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In India, *F. gigantica* Cobbold, 1885 is the principal aetiological agent responsible for causing fascioliasis. Infections with *F. hepatica* are not common and have been reported from animals in the hilly regions only.

The complexity of the life cycle of *F. gigantica* makes control of this parasite by antihelmintic treatment or management extremely difficult. This problem have encouraged investigation of control by vaccination. Several studies have shown such methods to produce a degree of protection against infection with adult flukes. No comparative studies on the damage produced by the immature stages of the parasite in vaccinated and non-vaccinated mice have been reported. Since this is an important consideration in the development of a useful vaccine, the aim of this study was to evaluate the potential problems associated with host response in the development of somatic fluke antigen for the control of *F. gigantica* using mice as a study model.
To accomplish this, three separate studies were performed. The first dealt with a comparison of pathogenesis of *F. gigantica* in hosts with existing infections and non-exposed controls. The second was an analysis of the response of infected hosts to selected *F. gigantica* antigen. The third was an investigation of the effect of pre-exposure to fluke antigens on the challenge infection.

The Thesis is divided into 4 PARTS. PART I deals with a detailed review of literature available on the subject. PART II deals with the pathogenesis of *F. gigantica* infection in mice, PART III with the studies on the influence of whole somatic antigen (WSA) on clinical disease produced by *F. gigantica* strains isolated from sheep and cattle, and PART IV with serological studies of *F. gigantica* infections in mice.

The studies conducted on the above lines and the findings thereon are summarized below:

1. The pathogenesis of *F. gigantica* in mice on both primary and secondary infections revealed that the lesions produced
were confined essentially to the parenchyma and the biliary system of the liver. The basic lesions of primary infection progressed from simple haemorrhagic tracts infiltrated with neutrophils to large tunnels whose walls were heavily infiltrated by eosinophils. The eosinophils probably started playing an active role after day 17 following initial exposure / infection.

In the parenchymal phase of migration, the flukes excavated hemorrhagic tunnels which were invaded by inflammatory cells.

There were two types of necrotic changes; the first type of was seen on day 23, whereas the second type was apparent after day 30 of initial exposure. The former was essentially secondary to thrombosis and partial occlusion of the blood vessels from the inflammatory response, resulting in ischaemic necrosis. This was observed as a lobar necrotic factor around day 30 of initial exposure. This occurred where large blood vessels supplying the lobe were obliterated.
The second type of multi-focal disseminated necrosis was not related to the effects on the blood vessels. These were eventually replaced by connective tissue and then regenerating hepatocytes. The inflammatory response and repair was more prompt and of shorter duration in animals challenged 40 and 50 days after primary infection than in animals upon initial exposure. Also there was a reduced death rate. Just prior to and following entry into the bile ducts, extensive hyperplasia occurred with thickening of all the layers of parasitized and nearby bile ducts. Severe periportal fibrosis was observed infrequently. With time, there was complete regeneration of the liver. However, hyperplasia persisted in bile ducts containing flukes.

Repair of ischaemic necrosis at the triads and the periphery of the bile lobe while that of bile necrosis always started at the periphery of the lesion. There was also a colour difference between the two blemishes. The ischamic
necrotic sites always stained deep pink, whereas those of bile stained light pink. The repair process involved entry of eosinophils.

In challenge mice, the spleen appeared larger than in initial infection, but there was a reduction in the size of germinal centres. This suggests lessened antibody production with subsequent decrease in the role played by antibodies in combating the disease. However, the increased lymphocytic serioes may suggest active involvement of these, probably T-cells in the cellular response. The enlargement of the spleen was associated with long-standing of challenge infection of *F. gigantica*. The presence of a spleen of two to three times the normal size in the absence of fluke lesions in the liver usually indicated a past infection. This was confirmed by the presence of precipitating antibodies against *F. gigantica*. The present work did not reveal whether the spleen participates in the production of protective immunity.
2. A drop in the packed cell volume (PCV) of erythrocytes was induced in mice inoculated with extracts of *F. gigantica* (cattle strain) or exposed to metacercariae of either cattle or sheep strains of *F. gigantica*. The drop in PCV following antigen administration was inconsistent. It apparently was dependent partly on the method used in preparation of the antigen and partly on intrinsic factors of individual mice.

The overall difference in mean PCV for all groups of mice (i.e. Group A to F) was significant (*p* < 0.05) if compared on a weekly basis or as an average for the entire period of the experiment.

The number of eosinophils in mice exposed to live *F. gigantica* metacercariae and those exposed only to antigen was compared. Animals infected with live organisms showed a rise in the percentages of eosinophils, while the animals injected with antigen extracts did not.
The weights of the mice in five different groups did not fall into a set pattern. There were no significant difference noted between the groups of within the groups for the entire experiment \((p < 0.05)\).

3. Comparative studies on the cattle and sheep strains of *F. gigantica* revealed differences in their virulence. The cattle strain induced a moderate drop in PCV while the sheep strain initiated more severe changes. Investigation of the cause of this anemia indicated that the cells from infected mice were Coombs' positive with anti-whole mouse serum during the anemic stage. Follow-up studies using anti-immunoglobulin and antisera to fluke antigens were negative. This suggested that the observed anemia may be partially due to attachment of serum protein, possibly complement, to erythrocytes. Tests on erythrocyte fragility were inconclusive. Administration of fluke antigen before exposure to metacercariae did not alter the course of anemia.
4. Immunization with *F. gigantica* antigens gave varied responses. It usually did not increase protection to challenge and sometimes produced a hypersensitivity type of reaction. These variations may be, in part, attributable to differences in antigens occurring in different preparations of fluke extracts.

5. Individual mice had considerable variation in serologic response. However, the majority of the mice which became serologically positive remained positive until the end of the experiment.

Serologic studies indicated that double gel immuno-diffusion assay was over 64.5% accurate and that indirect haemagglutination assay was 87-91% accurate for the diagnosis of *Fasciola* infections. The sera tested for IHA revealed 91% of the mice positive at week 7. The highest titer recorded was 1:2048 and the lowest was 1:64. Neither test showed false positives. The major problem with these techniques was that a few infected mice were not positive with either test. The percentage of positive mice
was slightly lower on week 10 (87%). The highest titer attained was 1 : 4096 and the lowest was 1 : 128.

The study and the results here indicate that DID is the most practicable method for use in the laboratory, whereas the IHA is the more sensitive of the two techniques but has the disadvantage of requiring freshly prepared antigen which has to be prepared in such a way to remove ingredients that agglutinate the sheep red blood cell. Both tests are easy to perform and do not require expensive instrumentation.

6. The study of pathogenesis of *F. gigantica* in mice thus has shown the association of inflammatory response with the migratory phase of the parasite inducing some type of immune reaction that exacerbates the damage to the liver particularly in the challenge infection and is apparently related to an immune hemolytic anemia. At present the data suggest the anemia of fascioliasis is caused by multiple factors
including probable attachment of complement to erythrocytes, deleterious of toxic substances produced by the parasite, and intrinsic factors involving both the host and the parasite.

7. The studies and findings are supported and explained by giving One photograph, 20 photomicrographs, 23 graphs, and 10 Tables.