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Medical Group

Journal of Vaccines and Immunology

DOI CC By

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**Dates: Received:** 21 December, 2016; **Accepted:** 29 December, 2016; **Published:** 30 December, 2016

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Review Article

# Immunoprophylactic Control Strategy for Tropical Fasciolosis: A Possibility

## Abstract

Fasciolosis is a wide spread economically important helminthosis caused by *Fasciola hepatica* and *F. gigantica* and considered as a limiting factor for domestic livestock production. The strategic control measures against fasciolosis mainly depend upon judicious use of the anti-fluke drugs. Repeated applications and need for skilled labour make chemotherapy for fasciolosis a more costly affair. Chemotherapy has certain limitations on account of development of resistance to anthelmintics. Since the cost of treatment impedes application of chemotherapy within the rural areas of developing countries, other control measures such as development of vaccines are therefore, warranted. Attempts made to confer acquired resistance in ruminants against the disease, using viable attenuated metacercariae, crude somatic antigens excretory/secretary products, native, recombinant and peptide antigens with different adjuvant formulations and / or passive transfer of immunity through serum / immune cells of infected host have been partly successful.

## Introduction

Fasciolosis is a wide spread economically important helminthosis caused by *Fasciola hepatica* and *F. gigantica* and considered as a limiting factor for domestic livestock production. It causes significant economic loss estimated at US $ 3.2 billion per annum worldwide [1], mainly due to condemnation of livers at abattoirs, mortality in infected herds, persistently depressed growth and feed conversion efficiency, loss of productivity, impaired fertility and also the cost of treatment [2,3]. Tropical fasciolosis has been reported as a disease of prime economic consequence by the Ministry of Agriculture, Government of India in its annual report for years 1996-1997, on account of large ruminants population being at the risk of infection. Fasciolosis is endemic in several parts of India, with 25-100% prevalence rate in the livestock [4]. The pathogenesis of tropical fasciolosis in the definitive hosts involves a liver migratory phase of the parasite causing traumatic hepatitis and an adult parasite phase in the bile ducts, causing hyperplastic obstructive cholangitis [5].

The strategic control measures against fasciolosis mainly depend upon judicious use of the anti-fluke drugs [6]. Other conventional methods for the control of fasciolosis include elimination of snail intermediate hosts using molluscicides or by applying biological methods. The use of anti-fluke drugs against fasciolosis has several drawbacks. Repeated applications and need for skilled labour make chemotherapy for fasciolosis a more costly affair. Very few anti-fluke drugs such as triclabendazole are efficient against both adult and immature flukes [7]. Chemotherapy has certain limitations on account of development of resistance to anthelmintics [8]. Since the cost of treatment impedes application of chemotherapy within the rural areas of developing countries, other control measures such as development of vaccines are therefore, warranted.

Progress in the development of effective vaccine for *F. hepatica* and *F. gigantica* control in ruminants has been relatively slow, despite intensive research efforts [9]. Development of a suitable immunoprophylactic control strategy for this parasite involves identification of new target antigens, adjuvant development and better understanding of the host immune pathways and parasite immune evasion mechanisms. The development of a suitable immunoprophylactic strategy involves evaluation of various target antigens of *Fasciola* for their potentiality in inducing immunoprotection in domestic animals. Various studies have already shown that *Fasciola* infection elicits immune responses that are effective at killing the parasite and conferring protection against fasciolosis [10]. These observations suggest that vaccination is an achievable goal to induce protective immune responses against fasciolosis in ruminants. But till date no successful vaccine has been developed against the fluke parasite. Attempts made to confer acquired resistance in ruminants against the disease, using viable attenuated metacercariae, crude somatic antigens excretory/secretary products, native, recombinant and peptide antigens with different adjuvant formulations and / or passive transfer of immunity through serum / immune cells of infected host have been partly successful.



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## Viable antigens

**Live attenuated metacercariae:** The live attenuated *F. gigantica* metacercariae (irradiated at 3 kr γ rays) were widely used an immunoprophylactic antigen in early nineties by the eminent workers with significant level of protection, ranging from 40-85% in the ruminants [11-13]. In spite of significant protection indices, this immunization procedure could not become popular on account of inherent limitations with live attenuated vaccines, such as short shelf life, low profit, non-patentable and imperfect attenuation leading to infection, besides difficulties associated with availability of antigen in bulk quantity etc.

## Non-viable antigens

**Somatic antigens:** Immunization efforts using somatic antigens of flukes could not confer desired level of protection for field use on account of complexity of somatic antigens and poor specificity, associated with a non-adequate quantity of specific protein molecule in somatic antigen, to confer desired level of protection [14].

## Passive transfer of immunity

Immunity can be transferred from infection exposed animal to a naive animal with immune sera / cells, suggesting the presence of an immune component to resistance. Sin Clair [15], found that homologous cells of lymph nodes and spleen from donor sheep infected with *F. hepatica* for 8 weeks did not confer resistance to homologous challenge in recipient sheep although retardation in fluke development was observed.

The limitations of this vaccine are short duration of immune response, absence of memory cells in the host, chance of hypersensitivity reaction in recipient animals and non-availability of sufficient quantity of immune sera. This vaccination method has not been accepted in the field due to low level of protection, chances of hypersensitivity reaction, besides non-availability of donor animals.

## Defined immunoprotective antigens

These include fatty acid binding proteins (FABPs), glutathione S-transferase (GST), cathepsin-L, fluke haemoglobin, paramyosin, myosin, leucine-aminopeptidases and saposin-like protein-II [10,16]. These molecules are involved in various metabolic processes that are crucial for the development and survival of the parasite. The most widely employed candidate antigens, FABP, GST and cathepsin-L were described here.

## Fatty acid binding protein (FABP)

Fatty acid binding protein was the first defined antigen to be tested as a vaccine candidate for fasciolosis [17]. These are a large family of proteins with affinity for fatty acids, present in vertebrates and invertebrates [18]. Trematodes are unable to synthesize their own sterols, saturated or unsaturated fatty acids de novo [19] and rely on the uptake of lipids (long chain fatty acids and cholesterol) from host serum, which are the main ingredients for the synthesis of the tegumental surface membrane. These parasites utilize cytoplasmic and plasmalemmal FABPs to traffic the precursors of fatty acids from the host serum [20]. The cytoplasmic FABPs are the best characterized class of FABPs which are between 12-16 kDa and 127-133 amino acids in length [21]. Estuningsih et al. [22], found that there is a conserved signature sequence at amino acids 5-22 which is found to have the fatty acid binding property.

An antigen complex named FhSmIII (m) was identified from a crude protective whole worm extract of *F. hepatica* [23] and Fh12, the protective component of the complex FhSmIII(m) was derived from this antigen complex. Fh12 is found to be expressed from the early stage after the excystment of metacercariae through to the adult stage [24]. Fh12 was used to screen a *F. hepatica* cDNA expression library and a 14.7 kDa protein coding gene termed Fh15 was isolated [24]. A similar protein isolated from *S. mansoni* was termed Sm14 and its recombinant form gave protection up to 67% in mice against *S. mansoni* challenge. The same protein induced complete cross-protection in mice against *F. hepatica* challenge [25]. Fh15 and Sm14 are homologues of a family of related polypeptides known as cytoplasmic fatty acid binding proteins (cFABPs). A native protein fraction of *F. gigantica* FABP was found to elicit low but significant protection (31%) against infection with the same parasite in cattle [22]. This was the first report of cattle vaccinated with purified antigen of *F. gigantica* found to give protection. The recombinant version of FgFABP1c protein failed to confer protection in cattle in the same trial. Muro et al. [26], immunized rabbits with native *F. hepatica* FABP in Freund's adjuvant and reported a 40% reduction in the fluke burden with reduced fluke size. An anti-fecundity effect was observed in a vaccine trial in sheep with native and recombinant FABP of *F. hepatica* in Freund's adjuvant, with no reduction in fluke burden [27]. Martínez-Fernández et al. [28], used adjuvant adaptation (ADAD) system of vaccination, utilizing *F. hepatica* FABP native antigen (Fh12) with saponin + Anapsos in mice (BALB/c and CD-1). It resulted in an overall protection rate of 40%. An immunization trial in buffaloes with *F. gigantica* rFABP was carried out resulting in a moderate level of protection, in terms of reduced fluke burden (35.8%), reduced liver damage and anti-fecundity effect of the parasite [29]. This constituted the first vaccine trial in buffaloes against fasciolosis using a recombinant antigen. López-Abán et al. [30], used Fh12 FABP with immunomodulator (lipidic aminoalcohol) alone or in combination with the hydroalcoholic extract of *Phlebodium pseudoaureum* (PAL) in the ADAD system in mice and sheep. Mice vaccinated with ADAD containing lipidic aminoalcohol (OA0012) + Fh12 or lipidic aminoalcohol (OA0012) + Qs + Fh12 had survival rates of 40-50%. Sheep ADAD vaccinated with lipidic aminoalcohol (OA0012) + Qs + Fh12 showed lower fluke recovery, less hepatic lesions and higher post-infection daily weight gain than *F. hepatica* infected control animals. Sheep ADAD-vaccinated with immunomodulator (OA0012) combined PAL and Qs + Fh12 showed lower fluke recovery (42%), lower adult worm count (57%), lower faecal egg count (38%), less hepatic lesions and higher post-infection daily weight gain than *F. hepatica* infected control animals. Thus, the addition of



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this immunomodulator to ADAD system with FABPs increased the protection against *F. hepatica*. Kumar et al. [16], recorded 23% protection in buffalo calves immunized with 400 µg of *Escherichia coli* expressed *F. gigantica* recombinant FABP dissolved in montanide 70 M-VG mineral oil-based adjuvant.

## Glutathione S-transferase (GST)

Glutathione S-transferase is a family of isoenzymes / multi-functional enzymes involved in the cellular detoxification and excretion of a variety of xenobiotic substances that are toxic to the organism. Representing an integral part of phase II biotransformation enzymes [31], these enzymes catalyze the S-conjugation of the thiol-group of glutathione to a wide variety of electrophilic substrates. This conjugation generally renders the toxin more water soluble, less toxic, which is more readily eliminated by the host. GSTs are found in most forms of life i.e. aerobic eukaryotes and prokaryotes and occur as multiple enzyme forms. The cytosolic classes of GST comprising of dimmers of 24-29 kDa monomeric units and are found widely in the plants and animals [32]. These enzymes exhibit a broad range of activities owing to their ability to bind many hydrophobic substances such as bilirubin, steroids and polycyclic aromatic hydrocarbons. Based on the substrate specificity and primary structure studies, cytosolic GSTs have been grouped into seven classes, Alpha, Mu, Pi, Sigma, Theta, Kappa and Zeta [33]. Purified GST of adult *F. hepatica* consists of five isoenzymes of molecular weight ranging from 24-29 kDa, exhibiting N-terminal sequence heterogeneity [34]. The GST enzymes from *F. gigantica* have also been isolated and characterized. The protein sequences predicted from different clones (GST-1, 7, 47 and 51) possessed 71-89% identity with each other and were a member of class of GST. Antisera raised against GST confirmed several important aspects of immunolocalization of GST. These studies primarily revealed that GSTs were distributed in parenchyma, tegument and gut tissues of adult *F. hepatica* [34].

Pioneering report of Smith et al. [35], indicated 26 kDa protein of *S. japonicum* recognized by resistant WEH1 129/J mice sera was a parasite glutathione S-transferase. Then extensive study on immunogenicity of that protein was done to protect mice against S. mansoni adult worm and found significant protection against experimental schistosomosis. Studies on the GST isoenzymes of *Fasciola* spp. confirmed this enzyme as a potent candidate antigen that can be exploited for immunoprophylaxis in a variety of host parasite systems [36].

First vaccine trial in rats using *F. hepatica* GST (FhGST) as immunogen was conducted by Howell et al. [37]. But he could not demonstrate protection against the parasite. Sexton et al. [38] reported GST as novel vaccine candidate that protected sheep against fluke infection. Glutathione S-transferase from adult *F. hepatica* was assessed as vaccine immunogen in cattle with several immunological adjuvants. Interestingly, Morrison et al. [39] obtained 19-69% protection in cattle inoculated with 400 µg GST and boosted a month later with 200 µg and challenged with *F. hepatica* metacercariae a month post immunization. However, some cattle under this experiment exhibited exceptionally higher protection (90%) with PLG/SM adjuvant formulation, despite the virtual absence of anti-GST antibody titres. Significant reductions in the fluke burden (19-69%) were only observed after challenge infection in calves vaccinated with GST in Quil A / Squalene montanide R (Sm) and poly-Dlactide co-glycolide (PLG) but there was no correlation between antibody titres and protection. The protection conferred in cattle was dependent on the choice of adjuvant. The protection conferred could not be correlated well with antibody titre against GST or the induction of neutralizing antibodies to GST [32]. Estuningsih et al. [22] observed low antibody titres in cattle vaccinated with *F. gigantica* (FgGST) and low to moderate titres were obtained with FgGST delivered in PLG microspheres in Squalene montanide. In India, Mandal [40] had isolated affinity purified FgGST and recorded encouraging results in terms of protection in rabbits against *F. gigantica*. Paykari et al. [41] evaluated FgGST in sheep using aluminium hydroxide or saponin as adjuvants and found 32% reduction in the fluke burden with GST-saponin, which was not statistically significant in comparison with the control group. Muro et al. [42] in an experimental study in rabbits found that recombinant *F. hepatica* GST induced reduction in the fluke burden and caused stunted growth of the fluke, as 64% of the flukes recovered were immature. Preyavichyapugdee et al. [43] reported strong protection (77-84%) against *F. gigantica* when recombinant FgGST26 (rFgGST26) was used as a vaccine in combination with Freund's adjuvant in mice. Mice were immunized via subcutaneous, intramuscular or intradermal routes and protection was observed in all routes. They observed that rFgGST26 is a good vaccine candidate against *F. gigantica* in mice and could also provide cross-protection against *S. mansoni*. Kumar et al. [16] recorded 30 and 35% protection in buffalo calves immunized with *Escherichia coli* expressed *F. gigantica* rFABP (400 µg) and combination of rFABP and rGST (400 µg each), respectively dissolved in montanide 70 M-VG mineral oil-based adjuvant.

## Cathepsin L (CL)

Parasite proteases play a significant role in determining the virulence, pathogenesis and finally govern in situ adult worm population establishment. The cysteine proteinase comprises a large family with a number of classes and cathepsin L and B in particular have been studied in relation to parasite invasion and vaccine potential [6]. In *Fasciola* the cathepsin L proteases play a number of functional roles including promoting tissue penetration [44], nutrient acquisition [45], egg production [46] and immune evasion [47]. The involvement of cathepsin L proteinase in such essential functions has made it attractive candidate against which vaccine could be targeted.

The utility of *F. hepatica* cathepsin L (FhCL) as a vaccine was first demonstrated in sheep using Freund's adjuvant. Though there was no reduction in worm burden in vaccinated animals, the faecal egg count was significantly reduced (69.7%) [48]. Vaccination of cattle with *F. hepatica* cathepsin L-1 in Freund's adjuvant resulted in reduction of fluke burden up to 69% (mean protection level 53.7%). When this molecule was used in combination with fluke haemoglobin, it elicited significantly higher level of protection (72.4%). Furthermore, a



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reduced viability was observed for fluke eggs recovered from all vaccinated groups. This anti-embryonation effect of vaccination was particularly evident in the group that received cathepsin L-2 and fluke haemoglobin combination where > 98% of eggs recovered did not embryonate to miracidia (Dalton et al., 1996). Estuningsih et al. [22], evaluated the *F. gigantica* cathepsin L (FgCL) as vaccine candidate in cattle. They observed that vaccination of cattle with the FgCL in DEAE adjuvant induced very high antibody titers but failed to reduce *F. gigantica* fluke burden or faecal egg count. Piacenza et al. [49], demonstrated that purified FhCL1 and FhCL2 in combination with parasite leucine aminopeptidase induced 78% protection in sheep and moreover, when purified liver fluke leucineaminopeptidase was used alone in vaccination trial, this protection increased to 89%. Golden et al. [50], used recombinant FhCL1 (rFhCL1) protease formulated in mineral oil based montanide ISA 70VG and ISA 206 VG as a vaccine and found significant reduction in fluke burden of 48.2 % in cattle against *F. hepatica* natural infection in both the groups. All vaccinated animals showed a sharp rise in total IgG levels which was significantly higher than levels reached in the control group. Arginase levels in the macrophages of vaccinated cattle were significantly lower than those of the control cattle, indicating that the parasite induced alternative activation of the macrophages was altered by vaccination. The data demonstrate the potential for recombinant FhCL1 vaccine in controlling fasciolosis in cattle under field conditions.

The new arrival of first commercial vaccine for sheep, Barbervax(®) against the haematophagous helminths parasites, *Haemonchus contorus*, it is fair to expect vaccine development to progress at an exponential pace against the liver fluke.

## Acknowledgements

The authors are thankful to the editor in chief for providing the opportunity to write a brief review on the asked topics.

The first authors pay sincere thanks to Dr. O. K. Raina, Principal Scientist, Division of Parasitology, IVRI, Izatnagar, 243122 for his exceptional guidance, constructive counsel, patience, constant encouragement and meticulous supervision all along the course of study during PhD programme.

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**Citation:** Kumar N, Maurya PS, Solanki JB, Mishra AK (2016) Immunoprophylactic Control Strategy for Tropical Fasciolosis: A Possibility. J Vaccines Immunol 2(1): 036-040.