IDENTIFICATION OF FASCIOLA DERIVED ANTIGENS INDUCING IMMUNE RESPONSES IN HUMAN FASCIOLIASIS

A Thesis

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DEDICATION

To Whom I Love " My Wife Nevine "

" My Sons Maykel and George "

To my First teacher in my life

" My Mother "

To my Father's Soul

List of Abbreviations

µg : Micro gram
µL : Micro liter
µm : Micrometer

ADCC : Antibody dependant cell mediated cytoxicity
AIDS : Acquired immunodeficiency virus
APS : Ammonium persulfate
ASC : Antibody secreting cell
BSA : Bovine serum albumin
C : control (unstimulated) cultures
CBC : Complete blood count
CD : Cluster differentiating
cDNA : Complementary Deoxy ribonucleic acid
Con A : Concanavalin A
CP : Cells producing
CPM : Count per minute
DAB : Diaminobenzidine
DDT : Dithiothritol
dH_2O : Deionized water
DTH : Delayed type hypersensitivity
E : experimental cultures
EDTA : Ethylene diamine tetra acetic acid
EIA : Enzyme immunoassay
EITB : Enzyme linked immunotransfer blot
ELISA : Enzyme linked immunosorbant assay
epg : Egg per gram
ERDC : Egyptian Reference Diagnostic Center
ES : Excretory/secretory
F : Female
FABP : Fatty acid binding proteins
FAST-ELISA : Falcon assay screening test enzyme linked immunosorbant assay

Fc : **Fraction constant**

FCA : **Complete Freund's adjuvant**

FEG : **Number of Fasciola eggs/g**

FhES : ***Fasciola hepatica*** **excretory secretory**

FhESP : ***Fasciola hepatica*** **excretory secretory products**

FheCL : ***Fasciola hepatica*** **purified cathepsin L**

FhTSE : ***Fasciola hepatica*** **total soluble extract**

Fhwwh : ***Fasciola hepatica*** **whole worm homogenate**

FP : **Fasciola patients**

FPLC : **Fast performance liquid chromatography**

g : **Gram**

gammaGT : **Gamma-glutamyl transferase**

GLDH : **Glutamate dehydrogenase**

GPX : **Glutathione peroxidase**

GST : **Glutathione S-transferase**

H_2SO_4 : **Sulfuric acid**

HAC : **Acetic acid glacial**

HCl : **Hydrochloric acid**

HEPES : **N-2 hydroxyethyl-piperazine-N-2-ethane sulfonic acid**

HID : **House identification number**

HIPH : **High Institute of Public Health Alexandria**

HLN : **Hepatic lymph node**

HMNC : **Hepatic mononuclear cells**

HPLC : **High performance liquid chromatography**

HPR : **Horse raddish peroxidase**

hr : **Hour**

IDR : **Intradermal reaction**

IFN-γ : **Interferon gamma**

IFS : **Sera from patients infected with Fasciola**

IgA : **Immunoglobulin A**

IgE : **Immunoglobulin E**

IgG : **Immunoglobulin G**

IgM : **Immunoglobulin M**

IL-10 : **Interleukin-10**

IL-2 : **Interleukin-2**

IL-4 : **Interleukin-4**

IL-5 : **Interleukin 5**

ISS : **Sera from patients infected with Schistosoma**

kDa : **Kilodalton**

kg : **Kilogram**

KPL : **Kirkegaard and Perry laboratories**

L : **Liter**

M : **Male**

MAMA : ***Schistosoma mansoni*** **adult microsomal antigen**

mg : **Milligram**

min : **Minute**

mL : **Milliliter**

MLN : **Mesenteric lymph node**

mm : **Millimeter**

Mol. Wt. : **Molecular weight marker**

mRNA : **Messenger ribonucleic acid**

NC : **Nitrocellulose**

ND : **Not Done**

NEJ : **Newly excysted juveniles**

µg : **microgram**

NHS : **Normal human sera**

NK : **Natural killer**

nm : **nanomiter**

^{o}C : **Degree centigrade**

OD : **Optical density**

OP : **Number of eggs/g in stool specimen for other parasites**

PBMC : **Peripheral blood mononuclear cells**

PBS : **Phosphate-buffered saline**

PHA : **Phytohaemagglutinin**

PID : **Patient number within the family**

PMSF : **Phenyl methyl sulfonyl fluoride**

PPD : **Purified protein derivatives**

PPI : **Post primary infections**

R_F : **Relative mobility**

rGST : **recombinant GST**

rpm : **Rotation per minute**

s.c. : **Subcutaneously**

S.m. : ***Schistosoma mansoni***

SAFA : **Soluble adult Fasciola worm antigen**

SDS-PAGE : **Sodium dodecyl sulfate poly-acrylamide gel electrophoresis**

SEC : **Skin eosinophil counts**

SD : **Standard deviation**

SLN : **Spleen lymph node**

SMEg : **Number of *Schistosoma mansoni* eggs/g**

SOD : **Superoxide dismutase**

sp. : **Species**

TCR : **T Cell receptor**

TEMED : **N,N,N,N-Tetramethyl ethylene diamine**

Th1/Th2 : **T helper1/T helper2**

TMB : **3, 3', 5, 5'-Tetra methyl benzidine**

TNF : **Tumor necrosis factor**

UK : **United Kingdom**

USA : **United States of America**

UV : **Ultraviolet**

VACSERA : **Egyptian Holding Company for Biological Products and Vaccines**

WFA : **Whole fluke antigens**

WHO : **World Health Organization**

WPI : **Week post infection**

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Review of Literature and Aim of The Work

1. Introduction to Fascioliasis:

Fascioliasis is a zoonotic disease caused by the hermaphroditic trematode *Fasciola hepatica*. This worm is a common pathogen of ruminants, especially sheep, goats and cattle. Humans can also be infected by injection of contaminated vegetables. The economic losses produced by this disease are often related to reduced production of meat **(Cawdery *et al.*, 1977; Genicot *et al.*, 1991)**.

Infection of domestic ruminants with *Fasciola hepatica* (Temperate Fluke) and *Fasciola gigantica* (Tropical iver Fluke) causes economic losses estimated at over US$2000 million per annum to the agricultural world wide, with over 600 million animals infected **(Boray, 1985; Hillyer and Apt, 1997).**

The estimated number of people currently having fascioliasis is 360,000 in Bolivia, 20,000 in Ecuador, 742,000 in Peru, 830,000 in Egypt, 37,000 in Yemen, 10,000 in Islamic Republic of Iran. The total estimated number of people infected is 2.4 million in 61 countries and that the number at risk is more that 180 million throughout the world **(Haseeb *et al.*, 2002)**. In Cajamarca, Fascioliasis is still an endemic illness **(Alban *et al.*, 2002)**.

Triclabendazole is recommended as the first line agent for the treatment of *Fasciola hepatica* **(Aksoy *et al.*, 2005)**.

2. Fasciola Life Cycle:

2.1. Life Cycle:

The adult fluke, which lives in the bile ducts, produce eggs which are passed in the faeces (Fig. 1a). In wet areas, under optimal conditions, the eggs separate from the faecal material, hatch, releasing the larvae or miracidia (Fig. 1b). The miracidia invade the lymnaeid snails in which they develop and multiply as sporocyst, rediae and cercariae (Fig. 1c). The tadpole like cercariae leave the snails (Fig. 1d) and swim until they encyst on vegetation, forming metacercariae (Fig. 1e), which are the infective stage of the fluke. The entire cycle of the liver fluke in the snail takes 2 - 3 months under favourable conditions in the field. If ingested by sheep, cattle or other hosts, including man (Fig. 1f), the metacercariae excyst in the small intestine and the released immature flukes penetrate the intestinal wall and enter the abdominal cavity. The young fluke penetrates the liver capsule and migrates through the liver tissue for 6 to 7 weeks before entering the bile ducts to become adult fluke (Fig. 1g). The fluke reachs sexual maturity and commence egg production 8 to 10 weeks after infection.

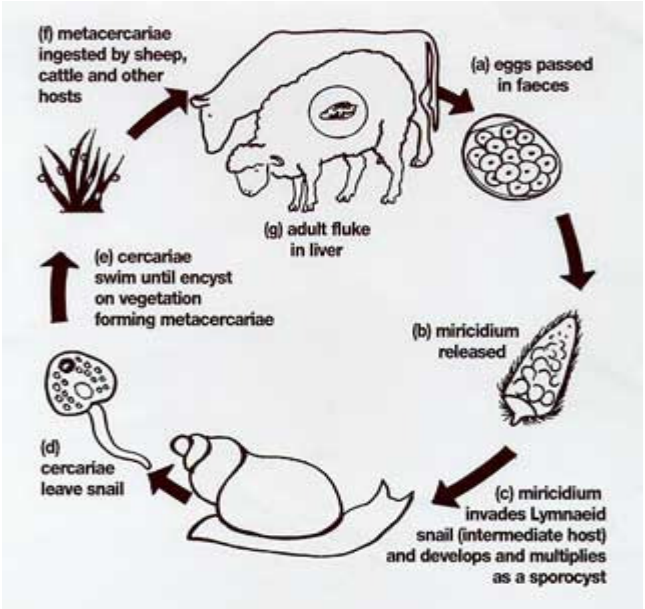

Figure 1: Life Cycle of *Fasciola Hepatica*.

Reproduced from Tropical Medicine an Parasitology 1989

2.2. Epidemiology:

Fascioliasis is enzootic worldwide in sheep raising regions. It is also found in goats, cattle, horses, camels, hogs, rabbits, deer, vicuna, and other herbivores, and even in dogs **(Hafeez, 2003)**. Human outbreaks have been reported in France, England and Cuba and sporadic cases from mainland United States, Hawaii, the West Indies, many European countries, the Middle East, China, Tibet, Siberia, north and east Africa, and South Africa.

Fasciola gigantica is a common parasite of cattle in Africa, Asia, and Hawaii and has been reported in humans in Gambia, Vietnam, Iraq, and Hawaii.

The most significant risk factor of acquiring fascioliasis in the family is eating salads in endemic areas **(Marcos *et al*., 2005)**.

2.3. Pathology and Pathogenesis:

The flukes live in both the intrahepatic and the extrahepatic bile ducts. Damage is a function of worm load, so that symptoms are usually mild in low-grade infections. In the rare cases of heavy infection, there may be ductal epithelial hyperplasia and necrosis, cellular infiltration, ductal dilatation, periductal fibrosis, new duct growth, and (rarely) calcification. Mechanical obstruction plus accumulation of fine biliary "sand" may lead to cholangitis, cholecystitis, and cholelithiasis **(Mas-Coma *et al.,* 1999)**.

Fibrosis may occur in the portal tracts, and adjacent liver tissue may be compressed.

The flukes may enter the liver parenchyma and cause additional damage, which is followed by fibrosis and parenchymal regeneration **(Behm and Sangster, 1999)**. In the most severe cases there is marked destruction of liver tissue, subcapsular hematoma, and even intra-abdominal hemorrhage. Flukes may also be found in the gallbladder. Although biliary cirrhosis occurs in herbivorous animals, it

is not known whether this also occurs in humans, leading to portal hypertension. Ectopic human fasciliasis is common and results in local necrosis, abscess formation, and fibrosis.

The most important clinical manifestations are: abdominal distention and flatulence, right upper quadrant pains, colicky abdominal pains, pallor, and etympanitic abdomen.

The most significant items in the Complete Blood Count (CBC) and liver function tests are significantly high eosinophilia, high alkaline phosphatase, and low haemoglobin. Besides, two fascioliasis patients may high serum bilirubin **(Hasseb *et al*., 2003)**.

2.3.1. Clinical Findings:

Many infections are asymptomatic. Symptomatic fascioliasis can be divided into acute and chronic forms.

2.3.1.1. Acute Stage:

Acute symptoms correspond to the period of larval migration from intestine through liver, to reach and then mature in the bile ducts. The incubation period for onset of acute symptoms is 2 to 6 weeks, and they may last for several months. Fever (sometimes high), headache, anorexia, nausea, vomiting, right upper quadrant and epigastric pain, and liver enlargement and tenderness are common. Anemia, leukocytosis to 35,000/µL, and eosinophilia to 90% may be present. Urticaria and other allergic manifestations may occur. In severe illness, the patient may be postrated, wasted and jaundiced, with hyper gammaglobulinemia and liver function abnormalities. Eggs are not found in the feces for 3 to 4 months, so diagnosis is difficult early in the acute stage **(Facey and Marsden, 1960)**.

2.3.1.2. Chronic Stages:

There may be pain in the right hypochondrium and epigastrium, hepatomegaly, dyspepsia, diarrhea, nausea, vomiting and jaundice. If the extrahepatic bile ducts are occluded, chronic obstructive phases may result in clinical findings similar to those of choledocholithiasis **(Mas-Coma *et al.,* 1999)**. Uncommonly, obstructive symptoms are the initial symptoms. Some patients with obstruction recover spontaneously as a result of evacuating flukes into the intestinal tract.

2.3.2. Ectopic Disease:

Young flukes may wander to other locations, such as the skin, or the intestinal wall, lungs, heart, brain, orbit, muscle and other tissues and may cause abscess formation **(Makay *et al.,* 2007)**. Halzoun in the Middle East, caused by eating raw liver of infected goats or sheep, has been attributed to pharyngeal fascioliasis, but it is now though to be chiefly a pentastomatid infection, though raw liver should nonetheless be avoided.

Zali *et al*. (2004) found a very unusual case of the disease, likely the first case involving the pancreas, spleen, and kidney, as well as the liver.

2.3.3. Diagnostic Tests:

2.3.3.1. Identification of Eggs:

Definitive diagnosis in the chronic stage rests on finding characteristic eggs in feces (repeated examination may be necessary) or in fluid obtained by duodenal or biliary drainage. In some instances, liver biopsy is needed to make the diagnosis. Spurious infections must be ruled out by placing the patient on a liver-free diet for a few days; if eggs continue to be passed, the infection is genuine.

Since eggs of *Fasciola hepatica* and *Fasciola buski* are similar, differentiation may be difficult.

2.3.3.2. Serologic Tests:

Chronic fascioliases are usually diagnosed by faecal and/or serologic studies **(Marcos *et al*., 2006)**.

2.3.3.3. Other Tests:

Marked leukocytosis and eosinophilia are usually present, hypergammaglobulinemia and abnormal liver function tests are often seen.

2.4. Differential Diagnosis:

In enzootic areas, the triad of fever, enlarged liver and eosinophilia, along with a history of eating raw water plants, suggests fascioliasis **(MacLean and Graeme-Cook, 2002)**.

2.5. Prevention and Control:

As a general role, the control strategies should be based on the education of the consumers, farmers and shepherds, the improvement of farming conditions, the improvement or the development of more sensitive methods to detect these parasites in slaughtered animals and in foodstuff, a control of sewage sludge on pastures and of drinking water resources, and the reduction of contacts between livestock and wild animals which frequently represent the most important reservoir of these pathogens **(Pozio, 2008)**.

2.6. Treatment:

Triclabendazole 20/kg is safe and efficient for patients with acute fascioliasis **(Dauchy *et al*., 2005)**.