

Determination of somatic and excretory-secretory antigens of *Fasciola hepatica* and *Fasciola gigantica* using SDS-PAGE

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(Received 18 Sept 2005; revised version 14 Mar 2007; accepted 7 Apr 2007)

Summary

Human fascioliasis due to unknown species and animal fascioliasis caused by both or one of *Fasciola* spp. are commonly seen in Iran. To compare electrophoretic patterns of somatic and excretory-secretory antigens of *F. hepatica* and *F. gigantica* by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), the adult flukes were collected from infected slaughtered bovine livers. E/S and somatic antigens were prepared by incubation and homogenizing of adult flukes, respectively. The antigens were electrophoresed using SDS-PAGE. Following SDS-PAGE, E/S proteins of *F. hepatica* and *F. gigantica* were characterized by the presence of 6 common major peptide bands with molecular weights of 15, 16, 20, 24, 33 and 42 kDa. Differences between *F. hepatica* and *F. gigantica* somatic proteins were noticed. *F. gigantica* had 11 major protein bands with molecular weights of 18, 22, 24, 33, 36, 42, 46, 57, 60, 62 and 68 kDa, whereas *F. hepatica* had proteins characterized by 8 distinct bands with molecular weights of 18, 22, 24, 33, 36, 42, 46 and 62 kDa.

Key words: *Fasciola hepatica*, *Fasciola gigantica*, Electrophores, Antigen

Introduction

In Iran, fascioliasis constitutes a major problem in breeding of 75 million sheep and goats and six million indigenous cattle (Eslami, 1998). The infection is not uncommon in equine (Eslami and Nadealian, 1987). It is also reported from wild animals including wild boar (Eslami and Farsad-Hamdi, 1992) and wild sheep (Eslami *et al.*, 1981). Because of high prevalence of human fascioliasis, particularly in northern parts of Iran (Rokni *et al.*, 2002), Iran is considered by WHO (1995), as an endemic focus of this infection. Mixed infection with both *Fasciola hepatica* and *F. gigantica* is common in ruminants in many parts of the country, especially in subtropical regions (e.g., Khuzestan province, along the Tiger river) (Sahba *et al.*, 1972). According to study of Ashrafi *et al.* (2004), *F. gigantica*

might be the parasite species most involved in human fascioliasis in Guilan. At present, parasitological methods including faecal egg count and autopsy constitute the major means for diagnosis of the infection in animals. In human, in addition to faecal examination, enzyme-linked immunosorbent assay (ELISA) is highly sensitive and specific for E/S antigen of *F. hepatica* (Espino *et al.*, 1987; Sampio Silva *et al.*, 1996; Khalili *et al.*, 2001; Rokni *et al.*, 2001).

Recently, detection of E/S or somatic antigens of *Fasciola* in urine, faeces and other fluids of the infected hosts by many researchers is considered as alternative methods for immunodiagnosis of the infection and for development of a vaccine (Spithill *et al.*, 1997; Rahman *et al.*, 1999).

The objective of the present investigation was to study different patterns of E/S and somatic antigens of *F. hepatica*

and *F. gigantica*.

Materials and Methods

Antigen preparation

F. hepatica and *F. gigantica* E/S antigens were prepared as described by Guobadia and Fagbemi (1995). Briefly, adult flukes were collected from infested bovine livers and washed 3-4 times at room temperature with 0.01 M phosphate buffered saline (PBS, pH = 7.2) (one worm per five ml) at 37°C for one hr. To remove particulate materials, the prepared antigens centrifuged at 10,000 g at 4°C for 20 min. Somatic antigens were prepared using the methods described by Hillyer and De Weil (1977) and Mansour *et al.* (1983). Adult worms were washed repeatedly with PBS, homogenized and centrifuged at 50,000 g for one hr at 4°C. The protein concentration of antigens was measured according to Bradford (1976). The prepared antigens were stored at -70°C until used.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Somatic and E/S antigens were separated by SDS-PAGE as described by Laemmli (1970), using a miniprotein II cell (Bio-Rad). The antigens were heated in a water bath at 100°C for 10 min. They then were added to each well of a 10% stacking gel and 12% separating gel. SDS-PAGE was carried out at 60, and 120 V, for 20, and 90 min, respectively. Gels were stained with 0.05% Coomassie brilliant blue and silver staining (Sigma Chem.). The molecular weights of proteins were determined by comparing their migration distance against that of a known molecular marker.

Results

We found a similar electrophoretic pattern for E/S proteins of *F. hepatica* and *F. gigantica* which included six common major protein bands with molecular weights of 15, 16, 20, 24, 33 and 42 kDa (Fig. 1). Nonetheless, SDS-PAGE analysis of somatic antigens of these two species revealed different patterns: *F. hepatica*, somatic proteins had eight major peptide

bands with molecular weights of 18, 22, 24, 33, 36, 42, 46 and 62 kDa, whereas *F. gigantica* somatic proteins had 11 bands (Fig. 2) with molecular weights of 18, 22, 24, 33, 36, 42, 46, 57, 60, 62 and 68 kDa.

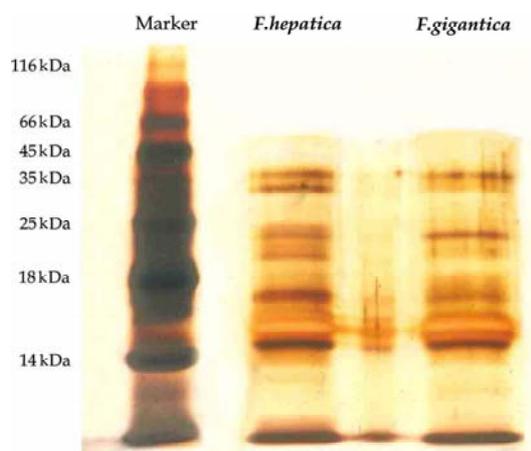


Fig. 1: SDS-PAGE pattern of *Fasciola* E/S antigens (silver staining)

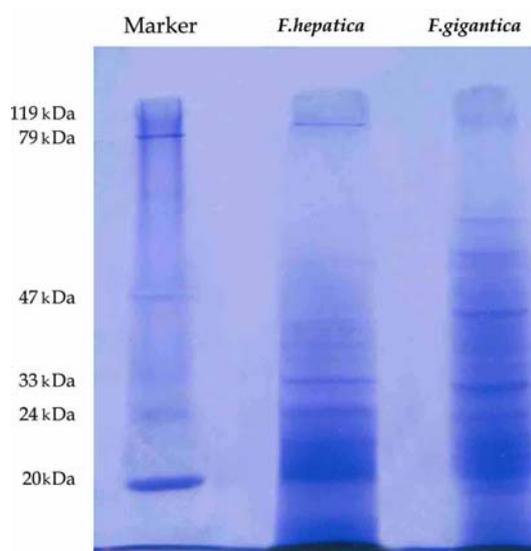


Fig. 2: SDS-PAGE pattern of *Fasciola* somatic antigens (Coomassie brilliant blue staining)

Discussion

Attempts have been made to use different techniques to separate various protein bands of the whole worm of *F. hepatica* and *F. gigantica* (Allam *et al.*, 2002); E/S and somatic antigens of *F. gigantica* (Upadhyay and Kumar, 2002; Gupta *et al.*, 2003); and E/S of *F. hepatica* (Sarimehmetoglu, 2002) for various purposes—mainly for immunodiagnosis of

the infection.

We showed the presence of six common E/S peptide bands with molecular weights of 15–42 kDa for both species, eight major protein bands with molecular weights of 18–62 kDa for somatic antigens of *F. hepatica*, and 11 bands with molecular weights of 18–68 kDa for *F. gigantica*. We also showed the presence of three common bands between E/S and somatic peptides of *F. hepatica* and *F. gigantica* (24, 33 and 42 kDa). Allam *et al.* (2002) showed the presence of eight and five protein bands in whole worm antigens with lower molecular weights ranging from 25.5–48 in *F. hepatica* and 27–57.6 kDa in *F. gigantica*. Our findings on SDS-PAGE analysis of *F. gigantica* were not in agreement with those reported by Upadhyay and Kumar (2002) on the number of E/S band (eight bands) and on their molecular weights (14–60 kDa and 13–62 kDa, respectively). We reported eleven somatic protein bands whereas they found seven. However, the range of molecular weights was almost similar. Upadhyay and Kumar (2002) reported four common bands (16, 30, 42 and 62 kDa) while we found three (24, 33 and 42 kDa) between E/S and somatic antigens of *F. gigantica*. The difference in the reported number of protein bands or molecular weights for *F. hepatica* and *F. gigantica* may be due to the existence of different isolates from different host species or geographic variations. Gupta *et al.* (2003) found six protein bands instead of 11 reported in the present study from *F. gigantica* collected from Indian cattle (*Bos taurus* and *B. indicus*). No matter the difference exists in the number of protein bands or molecular weights of somatic and E/S polypeptides of *Fasciola* spp., the findings of various researchers suggest existence of antigens with promising diagnostic value in human and animals. Silva *et al.* (1996) showed 11 polypeptides in E/S of adult *F. hepatica* of which five were detected in sera of 20 patients infested with this parasite. According to their results, 25- and 27-kDa bands were antigenic components and may be sensitive and specific for detection of human fascioliasis. Intapan *et al.* (1998) and Rokni *et al.* (2004) also showed that the 27-kDa antigen is potentially useful for the diagnosis of human

infection with *F. gigantica* and *F. hepatica*. In Gupta *et al.* (2003) report, somatic antigen of *F. gigantica* resolved by SDS-PAGE contained six proteins of 27.7–37.5 kDa. Rokni and Ghraivi (2002) in comparison of adult somatic and cysteine proteinase antigens of *F. gigantica* for serodiagnosis of human fascioliasis, showed a higher specificity of cysteine proteinase than somatic antigen. We found differences between E/S and somatic antigens of *F. hepatica* and *F. gigantica* which can help us in differential immunodiagnosis of these two species.

References

- Allam, AF; El-Agamy, ESI and Helmy, MH (2002). Molecular and immunological characterization of *Fasciola* species. Br. J. Biomed. Sci., 59: 191-195.
- Ashrafi, K; Massoud, J; Holakouei, K; Mahmoodi, M; Joafshani, MA; Valero, MA; Fuentes, MV; Khoubbane, M; Artigas, P; Bargues, MD and Mas-Coma, S (2004). Evidence suggesting that *Fasciola gigantica* might be the most prevalent causal agent of fascioliasis in northern Iran. Iranian J. Public Health. 33: 31-37.
- Bradford, MA (1976). A rapid and sensitive method for the quantification of microgram quantities of protein principles of protein dye binding. Anal. Biochem., 72: 248-254.
- Eslami, A (1998). *Veterinary helminthology*. Vol. III, Nematoda and Acanthocephala. 2nd. Edn., Tehran University Publications. P: 395.
- Eslami, A and Farsad-Hamdi, S (1992). Helminth parasites of wild boar, *Sus scrofa* in Iran. J. Wildlife Dis., 28: 318-319.
- Eslami, A and Nadealian, MGH (1987). A report on the trematode infection of equine in Iran. J. Fac. of Vet. Med., University of Tehran, Iran. 42: 33-38 (with English abstract).
- Eslami, A; Rahbari, S and Meydani, M (1981). Cestodes and trematodes of wild sheep, *Ovis ammon orientalis* and goitered gazelle, *Gazella subgutturosa* in Iran. Vet. Parasitol., 8: 99-101.
- Espino, AM; Dumenigo, BE; Fernandez, R and Finlay, CM (1987). Immunodiagnosis of human fascioliasis by enzyme-linked immunosorbent assay using E/S products. Am. J. Trop. Med. Hyg., 37: 605-608.
- Guobadia, EE and Fagbemi, BO (1995). Time-course analysis by EITB and ELISA of immune response of sheep infected with *F. gigantica* before and after chemotherapy.

- Vet. Parasitol., 58: 247-253.
- Gupta, SC; Ghosh, S; Joseph, D and Singh, BP (2003). Diagnosis of experimental *Fasciola gigantica* in cattle by affinity purified antigen. Indian J. Anim. Sci., 7: 963-966.
- Hillyer, GV and De Weil, NS (1977). Partial purification of *F. hepatica* antigen for the immunodiagnosis of fascioliasis in rats. J. Parasitol., 63: 430-433.
- Intapan, PM; Maleewong, W; Wongkham, C; Tomanakarn, K; Ieanviteevanich, K; Pipitgool, V and Sukolapong, V (1998). Excretory-secretory antigenic components of adult *Fasciola gigantica* recognized by infected human sera. Southeast Asian J. Trop. Med. Public Health. 29: 579-583.
- Khalili, G; Partovi, F and Ajdari, S (2001). Immunodiagnosis of human fascioliasis by ELISA using excretory-secretory products as antigen. Iranian J. Med. Sci., 26: 127-132.
- Laemmler, UK (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage, T4. Nature. 227: 680-685.
- Mansour, NS; Youssef, FG; Mikhail, EM and Boctor, FN (1983). Use of partially purified *F. gigantica* worm antigen in the serological diagnosis of human fascioliasis in Egypt. Am. J. Trop. Med. Hyg., 32: 550-554.
- Rahman, SA; Oreilly, KL and Malone, JB (1999). Biochemical characterization and localization of *F. hepatica* 26-28 Kda diagnosis coproantigen. Parasite Immunol., 21: 279-286.
- Rokni, MB; Baghernejad, A; Mohebbi, M and Kia, EB (2004). Enzyme-linked immunotransfer blot analysis of somatic and excretory-secretory antigens of *Fasciola hepatica* in diagnosis of human fascioliasis. Iranian J. Public Health. 33: 8-13.
- Rokni, MB and Gharavi, MJ (2002). Evaluation of a pre-selected epitope of *Fasciola hepatica* cathepsin-L1 for the diagnosis of human fascioliasis by IgG-ELISA test. Iranian J. Public Health. 31: 80-82.
- Rokni, MB; Massoud, J and Dalton, JP (2002). Comparison of adult somatic and cysteine proteinase antigens of *Fasciola gigantica* in enzyme linked immunosorbent assay for serodiagnosis of human fascioliasis. Iranian J. Public Health. 31: 47-50.
- Rokni, MB; Massoud, J; Pezeshki, M and Jalali, M (2001). Purification and evaluation of somatic, excretory-secretory and cysteine proteinase antigens of *Fasciola hepatica* using IgG-ELISA in diagnosing fascioliasis. Acta Med. Iranica. 39: 213-218.
- Sahba, GH; Arfaa, F; Farahmandian, I and Jalali, H (1972). Animal fascioliasis in Khuzestan, southwestern Iran. J. Parasitol., 4: 712-716.
- Sampio Silva, ML; Correia Da Costa, JM; Viana Da Costa, AMV; Pires, MA; Lopes, SA; Castro, AM and Monjour, L (1996). Antigenic components of E/S products of adult *F. hepatica* recognized in human infections. Am. J. Trop. Med. Hyg., 54: 146-148.
- Sarimehmetoglu, HO (2002). Application of western blotting for the immunodiagnosis of *F. hepatica* in cattle using excretory/secretory antigens. Turk Veterinerlik Ve Hayvancilik Dergisi. 26: 1061-1065.
- Silva, MLS; Costa, JMC; da'Costa, AMV; da'Pires, MA; Lopes, SA; Castro, AM and Monjour, L (1996). Antigenic components of excretory secretory products of adult *Fasciola hepatica* in human infections. Am. J. Trop. Med. Hyg., 54: 146-148.
- Spithill, TW; Pietrafita, D and Smooker, PM (1997). Immunological approaches for the control of fascioliasis. Int. J. Parasitol., 27: 1221-1235.
- Upadhyay, AK and Kumar, M (2002). SDS-PAGE analysis of *Fasciola gigantica* antigen. J. Immunol. Immunopathol., 4: 91-92.
- World Health Organization (1995). Control of foodborne trematode infections. WHO Technical report series. 849: 1-157.