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

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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.

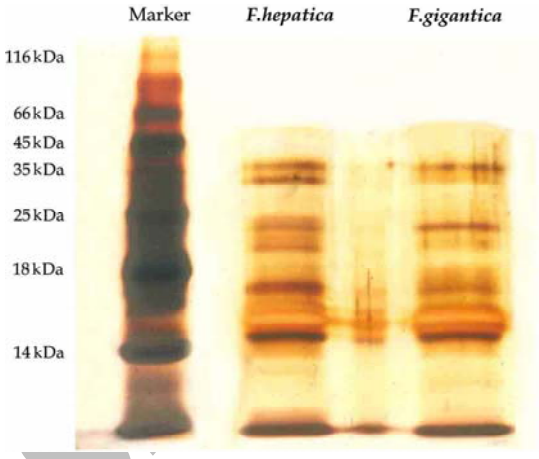

Fig. 1: SDS-PAGE pattern of *Fasciola* E/S antigens (silver staining)

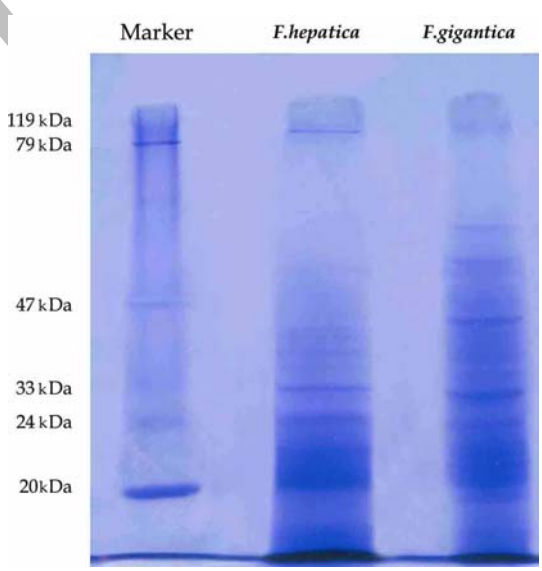

Fig. 2: SDS-PAGE pattern of *Fasciola* somatic antigens (Coomassie brilliant blue staining)

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 Ghravi (2002) in comparison of adult somatic and cysteine proteinase antigens of *F. gigantica* for sero-diagnosis 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.

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