and ES antigens of *F. hepatica* for serodiagnosis of fascioliasis in naturally or experimentally infected sheep and reported the specificity of 98% and 95% with somatic and ES antigens, respectively. Lehner and Sewell (1980) reported no difference when comparing a crude ES with somatic antigen in the serodiagnosis of fascioliasis in sheep by ELISA. Maleewong *et al.*, (1996) compared somatic and ES antigens of *F.*

gigantica for serodiagnosis of human fascioliasis and reported that the absorbance values in ELISA using somatic antigen are not significantly different from the values using ES antigen, therefore both somatic and ES antigens are effective for use in the diagnosis of human fascioliasis due to *F. gigantica*. Moazeni and Gaur (2003) found no difference between the antigenicity of ES and somatic antigens of *Fasciola spp* in gel diffusion test.

Although the results of our study showed a good agreement with the findings of Lehner and Sewell (1980), Cornelissen *et al.*, (1992), Maleewong *et al.*, (1996) and Moazeni and Gaur (2003), it is obvious that there is some differences between the antigenicity of ES and somatic antigens of *Fasciola spp* in ELISA test, because the results clearly indicated higher ELISA values in homologous combination of antigens and antisera in comparison with heterologous combination. So further investigations is needed for identification, detection and purification of antigenic materials in excretory-secretory products and somatic materials of the parasite.

References

- 1- Cornelissen, JB; Deleew, WA and Hejden, PJ (1992). Comparison of an indirect haemagglutination assay and an ELISA for diagnosis of *Fasciola hepatica* in experimentally and naturally infected sheep. *Vet. Quarterly*. 14(4): 152-156.
- 2- Dalton, JP (1999). *Fasciolosis*. 1st. Edn., CAB International. P: 545.
- 3- Fagbemi, BO; Aderibigbe, OA and Guobadia, EE (1997). The use of monoclonal antibody for immunodiagnosis of *Fasciola gigantica* infection in cattle. *Vet. Parasitol.*, 69: 231-240.
- 4- Ferre, I; Mora, LMO and Vazquez, FAR (1997). Serum and bile antibody responses (IgG and IgA) during subclinical *Fasciola hepatica* infection in sheep. *Vet. Parasitol.*,

- 68: 261-267.
- 5- Guobadia, EE and Fagbemi, BO (1997). The isolation of *Fasciola gigantica* specific antigens and their use in the serodiagnosis of fascioliasis in sheep by the detection of circulating antigen. *Vet. Parasitol.*, 68: 269-282.
 - 6- Hillyer, GV; Galanes, MSD; Buchon, P and Bjorland, J (1996). Herd evaluation by enzyme linked immunosorbent assay for the detection of *Fasciola hepatica* infection in sheep and cattle from Altipano of Bolivia. *Vet. Parasitol.*, 61: 211-220.
 - 7- Itagaki, T; Ohta, N; Hosaka, Y; Iso, H; Konishi, M; Chinone, S and Itagaki, H (1989). Diagnosis of *Fasciola spp* infections in cattle by enzyme linked immunosorbent assay. *Jpn. J. Vet. Sci.*, 51(4): 757-764.
 - 8- Lehner, RP and Sewell, MMH (1980). A study of the antigens produced by adult *Fasciola hepatica* maintained in vitro. *Parasite Immunol.*, 2: 99-102.
 - 9- Lowry, OH; Rosebrough, NJ; Farr, AL and Randall, RJ (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193: 265-275.
 - 10- Maleewong, W; Intapan, PM; Wongkham, C; Tomanakan, K; Daensekaew, W and Sukeepaisarnjaroen, W (1996). Comparison of adult somatic and excretory secretory antigens in enzyme linked immunosorbent assay for serodiagnosis of human infection with *Fasciola gigantica*. *South East Asian J. Trop. Med. Public Health.* 27(3): 566-569.
 - 11- Martinez, A; Martinez-Cruz, MS; Martinez, FJ and Gutierrez, PN (1996). Detection of antibodies to *Fasciola hepatica* excretory-secretory antigen in experimentally infected goats by enzyme linked immunosorbent assay. *Vet. Parasitol.*, 62: 247-252.
 - 12- Mbuh, JV and Fagbemi, BO (1996). Antibody and circulating antigen profile before and after chemotherapy in goat infected with *Fasciola gigantica*. *Vet. Parasitol.*, 66: 171-179.
 - 13- Moazeni, M and Gaur, SNS (2003). Comparison of excretory-secretory (ES) and somatic antigens of *Fasciola spp* in gel diffusion test. *IJVR.*, 4(2): 176-179.
 - 14- Santiago, N; Hillyer, GV; Garcia-Rosa, M and Morales, MH (1986). Identification of functional *Fasciola hepatica* antigens in experimental infections in rabbit. *Am. J. Trop. Med. Hyg.*, 35: 135-140.