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RESEARCH PAPER

Study on immunoprotection against *Fasciola gigantica* by single immunization with an isoenzyme, glutathione-S transferase (GST) in rabbit

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ABSTRACT

The present study revealed that a single immunization dose of 350µgGST in combination with Freund's adjuvant conferred higher degree of acquired immunity (peak titre 800) than those receiving the same immunization does of 350?g along with saponin(titre 400) in rabbit .Other findings were lesser fluke weight and lower mean score of lesion of liver found in the animals receiving 350µgGST in combination with Freund's adjuvant. As far as the clinical findings are concerned, after challenge infection, this group of animals behaved almost like healthy animals. Higher protection(40.9%) in animals receiving GST with Freund's adjuvant was synchronous with antibody titre (800) which was distinguishing during pre challenge period from animals receiving GST with saponin (titre 400).The protection was lower (33.36 %)in the animals receiving the immunogen along with saponin. The protection was also supported by the fluke size, fluke weight etc.

Key words : *Fasciola gigantica*, GST, immunization, ELISA, Protection

The digenetic trematode parasite of liver, specially *Fasciola hepatica* and *Fasciola gigantica* are important pathogens of ruminants as these parasites are known since decades for causing incessant, unnoticed but significant production losses to livestock owners (Morrison *et al.,* 1996). The chemical control of the disease had its own inherent drawbacks. Incomplete elimination of the infection results in subclinical fasciolosis – a continuous source of contamination of grazing lands (Ollerenshaw,1971).Therapeutic and toxic doses of these anthelmintics are very closed (Gibson, 1964), above all, employment of skilled labour for careful administration adds to the higher treatment cost, particularly in endemic areas where herd treatment seems unavoidable. Alternative control approach against fasciolosis has been pinpointed towards vaccination. No successful vaccine is available till date. Intensive efforts have been directed towards the search for novel immunogens which can confer significant level of protection. The immunogens which have been recognized for conferring protection are F ABP, GST and Cathepsin L etc. However, selection of adjuvant is the prime criteria while designing any vaccine regimen. To develop a vaccination protocol of multiple immunization ,observation of antibody response by single immunization is obligatory. In the present study comparative evaluation was done between Freund's adjuvant and saponin adjuvant for vaccination by single immunization against fasciolosis in rabbit model.

MATERIALS AND METHODS

The parasite:

Adult *Fasciola gigantica* flukes were recovered from the biliary system / gall bladder of bubalian liver, at necropsy of animals slaughtered at buffalo slaughter house, Bareilly(U.P).The flukes were thoroughly washed several times with chilled PBS (pH 7.2) till these were free from host tissues adhering therewith and / or host secretions (bile etc.). The flukes were thereafter processed for isolation of GST.

F. gigantica homogenate:

Scroupulously PBS washed 100 flukes were subjected to homogenization in Trition-X-100 containing buffer (100mM EDTA, 2mM PMSF, 0.15 M NaCl, 50mM Tris, pH 7.8 containing 0.5% Triton-X-100). The homogenate was centrifuged at 4^0C in refrigerated centrifuge (Hitachi, Japan) at 12000g for an hour. Supernatant was stored at -20^0C in deep fridge and was designated *F. gigantica* homogenate.

Isoaltion of GST by affinity chromatography:

Isolation of GST from adult fluke homogenate (s) was effected following technique described by Wijffels *et al.* (1992). 5 mg of protein in fluke homogenate was charged and allowed to pass through the GST affinity column (Sigma, G4510) containing the glutathione-agarose affinity matrix and washed by Triton X-100 containing buffer, till the fractions were free from protein. The