**FASCIOLA GIGANTICA: IMMUNIZATION OF RABBITS WITH PROTEINS ISOLATED FROM COPROANTIGEN**

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**Abstract**

Two fractions were isolated from coproantigen by ion-exchange chromatography in which DEAE cellulose was utilized. Both fractions and crude antigen were characterized by SDS-polyacrylamide gel electrophoresis which revealed 13 bands of molecular weight ranged from 205-31 in crude coproantigen. While fraction I resolved into six bands of molecular weight 198, 178, 148, 111, 101 & 45. Fraction II showed seven bands of 191 KDa, 178KDa, 166KDa, 118KDa, 98.5 KDa, 72KDa & 32KDa. Fraction II was higher immunoreactivity than fraction by ELISA. Three immunoreactive bands of 191 KDa, 118KDa & 98.5KDa were identified in fraction II using immunoblot assay. Five bands of 178 KDa, 148KDa, 111KDa, 101 KDa & 45KDa were detected in fraction I. Immunization of rabbits twice with fraction II in Freund’s adjuvant with two weeks interval followed by challenge with *F. gigantica* metacercariae resulted in 66.6% protection from infection. The protection was assessed by detection of hepatic damage, worm recoveries and antibody response. High level of IgG response in vaccinated rabbits than control infected ones occurred and being responsible for the recorded protection.

**Introduction**

*Fasciola gigantica* is an economically important parasite of domestic animals and cause economic losses in the animal
husbandry industry (Boray, 1985 and Sexton et al., 1991). The presence of eggs in stool is the standard method for fascioliasis diagnosis. However, it lacks sensitivity since parasite eggs do not appear during acute fascioliasis and are intermittently shed during a chronic disease. As antibody titers persist after curing, serological tests are of limited diagnostic value (Garcia-Rodriguez et al., 1985; Espino et al., 1992). In addition to lack of specificity due to cross-reaction between Fasciola and other helminthes (Thaumaturgo et al., 2001, 2002; Abdel-Rahman et al., 2003; Ramos et al., 2003). Detection of F. gigantica antigens in the feces of infected hosts offers several potential advantages over serologic assays; there is less possibility of immunocomplex formation; and animal handling is not required to obtain samples because fresh fecal specimens can be collected from pasture (Abdel-Rahman et al., 1998). Reports on the diagnosis of fascioliasis by detection of coproantigen in feces of different hosts included use of polyclonal sera and monoclonal antibodies developed against a 26-28KDa coproantigen (El-Bahy et al. 1992; Espino and Finlay, 1994; Espino et al., 1997, 1998, 2000; Abdel-Rahman et al., 1998; Moustafa et al., 1998; Dumenigo and Mezo, 1999; Paz-silva et al., 2002). The usefulness of a 26-28Kda coproantigen of F. hepatica in the diagnosis of fascioliasis encouraged scientists to further investigate the characters and localization of this antigen. Abdel-Rahman et al. (1999) proved that this antigen is monomeric, highly glycosylated glycoprotein composed of 8 KDa protein core which still contained the epitope recognized by the MoAb. The antigen did not posses protease activity but could be cleaved by trypsin without altering the reactive epitope. Indirect immunofluoresence of tissue sections of adult fluke indicated that 26-28KDa coproantigen was present in gut cells and tegument (Abdel-Rahman et al., 1999). Despite of the previous studies proved and confirmed the potency of coproantigen in diagnosis in addition to biochemical characterization and localization of copro-purified antigen no available literatures, up to our knowledge, probed the protective value of coproantigen against fascioliasis. Although several successful vaccination trials adopted many other candidates as crude antigen or fractions isolated either from the fluke itself or its products were reported as fatty acid binding protein, glutathione-S-
transferase, cathepsin L-proteinases and liver fluke haemoglobin (Morrison et al., 1996; Muro et al., 1997; Spithill and Dalton; 1998 Mulcahy et al., 1998, 1999; Cervi et al., 2004).

The current research aims to purify *F. gigantica* coproantigen, characterize the pure fractions and evaluate the most immunogenic fraction as an immunoprophylactic candidate against fascioliasis in rabbits.

**Materials and Methods**

Preparation of coproantigen: Fecal supernatant of infected *F. gigantica* sheep was prepared (Allan et al., 1990). In brief, fecal samples were vigorously shaking in an equal volume of 0.15M phosphate buffer saline containing 0.3% tween 20 until a slurry formed followed by centrifugation at 2000g at room temperature for 30 min. The supernatant was examined for protein content by the method of Lowery (1951) and stored at -20°C until use.

Preparation of hyperimmune serum: About 100 mg of *F. gigantica* excretory-secretory antigen (FgESA) was mixed with an equal volume of Freund’s complete adjuvant and injected subcutaneously into each rabbits of 3 rabbits used according to Guobadia and Fagbemi (1997). Booster doses were administered at week 2,3 and 4 after the initial dose according to Fagbemi et al. (1995). Sera were collected 4 days post last injection from ear vein.

Ion-exchange chromatography: Purification of corproantigen was performed by ion-exchange chromatography in which DEAE cellulose was adopted. The elution pattern was recorded by UV transmission at 280nm (Gomez-Munoz et al., 1996). Eluted fractions were lyophilized and stored until use.

Enzyme-linked immunosorbent assay (ELISA): Coproantigen pure fractions were tested using ELISA based on hyperimmune rabbit serum rasied against FgESA as described by Santiago et al. (1986) and the cut off point of optical density values was determined (Allan et al., 1992).

Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE): Crude and pure fractions of coproantigen were separately mixed with reducing sample buffer and electrophoresed on SDS-polyacrylamide slab gels according to
procedures of Laemmli (1970). After separation, gels were fixed in 50% methanol and stained with silver stain according to Wray et al. (1981). Bio-Rad High and low molecular weight standards were electrophoresed on the same gels to calculate the relative molecular weights of examined antigens.

Immunoblotting: Protein bands of crude as well as pure fractions of coproantigen were electrophoretically transferred from SDS-polyacrylamide gel to nitrocellulose sheets according to Towbin et al. (1979) in a blotting system. Nitrocellulose paper was incubated with anti-FgESA antisera raised in rabbits. After washing, the paper was incubated with alkaline phosphatase-conjugated anti-rabbit IgG, then exposed to substrate solution for 30 min. Nitrocellulose was washed with distilled water to stop the reaction.

Vaccination procedures: Six rabbits (1.5 kg each) were equally divided into two groups: control infected group and vaccinated infected group. Fraction I1 of coproantigen was reconstituted in physiological saline and the vaccine formulated by mixing 0.5 ml (40 µg) of the reconstituted antigen with an equal volume of Freund’s complete adjuvant. This dose was injected subcutaneously in each rabbit of the vaccinated group. Two weeks later, booster dose of the same amount in Freund’s incomplete adjuvant was administered (Muro et al., 1997). All rabbits were necropsied at 10 weeks after infection to determine worm burdens.

Parasite challenge: *F. gigantica* metacercariae were purchased from Theoder Bilharz Research Institute. Viability of the metacercariae was checked by microscopy on arrival. Each rabbit was infected orally with 30 metacercariae 14 days post last vaccination (Muro et al., 1997).

Assessment of protection by post mortem examination of all animals: The examination included evaluation of hepatic damage and fluke recoveries (Muro et al., 1997). Antibody detection in sera of all rabbits by ELISA as given by Santiago et al. (1986).

**Results**

Purification of *F. gigantica* coproantigen (Fig. 1) by ion exchange chromatography using DEAE cellulose resulted in two
fractions (FI & FII). The antigenic activities of fractions were evaluated by ELISA utilizing hyperimmune sera against excretory-secretory antigens of *F. gigantica* which revealed potency of FII than FI. The crude extract (Fig. 3) resolved into 13 bands of molecular weight ranged from 205 KDa to 31KDa while fraction I showed 6 bands of molecular weight 198, 178, 148, 111, 101 & 45. Fraction II revealed 7 bands of 191 KDa, 178KDa, 166KDa, 118KDa, 98.5KDa, 72KD & 32KDa. Protein bands responsible for the immunogenicity in crude coproantigen and pure fractions were identified by immunoblot assay (Fig.4). Eight immunogenic bands were observed in crude extract, five of them were reactive in FI of molecular weight 178, 148, 111, 101 & 45KDa while the immunogenicity of FII reside in only 3 bands of 191KDa, 118KDa & 98.5KDa. Post mortem resulted in alterations in the liver of non-immunized infected rabbits that include change in the colour to grayish white increase in the size and increase in the thickness of bile ducts. Livers of vaccinated animals looked normal and healthy. Concerning worm recoveries from non-vaccinated infected rabbits, it was ranged from 4-5 worms per animal while no flukes were detected in vaccinated rabbits recording 66.6% protection. In one case of vaccinated infected animals, two worms were recovered with reduction in worm burdens reached to 56%. High antibody level was observed in immunized rabbits 2 weeks post first immunization and increased to reach its maximum value 8 weeks post infection (Fig.5). At the infection time the antibody titer in vaccinated animals was higher than non vaccinated ones and remained higher to end of experiment.

**Discussion**

Reports of purified antigens for the immuno-prophylaxis of fascioliasis have led to an optimism in respect of the prospects for the development of effective vaccines against fascioliasis (Spithill and Dalton, 1998). The present study probed the protective effect of coproantigen that was not evaluated against fascioliasis despite of its potency in diagnosis. In the present study, a purification of coproantigen was performed utilizing ion-
Fig. 1: Purification of *Fasciola gigantica* coproantigen by DEAE cellulose ion-exchange chromatography.

Fig. 2: Evaluation of antigenic activities of *Fasciola* coproantigen fractions, resulted from DEAE-cellulose column, by ELISA.
Fig. 3: SDS-poly-acrylamide gel electrophoresis of *F. gigantica* crude coproantigen (lane B), FI (Lane C) & FII (lane D). Molecular weight standards (lane A) indicated in KDa.

Fig. 4: Western blot analysis of immunoreactive proteins in crude coproantigen (lane A) in FI (lane B) & in FII (lane C) probed by antisera-raised against excretory-secretory antigen of *F. gigantica*. (No. corresponds to arrangement of bands in electrophoretic profile).
exchange chromatography resulted in two fractions. The most potent of them by ELISA was a fraction of 3 immunoreactive bands of molecular weight 191, 118 & 98.5. The purification of coproantigen was previously probed by chromatography (Abdel-Rahman et al., 1999) and the most potent component in the diagnosis was that of 26-28KDa (Paz-silva et al., 2002). The difference between our results and the previous one may be attributed to the difference of Fasciola species in both studies or in the antibodies utilized in evaluating the potency of each antigen. The results presented in this paper show that vaccination of rabbit with FII of coproantigen resulted in protection level reached to 66.6% and reduction in worm burden of about 56% in only one vaccinated rabbits. Significant reductions in fluke burdens (49%-69%) were observed in cattle vaccinated with glutathione S-transferase in Quil A (Morrison et al., 1996). In rabbits, Muro et al. (1997) recorded a reduction of 40% in F. hepatica worm burden after immunization with native Fasciola 12KDa fatty acid binding protein. Immunization of cattle with a combination of cathepsin L2 and haemoglobin induced 70% protection against challenge infection with F. hepatica (Mulcahy et al., 1998). Partial protection against challenge infection with F. hepatica was achieved in calves vaccinated with either cathepsin L2 plus fluke derived haemoglobin or cathepsin L1 plus cathepsin L2 with the greatest level of protection (55% reduction
in worm burdens) in the group vaccinated with cathepsin L1 plus cathepsin L2 (Mulcahy et al., 1999). All the previous studies tried to develop vaccines against *F. hepatica* infection while in the current study the vaccine was evaluated against infection with *F. gigantica*. On the other hand, a close protection level to that induced in previous trials was recorded here although the vaccine candidate was developed from coproantigen which was not probed before in vaccination.

In the current study, control animals produced low level of IgG in response to challenge. While animals vaccinated with coproantigen fraction produced high levels of IgG. This observation explained the reason behind the protection recorded or the mode of action of vaccine candidate and was expected on the basis of previous work (Clery et al., 1996; Mulcahy et al., 1998, 1999). Work remains to be done on elucidating mechanisms of protection evoked following vaccination. Although Mulcahy et al. (1998) clarified that the type of immune response produced by vaccination with cathepsin L2 and fluke haemoglobin differs qualitatively and quantitatively from the following infection.

It is concluded that vaccine against fascioliasis can be developed using purified antigens. Future studies with sub-unit epitopes will offer a supplement to the present trial.

**References**


