Immunodiagnosis as an aid for early detection of *Fasciola gigantica* by glutathione S-transferase (GST)

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Abstract In the present study, effort to isolate purified GST from *Fasciola gigantica* homogenate and its early detection for confirmation of infection has been successful. In this study, immunoprotection studies with GST and antibody titer post-infection has revealed GST to be an immunogenic protein with high sensitivity to detect anti-*Fasciola* antibody as early as two weeks post-infection in large ruminants.

Keywords GST · *Fasciola gigantica* ·
Immunoprotection · Immunogenic ·
Anti-*Fasciola* antibody

Introduction

Tropical fascioliasis in domestic ruminants is mainly caused by a digenetic trematode *Fasciola gigantica* and is a disease of immense economic significance. It cause losses not only through condemnation of livers at abattoirs but also through high mortality rates in infected herds, persistently depressed

growth and productivity losses including impaired reproduction in the infected host animal (Sarkar et al. 2003; Yokananth et al. 2005). Although coprological examination for detecting eggs of parasites is most reliable diagnostic method, but this technique is only useful three months post-infection (Soulsby 1982). Pathology of the disease become evident two weeks after the onset of infection. So, alternative methods such as serodiagnosis are needed to be investigated for early confirmed diagnosis of the infection.

Several detailed studies have been performed on immunodiagnosis of *F. hepatica* in both man and animal (Richard 1995; Dumenigo et al. 1999). Despite of satisfactory sensitivity, immunodiagnostic assays for helminth are hampered due to lack of specificity which is attributed to possession of common antigens by different helminthes leading to cross reaction among *F. gigantica*, *Dicrocoelium hospes* and *Schistosoma bovis* (Fagbemi and Obarisiagbon 1991). The phenomenon poses a problem in the serodiagnosis of fascioliasis and attempts have been made for isolation and identification of specific immunodominant antigen of *F. gigantica* for early serodiagnosis.

The available fasciolicidal drugs do not possess adequate efficacy against immature *F. gigantica* and causes damage to host organs thereby impairing their normal physiological mechanism. So, development of alternative approach based on immunization of host seems obligatory. Till date, no successful vaccine is available against the disease.

Keeping this in view for exploitation of defined antigens of the fluke, glutathione S-transferase (GST) has been selected in the present study for diagnosis of the infection and immunoprotection studies against *F. gigantica* within experimental evaluation of immunogenicity of the enzyme GST and standardization of optimum dose and immunization protocol.

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Materials and methods

Adult *F. gigantica* flukes were recovered from buffalo livers after slaughter followed by necropsy. The flukes were thoroughly washed in PBS for several times and thereafter processed for preparation and purification of somatic antigen (SAg) and for isolation of GST using the standard procedures as described by Oldham (1983) and Wijfell et al. (1992) respectively with slight modifications.

GST in elute fraction was confirmed by detection of its activity following spectrophotometric conjugation of glutathione and 1-chloro 2,4 dinitrobenzene at 37°C according to the method of Habig and Jacoby (1981) with some medication.

Characterization of referral antigen was done using technique of SDS-PAGE, western blotting and immunoelectrophoresis, respectively. Evaluation of antigens were done by standard serological techniques like double immunodiffusion test and counter immunoelectrophoresis based on antigen–antibody interactions.

Immunodiagnostic technique for early detection of *F. gigantica* infection by GST was done by ELISA as described by Engvall and Perlman (1971) with slight modification.

Immunoprotection studies of GST

Immunoprotection studies were carried out to standardize the dose of immunogen for conferring optimum level of protection. Twenty-five rabbits of either sex were randomly divided to five groups of five rabbits in each group. Each animal in Group 1, 2 and 3 were subsequently immunized with 50, 100 and 200 µg of GST, respectively with saponin as adjuvant. Animals in Group 4 served as challenge control and Group V as non-infected control. Except for Group V, each animal in Groups 1, 2, 3 and 4 were challenged orally with fifty viable *F. gigantica* metacercariae on 4th week of start of experiment. All animals were sacrificed on 12th week of start of experiment for collecting necropsy data.

Titer of anti *F. gigantica* antibody titer

The protection conferred by GST in immunized animals was monitored through measurement of titer of anti-*Fasciola* antibody using ELISA and immunoblotting. Sera samples collected from animals belonging to respective groups were pooled at weekly interval and antibody titer was measured using GST as antigen, and hyperimmune sera against GST as standard positive control. Sera from non-infected non-immunized animals served as negative control.

Results

Fluke recovery in three groups was taken into consideration for assessing protection conferred by GST (Table 1). Animals belonging to Group 3 revealed the lowest mean number of flukes and corresponding to the highest protection when compared with rabbits belonging to Groups 1 and 2 respectively, whereas animals belonging to Group 4 showed higher fluke recovery. There was no fluke recovery from Group 5 (healthy control). Though mean fluke weight in different groups did not differ significantly, yet it was lowest in Group 3 exhibiting highest level of protection followed by Groups 2 and 1, respectively. An analogous result was obtained when comparison of mean fluke size was observed. The data generated on mean fluke weight and size was strongly suggestive of stunted growth of the parasite in animals due to resistance acquired from GST sensitization from Groups 1–3 exhibited an increasing trend.

Post challenge titers

Among immunized groups, Group 3 exhibited highest titer of 400 Anti-GST antibody titer profile during post-challenge period followed by Groups 2 and 1, respectively with titer of 200 and 100, respectively on 6th week post infection. On 8th week post infection, elevated immune response was exhibited by challenged and experimental; groups with highest titer of 800 in Group 3 and titers of 200 and 400, respectively in Groups 1 and 2, respectively.

Discussion

GST in somatic extract of *F. gigantica* and proteinases in fluke regurgitates have been claimed as potential candidate antigens by Dalton et al. (1996). In the present study, ELISA showed 96.83% sensitivity in naturally infected buffaloes which is similar to the finding of Swarup et al. (1987) and Hillyer et al. (1992). Present finding on antibody titer response was in confirmation with findings of Hillyer et al. (1992).

Table 1 Experimental parameters

Group	No. of animal (n)	Immunization dose (µg)	Mean fluke weight (mg)	Mean fluke size (mm) ^a
1	5	50	8.00 ± 0.03	10.16 ± 0.03
2	5	100	7.30 ± 0.09	9.33 ± 0.10
3	5	200	7.07 ± 0.08	9.00 ± 0.09
4	5	–	8.08 ± 0.09	10.52 ± 0.04
5	5	–	–	–

^a Mean length/mean breadth; – not detected

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