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Evaluation of Some *Fasciola gigantica* Antigens as Vaccines Against Fasciolosis in Goats

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Abstract: Vaccine trials were conducted in goats evaluating the efficacy of three antigens of adult *F. gigantica*, as vaccines against fasciolosis. The antigens tested were crude worm, excretory-secretory material and glutathione S-transferase. Each antigen was emulsified in Freund's adjuvant. ELISA was used for monitoring of antibody levels in all immunized goats, before and after infection with 125 *F. gigantica* metacercariae. All goats were slaughtered 14 weeks after challenge. The results indicated that the antibody titer was not elevated after challenge in all immunized goats. The highest reduction in eggs per gram faeces (EPG) and fluke burden was observed in goats immunized with purified GST antigen (90.7 and 66.1%, respectively). Besides, this purified antigen induced the highest effect in minimizing fluke size among the tested antigens. This protection level in goats supported the notion of variable effect of vaccination with trematode GST in various ruminant species. This was the first report of protective vaccination of goats against *F. gigantica* with purified GST antigen.

Key words: *Fasciola gigantica* · vaccine · GST · goats

INTRODUCTION

Fasciolosis in domestic ruminants, due to infection with the parasites *Fasciola gigantica* (tropical liver fluke) and *Fasciola hepatica* (temperate liver fluke), causes significant economic loss estimated at US$2000M per annum to rural agricultural communities and the commercial sector worldwide [1]. Fasciolosis is increasingly recognized as causing significant human disease, with 2.4 million people infected [2, 3].

In Egypt, *Fasciola gigantica* remains one of the single most important helminth parasites of livestock. It is considered to be among the main causes of reduction livestock productivity. According to the report of Central Organization of Mobilization and Computation, Cairo, (2000), direct and indirect losses ascribed to fasciolosis were estimated at 484.5 million LE per year. Ruminants are considered to be the main source and reservoir of infection to man. In the last decade, fasciolosis had imposed itself as an important zoonotic disease in Egypt posing a clinical and epidemiological health problem. A figure of 830,000 individuals was the estimated to be the number of fasciolosis cases in Egypt [4].

Its control is, almost exclusively, carried out by the strategic application of anthelmintics, Moreover, although there are a great number of efficacious pharmaceuticals against adult stages, only triclabendazole is efficient against both adults in the biliary tract and pre-adults in the hepatic parenchyma [5]. However, this control has problems related to parasite resistance [6]. In the search for an alternative to chemotherapy the development of an efficient and commercially viable vaccine is considered a priority. There have been many attempts to vaccinate animals with various liver fluke extracts, such as crude somatic antigens and excretory/secretory antigens, but also with irradiated attenuated vaccines and various defined antigens [7]. The mean level of reduction in worm burdens observed in cattle immunized with different antigens was in the range of 43-72%, suggesting that the control of fasciolosis by immunological intervention may be an achievable goal [7]. Recently, the search for the development of an effective vaccine against *Fasciola* has focused on essential enzymes. One of the most promising candidates has been glutathione S-transferase (GST) [8]. The GST belongs to a family of enzymes that are involved in the cellular detoxification process. It primarily functions

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by catalyzing the conjugation of the glutathione to a wide variety of electrophilic toxic substrates [9]. GSTs of helminths act as immune defense proteins and have significant activity with lipid peroxidation-derived carbonyls. They also have the potential to neutralize exogenously derived toxins such as anthelminthics [10]. Moreover, GSTs have been highly conserved throughout evolution and are particularly abundant in parasitic helminthes [10]. These molecules have been used as likely vaccine candidates against *Schistosoma* spp. [11]. Whereas the homologous GST fraction purified from *F. hepatica* proved to be ineffective in a vaccination study in rats [12]. Previously, a trial in sheep indicated that a mean 57% reduction in worm burdens was possible [13]. However, a preliminary trial in cattle, using native GST from adult fluke emulsified in Freund's complete adjuvant, was not successful in inducing protection against fluke challenge. The lack of protection in this experiment was attributed to the production of considerably lower titres of anti-GST antibodies than in the sheep study [8]. Indeed, a significant reduction in fluke burdens (49-69%) was observed in cattle vaccinated with GST in Quill A/Squalene Montanide [8].

The present work had been performed to study the protective value of *F. gigantica* crude, excretory/secretory and purified GST antigens against fasciolosis in goats as a highly susceptible host of *F. gigantica*.

MATERIALS AND METHODS

Animals: Twelve goats, 5-6 months old, were used in this study. These animals were from a farm free of fasciolosis. Nonetheless, faecal samples were examined to confirm the absence of infection of *F. gigantica* as well as other helminthes.

Preparation of antigens

***F. gigantica* crude worm antigen:** *F. gigantica* crude worm antigen was prepared according to [14] from the oral cone of fresh extracted adult *F. gigantica* worms collected from fresh condemned buffaloes' livers.

***F. gigantica* excretory-secretory antigen (ES antigen):** *F. gigantica* ES antigen was prepared from living flukes collected from fresh condemned buffaloes' livers at Cairo abattoir according to [15].

***F. gigantica* GST antigen (FgGST):** Adult *F.gigantica* worms were collected from infected buffaloes, slaughtered at Cairo abattoirs and were used for GST preparation according to [16]. Adult worms were homogenized on ice in triton-EDTA buffer, pH 8. The suspension was centrifuged at 15000 xg for 30 min and the supernatant was used for the purification of GST by affinity chromatography on glutathione sepharose 4B (Pharmacia Biotech). The worm extract was filtered through the column and the bound FgGST was eluted with 5mM glutathione in 150mM tris buffer; pH 9.6. FgGST was neutralized to pH 7.0 by adding drop wise 2Mtris, pH 6.0 (5 drops per 1.5 ml fraction) and dialyzed against PBS and protein concentration was determined [17]. For molecular determination, the FgGST was analyzed under reducing conditions on 12% SDS-PAGE with lanes loaded with 100 µg protein and subsequently stained with coomassie blue [18].

Immunization protocol: The goats were divided into four groups, each with three animals. Groups 1, 2 and 3 were immunized twice at 4 weeks intervals with crude, ES and GST antigens, respectively, each with 100 µg of antigen in Freund´s adjuvant (first complete and the same amount of antigen in incomplete 4 weeks later). While, Group 4 was kept as non-immunized, infected control.

Challenge: One hundred and twenty five *F.gigantica* metacercariae were orally inoculated to each goat two weeks post second immunization. The metacercariae were prepared in *Lymnaea cailliaudi*, intermediate host of *F.gigantica* previously infected with *F.gigantica* miracidiae at Parasitology and Animal Diseases Department, National Research Center Cairo, Egypt. Serum samples from immunized goats were collected weekly during the immunization schedule for determination of antibody responses. Meanwhile, faecal samples were collected weekly for egg counts after elapse of 60 days post-infection. Faecal egg counts were determined using the Fluke finder technique and expressed as number of eggs per gram faeces (EPG) as described by [19]. All goats were humanely slaughtered for fluke burden, 14 weeks after parasite challenge. Fluke burden was determined for each animal according to [20] and the numbers recovered from each group were compared and statistical differences analyzed by student's t test.

Antibody detection assays by ELISA: The response of antibodies against each of the tested antigens (crude, ES and GST) was analyzed by ELISA in accordance with the procedure described by [21].

RESULTS

Purification of *F. gigantica* GST: *F. gigantica* GST was purified by affinity chromatography and the eluent was migrated in SDS-PAGE as a single band at a molecular weight of 23.2-24.2 KDa (Fig. 1).

Monitoring of antibody levels by ELISA: Antibody levels in all immunized goats; G1, G2 and G3, before and after infection were measured by ELISA using crude, ES and GST antigens, respectively. As could be observed in Fig. 2, antibodies were detected as early as 2 weeks of immunization where, mean ± standard deviation (SD) OD values were 0.240±0.003 in G1, 0.309±0.008 in G2 and 0.314±0.004 in G3. They were reaching a maximum level at 4th week post-infection (wpi) in G1 (0.490±.0.006), 5th wpi in G2 (0.652±0.013) and 6th wpi in G3 (0.358±0.002). After challenge, there was a no significant increase of antibody titers in all immunized goats from day of infection till the end of experiment (14 wpi). But, the sera from animals vaccinated with ES antigen gave higher absorbance values than crude and GST antigens.

Faecal egg counts (EPG): *F. gigantica* eggs began to appear in faeces of goats at 12th wpi. The faeces were examined from immunized and control animals for 3 successive weeks (12th, 13th and 14th week) after detection of eggs for counting EPG. The egg counts rose gradually from first appearance until the end of the experiment at 14th wpi. Fluke EPG (mean±SD) for each of experimental groups were shown in Table 1. It was observed that the means of EPG at the interval of 12th-14th wpi for goats immunized with *F. gigantica* crude, ES and GST antigens were 100.5±4.0, 71.7±0.81 and 14.2±2.3 eggs, respectively. However, it reached 152.9±2.9 eggs in goats of control group. From these data, the EPG parameter expressed important reductions in the immunized animals on comparison with the control group. The highest reduction was recorded with GST (90.7%), followed by ES and crude antigens (53.1 and 34.3%, respectively).

Fluke burdens: Fluke burdens were assessed in all animals 14 weeks post challenge (Table 1). All immunized groups (G1, G2 and G3) exhibited fluke burdens which were lower than that of the control group. The highest level of protection was achieved with GST (G3, 66.1%), this protection level was significantly higher ($p<0.01$) than that observed in the other groups. It was noted that flukes recovered from the immunized animals tended to be shorter and narrower than those recovered from the control animals (Fig. 3). The GST antigen induced the highest effect in minimizing of fluke size.

DISCUSSION

The previous recent works have led to optimism in respect of the prospects for the development of effective

Table1: Eggs per gram of faeces and fluke burdens in immunized and non-immunized experimentally infected goats

			EPG						
Group	Tested antigen	Goat	12th wpi	13th wpi	14th wpi	Mean/gm	Reduction (%)	[a]Fluke burdens	Reduction (%)
G1	Crude	No 1	50	97	143	96.6	34.3	19	
		No 2	56	104	154	104.6		17	
		No 3	53	100	148	100.3		18	23.7
		Average	53.0±3.0	100.3±3.5	148.3±5.5	100.5±4.0		18.0±1.0	
G2	ES	No 4	24	70	119	71.0	53.1	17	
		No 5	22	73	123	72.6		15	
		No 6	23	71	121	71.6		16	32.2
		Average	23.0±1.0	71.3±1.5	121.0±2.0	71.7±0.81		16.0±1.0	
G3	GST	No 7	3	15	18	12.0	90.7	4	
		No 8	6	21	23	16.6		12	
		No 9	4	18	20	14.0		8	66.1
		Average	4.3±1.5	18±3.0	20.3±2.5	14.2±2.3		*8.0±4.0	
G4	Infected control	Nl 10	90	167	211	156.0	0.0	19	
		Nl 11	89	156	206	150.3		28	
		Nl 12	89	161	208	152.6		24	0.0
		Average	89.3±0.6	161.3±5.5	208.3±2.5	152.9±2.9		23.7±4.5	

a. at 14th week post infection EPG, Eggs per gram of faeces, wpi. Week post infection,*P<0.01 (Student's t-test)

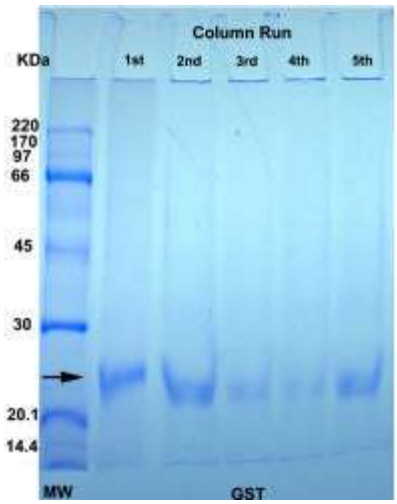

Fig. 1: SDS-PAGE of *F. gigantica* GST antigen, MW. Molecular weight marker

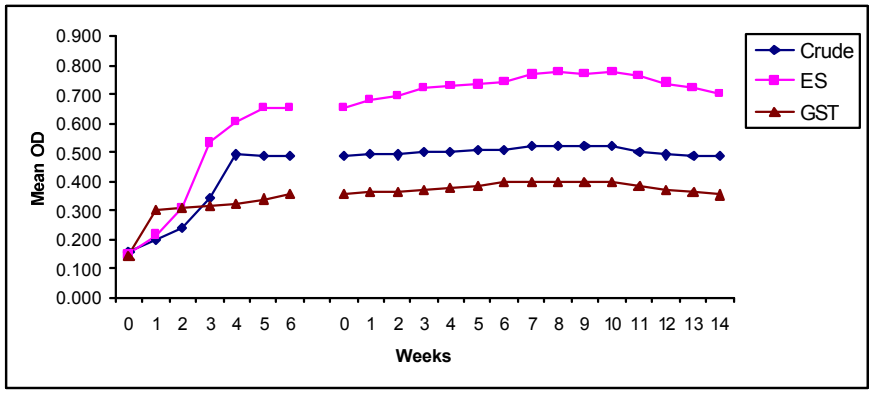

Fig. 2: Mean OD of antibody level detected by ELISA in immunized groups. The ODs of negative group were all below 0.160

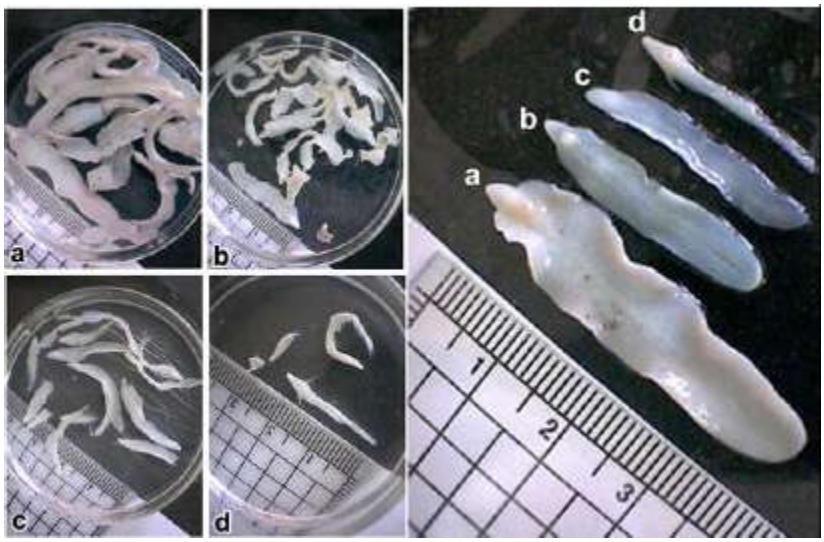

Fig. 3: Comparative size of adult flukes in immunized and non immunized infected goats. A: Infected control, B: Crude antigen immunized goat, C: ES antigen immunized goat, D: GST antigen immunized goat

vaccines for the control of fasciolosis. The results presented in this study showed that it was possible to induce protection levels against *F. gigantica* in goats using crude and purified antigens. It was evaluated three antigens of *F. gigantica* (crude worm, ES and GST) in a vaccination trial in goats. These antigens were chosen for this study based on their previous successful use against *F. hepatica* [7, 8, 13, 22]. The *F. gigantica* GST antigen defined in this study by SDS-PAGE gave one band at molecular weight of 23.2-24.2 KDa. This result appeared to be in line with the only report of analysis of *F. gigantica* GST by SDS-PAGE obtained by [23].

The data obtained in ELISA comparing the reactivity of sera from immunized goats at various intervals of immunization, before and after infection, with crude, ES and GST antigens showed that the antibody titers in all immunized goats were not elevated after challenge. This indicated that infection of *F. gigantica* was not able to induce any antibody titre against tested antigens. This result confirmed that these antigens were immunogenic [16, 22]. No boosting from the infections might be attributed to suppressive activities that were exaggerated by the highest antibody response [24].

The current study demonstrated the reduction in EPG (anti-fecundity effect) and fluke burden (anti-fluke effect) by the previous tested antigens in goats. In comparison with non-immunized control group, the GST immunized group showed the highest reduction in EPG and fluke burden (90.7 and 66.1%, respectively) followed by ES and crude antigen immunized groups. To date and contrary to these results, there are only two reports of the use of defined antigens as vaccine against *F. gigantica*. When the efficacy of GST from *F. gigantica* was assessed in Brahman cross cattle, no significant reduction in fluke burdens or faecal egg counts was recorded [23]. Paykari *et al*. [16] failed to obtain significant protection in sheep vaccinated with native, purified *F. gigantica* GST. There is a possible explanation as to why *F. gigantica* GST did not induce the same high levels of protection of the current study. This might be attributed to host species. Indeed, GST isolated from trematodes had been shown previously to induce a significant reduction in parasite infection and/or fecundity in vaccinated ruminants [16]. The fact that certain molecules could protect some hosts and not others against *F. hepatica*, in terms of the reduction of adults, had been reported for almost all the antigen candidates for vaccines which had been assayed [25]. In addition, the positive results achieved in some experiments had not been confirmed in successive repetitions [7]. These results highlight the variability in the effect of vaccination with trematode GST in various ruminant species. In accordance with those previous results, this study was believed that each host/parasite system was unique and could require its particular vaccination formulation and application protocol. This finding was considered as a first report of protective vaccination of goats against *F. gigantica* using a purified GST antigen. Additionally, it was noted that the GST antigen induced the highest effect in minimizing of fluke size among the tested antigens. The reduction in the size of the worms in the vaccinated animals must be interpreted as a sign of protection because it implied a deficiency in their normal physical development. These deficiencies had also been observed by [23] in *F. gigantica* in bovines immunized with FABP homologues. This statement was also detected in *F. hepatica* in rabbits vaccinated with nFh12 and to a greater degree with rFh15 [26]. The less developed worms, smaller in size, produced either very little or no eggs. Both the lower number of eggs, coupled with the observed reduced fluke size, was compatible with the anti-fecundity effect described in this study. On the other hand, there was no doubt that Freund's adjuvant could non-specifically modulate immune responses. However, the high total antibody titres were not sufficient for expression of immunity against *F. gigantica* [23]. Although, GST actually induced the lowest antibody titre in this study, it elicited the highest level of protection against *F. gigantica*.

In conclusion, the result of candidate vaccines indicated that it not only reduced fluke burdens, but produced also smaller flukes, fewer eggs and less liver pathology [27]. Moreover, the current study and those cited above demonstrated that a vaccine against *F. gigantica* in goats could be developed using purified GST antigen. It induced significant reductions in fluke burden and fecundity in vaccinated animals, with striking differences in effects observed in different animal species.

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