A COMPARATIVE STUDY OF MULTIPLE VERSUS SINGLE INFECTION DOSES OF *SCHISTOSOMA HAEMATOBIUM* IN GOLDEN HAMSTERS (*MESOCRICETUS AURATUS)*

BY

NJOROGE VINCENT KOMO (BSc. Hons)

A THESIS SUBMITTED TO THE GRADUATE SCHOOL IN PARTIAL FULFILMENT FOR THE REQUIRMENTS OF THE AWARD OF A MASTERS OF SCIENCE DEGREE IN BIOCHEMISTRY OF EGERTON UNIVERSITY.

EGERTON UNIVERSITY, NJORO

OCTOBER 2005.

DECLARATION AND RECOMMENDATION

This thesis is my original work and has not been submitted in any University/Institution for any other award.

NAME OF CANDIDATE: NJOROGE VINCENT KOMO.

SIGNATURE: _____ DATE 4TH/09/2006.

This thesis has been submitted with our approval as supervisors

SIGNATURE: _____ DATE 13 Sept 2006

Prof. MOSES LIMO.

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

EGERTON UNIVERSITY.

SIGNATURE: _____ DATE 4 September 2006

Dr. DORCAS YOLE.

THE HEAD. DEPARTMENT OF PARASITOLOGY. INSTITUTE OF PRIMATE RESEARCH (IPR).

ACKNOWLEDGMENTS

I thank the Almighty God for seeing me through the hard times of this study. I would like to extend my sincere gratitude to Dr Yole, my field supervisor for her tireless efforts, material support and constructive advice throughout this study. I am whole-heartedly grateful to Prof Limo, my University supervisor for his academic input in this work.

I am greatly indebted to the technical staff of Parasitology Department, Institute of Primate Research, particularly Dr F. Maloba, S. Kisara, F. Nyundo and Kithome Kiio for their unreserved willingness to work even at odd hours to see this work succeed. Input by Mary Nzuve and the entire section of Histopathology and Diagnostics Deprtment was greatly recognized and appreciated.

I would also like to sincerely thank my family members for their undying financial and moral support during the study, particularly my sister Salome for her resolute financial support throughout my entire study. Special thanks to the family of Mr. Kenda, for the comfortable accommodation they gave me during the study at Kilifi. I also extend my gratitude to Mesters stationers for their support in printing and photocopying of this work.

I would like to sincerely thank the IPR administration and staff for giving me the chance to carry out the research in their laboratory and their continued support during the study. Many thanks also go to the University of Nairobi, Department of Zoology for their assistance in breeding of hamsters.

DEDICATION

This work is dedicated to my parents, my late Dad Josiah and my Mum Mary for their struggle to provide me with quality education, their limited resources notwithstanding.

ABSTRACT

Schistosomiasis is a parasitic disease that ranks second behind malaria in terms of socio-economic importance globally. An intermediate fresh water snail host, of the genus *Bulinus*, transmits *Schistosoma haematobium*. It is not possible to study in humans, the low infection rates over prolonged periods of time, characteristic of *S. haematobium* infections of people living in endemic areas. The only option is to use appropriate animal models in an experimental setup for the study. In the present study two groups of hamsters each composed of twenty animals were obtained, the singly infected group (SI) simulated laboratory experimental procedure while multiply infected group (MI) represented what happens in endemic areas during schistosome infections. The naive group contained ten hamsters (NC). Hamsters in the MI group were infected by abdominal skin exposure with 50 cercariae every week, for four weeks. At the fourth week each of SI hamsters were infected with a single dose of 200 *S. haematobium* cercariae. Five hamsters from each group were sampled at weeks 3, 6, 9 and 12 post-SI for gross pathology and histopathological assays. 18-hour schistosomula soluble protein (SSP), schistosoma adult worm preparation (SWAP) and schistosome egg antigen (SEA) were used for lymph node cells, spleenocytes proliferation assays and also for specific immunoglobulin G (IgG) assays. Perfusion was done at week 12 post-SI.

Lymph node cells stimulation by SSP in the SI group was higher than in the MI group at week 3 post-SI while the MI group had significantly higher responses at weeks 6 and 9 post-SI. Peak lymph node cells stimulation by SWAP and SEA was at week 9 post-SI in the two groups, with the SI group showing higher responses than the MI group. Peak spleenocytes stimulations by SSP, SWAP and SEA were during the 3rd week post-SI in the two groups, though MI responses were higher than the SI responses. IgG levels specific to SSP in the SI group were higher than MI responses at week 3 post-SI; the trend was reversed at week 6 and 9 post SI. At all sampling points, SWAP specific IgG was higher in MI group than SI group. A similar trend was also recorded for the SEA specific IgG levels. The mean number of worms was 42.2$\pm$2.71 and 24.0$\pm$2.59 in the MI and SI groups respectively. At all sampling points MI group registered more severe gross pathology and histopathology of the liver and the urinary bladder. An indication that the trickle infections MI did not elicit an immune response strong enough to clear subsequent infections while the single infection SI elicited an immune response that was able to reduce the worm burden and consequently lowering oviposition and hence reducing pathology.

TABLE OF CONTENTS

LIST OF FIGURES

TITLE **PAGE**

LIST OF PLATES

LIST OF SYMBOLS AND ABBREVIATIONS

CON-A- Concanavalin A

C.P.M- Counts Per Minute

D.C.P.M - Differential counts per minute

DTH-Delayed Type Hypersensitivity

ELISA-Enzyme Linked Immunosorbent Assay

GSM-CSF-Granulocytes Macrophages Colony Stimulating Factor

Ig G- Immunoglobulin G

IL- Interleukin

KDa- Kilo daltons

KHz-Kilohertz

Krad- Kilo radiations

MI –Multiple Dose Infection

PBS-Phosphate Buffered Saline

RPM-Revolutions per minute

RPMI-Rosewell Park Memorial Institute

SEA- Soluble Egg Antigen

SI- Single Dose Infection

SSP – Schistosomulae Soluble Protein

SWAP- Soluble Worm Antigen Preparation

Th-T-Helper Cells

µm-Micrometers

UV-Ultraviolet Light

CHAPTER ONE

1.0 INTRODUCTION

1. 1 Background Information

The common name for schistosomiasis is Bilharziasis, named after a German Scientist, Theodore Bilharz, who discovered the schistosome parasite in Egypt in 1852. The schistosome parasites are digenetic trematodes belonging to the super family Schistosomatoidea of sub-order Strigeata. Three important species cause human schistosomiasis with *S. japonicum* and *S. mansoni* causing hepatosplenic schistosomiasis and *S. haematobium* causing genito-urinary schistosomiasis. *S. intercalatum*, *S. matheei*, *S. bovis* and *S. mekongi* have been demonstrated to cause the disease in humans (Grove, 1990).

Schistosomiasis is a parasitic disease of both humans and domesticated animals. It is principally a disease of tropical and sub-tropical regions and is found in South and Central America, Africa, Asia and South East Asia. Schistosomiasis infects in excess of 200 million people in 74 countries and results in severe morbidity and mortality, with genito-urinary schistosomiasis infecting more than a 100 million people (King *et al.*, 2004). It is estimated that 500-600 million people are at risk of infection (Birgitte and David, 2004).

The distribution of the infection corresponds to the distribution of the snail hosts. Within endemic areas transmission may be focal and can be localized to some specific water sources (Berquist, 1995). The intensity and the frequency of exposure to contaminated freshwater determine the occurrence of the heavy infection that leads to disease. Prevalence and intensity of infection are usually correlated in endemic areas and especially in children (Kabatereine *et al.*, 2003).

Death may be caused by urinary tract disease infections in *S. haematobium* infection (Berquist *et al.*, 1996). The infection with *S. haematobium* is not synonymous with clinical diseases, and many infections are asymptomatic. The outcome of the infection is influenced by genetic factors, the immune response of the host and concomitant infections. Clinical manifestation is a sequel of heavy infection (Berquist, 1995). The life –cycle of the schistosomes require a fresh water snail intermediate host with planorbid snails of the genus *Biomphalaria* for *S. mansoni*, complex amphibious snails of the genus *Oncomelania* for *S. japonicum* and pulmonate snails of the genus *Bulinus* for *S. haematobium*. In the field, people are infected by a few parasite larvae (cercariae) at a time over a long period of time. However, work in the

laboratories has normally concentrated on a single infection, normally with higher numbers of larvae than would happen in the field. It is important to know if there is any difference between what happens in the field versus what happens in the laboratories in terms of immunological, pathological and parasitological aspects.

1.2 Statement of the Problem

In the study of protective immune responses in *S. haematobium* infections and attenuated cercariae studies in experimental animals, mainly baboons and hamsters, high single dose cercariae infections have been used. In nature, humans are exposed to multiple small doses of infections every time they are in contact with schistosome contaminated water bodies. Experimental work outlining the protective immune responses using the natural course of infection has not been done, thus drug efficacy tests and vaccine development in laboratory studies routinely use the single infection dose which may not give valid results as it does not reflect the situation in *S. haematobium* endemic areas.

1.3 The Objectives

General objective

To investigate the immune responses, pathology and parasitological patterns of multiple *S. haematobium* infected golden hamsters (MI) versus single dose infected hamsters (SI).

Specific objectives

1. To compare lymph node and spleen cells proliferation responses stimulated by SSP, SWAP, and SEA in both SI and MI group of hamsters at different time points post-infection.
2. To determine IgG levels, specific for SSP, SWAP and SEA antigens as induced by single or multiple infections at different time points post-infection.
3. To evaluate both gross and histopathology of the liver and urinary bladder in the two groups of hamsters at the specific time points post-infection.
4. To determine the worm burden in the two modes of infection.

5. To correlate the immune responses with pathology and worm burden as elicited by the two modes of infection.

1.4 Hypothesis.

The single infections (SI) and multiple infections (MI) of hamsters with *S. haematobium* parasites elicits different protective immune responses, and exhibits different gross pathology, histopathology and worm burdens at different time points post-infection.

1.5 Justification.

In the present study both single and multiple infections were compared. The aim of this work was to outline the protective cellular and humoral immune responses, parasitological and pathological patterns as induced by single or multiple *S. haematobium* infection doses in golden hamsters (permissive hosts).

It is important to know if the two modes of infections result in different disease patterns. This is crucial if the data generated in the laboratories from single infections, will be useful in the field. This work intends to validate the single infections and/or multiple infections of experimental animals used during drugs development and vaccine trials.

CHAPTER TWO

2.0 LITERATURE REVIEW

2. 1 History and Geographical Distribution

Theodore Bilharz in 1852 in the Nile valley in Egypt found a trematode in the blood of mesenteric veins of a man on autopsy; it was not a hermaphrodite like other flukes known at that time. He named the worm *Distomum haematobium* and described this as the cause of haematuria. In 1859 Cobbold used a new genus name *bilharzia* for the worm he found in the portal vein of a sooty monkey, although Weinland had already used the name Schistosoma meaning split body in 1858. Late 1903, Manson observed lateral spined eggs in the feaces of a patient who had no haematuria. In 1907, Sambon suggested that these eggs were the cause of intestinal infection and named it *Schistosoma mansoni* (Topley and Wilson, 1998).

Today schistosomes are described as digenetic trematodes of super family Schistosomatoidea, sub-order Strigeata. Unlike other trematodes schistosome adults are dioecious, they lack muscular pharynx, they produce non-operculate eggs and they parasitise blood vessels (Sturrrock, 1993). Four species, *S. mansoni*, *S. haematobium*, *S. japonicum* and *S. mekongi* are the most important parasites causing human schistosomiasis, although other species like *S. intercalatum*, *S. bovis* and *S. matheei* which are parasitic to other mammalian hosts may cause infection in humans (Grove, 1990).

Both *S. mansoni* commonly found in Asia and Africa and *S. japonicum* that is rampant in Asia parasitise the mesenteries and the liver. *S. haematobium* widely distributed in Africa and Middle East, parasitise the veins of vesicle and pelvic plexus. The disease is known as genito-urinary schistosomiasis.

In terms of social, economic and public health importance among parasitic diseases schistosomiasis ranks second behind malaria with more than 200 million people infected in 74 countries. 20 million people suffer severe consequences from schistosomiasis with 800,000 dying per annum. A further 500-600 million people are at great risk of infection (WHO, 2000). Of these, 100 million people are infected with *S. haematobium* in 53 countries (WHO, 1998).

2.2 Distribution in Kenya

In Kenya the presence of schistosomiasis has been attributed to rapid growth of human population. This has led to utilization of the natural resources through manipulation of the environment such as deforestation and damming of the flowing rivers. Expansion of irrigation schemes has also aggravated the situation. Kenya, as many other African countries, is plagued by several fresh water snails, which are intermediate hosts for the schistosome parasites (Goll *et al*, 1996). *S. haematobium* is mostly found in the lower and upper regions of the Coast, Lake Victoria region, Central province, Kano plains and parts of Western province (King *et al*., 2004).

2.3 Control of Schistosomiasis

Several approaches have been used to control the spread of schistosomiasis. Combined measures to reduce human contact with the snail infested water and to reduce the contamination of such bodies with schistosome eggs have been opted for. Different approaches have been used which include the elimination of the intermediate host by use of seasonal focal molluscides (Chu and Klump, 1987). Among the many molluscides, Niclosamide is most readily available. However it's very expensive and detrimental to the environment.

Biological control methods involving use of living organisms, which reduce the density of the target snail vector, are under test worldwide. These organisms may be categorized as predators, parasites, parasitoids, pathogens and competitors. The most promising approach is the introduction of non-susceptible snail species that act as competitors of intermediate host (Madsen, 1990). The most commonly used competitors are; *Marisa cornurietis, Helisoma duryi, Thira granifera* and *Melanoides tuberculate*, which have successfully been used against *T. granifera* and *M. tuberculata* in the Caribbean (Mac Cullough and Pointer, 1989).

Health education and sanitary awareness are also being employed to curb the spread of the disease (Bergquist, 1995). Chemotherapy has also been very successful using drugs such as praziquantel, a heterocyclic pyrazine isoquinoline that is effective against all species of schistosomes (Utzinger and Keiser, 2004). It induces an influx of calcium ions across the tegument of the schistosomes causing an immediate muscular contraction resulting to death of adult worms (Coili *et al*., 1996). Adult schistosome worms resistance to this drug has been reported (Fallon *et al*., 1995). The major drawback is that the drug is very expensive, continued surveillance is a requirement and re-infection always occurs (Fenwick *et al*., 2003).

An efficient method to curb the disease spread is dependent on the development of an effective and cheap vaccine. Schistosomes do not multiply inside the host and thus a completely sterilizing vaccine is not a necessity (Capron *et al.*, 1992). The current vaccine candidates include stage specific antigens, which include: glutathione-s-tranferase (Sm28GST), a 28 KDa enzyme found in schistosomula and adult stages (Balloul *et al.*, 1987; Capron *et al.*, 1992); Paramyosin (Sm 97), a 97 KDa muscle protein found in schistosomula and adult stages (Pearce *et al.*, 1986). Other approaches include creating anti-idiotype vaccine against carbohydrate antigens, interfering with egg production, and blocking sex pairing (Wynn, 1996).

In addition to these protein candidate antigens for vaccine, another interesting approach to schistosomiasis vaccine development involves irradiation of the cercariae. By optimally irradiating the cercariae of *Schistosoma mansoni* (20 Krad of gamma) they become attenuated and when injected in mice can induce up to 70% protection against a challenge with normal parasites (Allen and Maizels, 1996). To date there is no single schistosome vaccine that is on clinical use, therefore there is need for continued research to develop an effective vaccine. Shedding light in the immunity impacted by either multiple and single infection doses will elucidate whether use of several doses of vaccines during field trials and clinical applications will be a requirement.

2.4 Life Cycle of *Schistosoma haematobium*

The life cycle of *S. haematobium* is similar to other species. It starts with sexual reproduction of adult schistosomes within the vascular system of the definitive host (humans, rodents and primates) to asexual reproduction in the intermediate hosts, which are fresh water pulmonate snails of the genus *Bulinus*. The *Bulinus* species are divided into four groups: *B. africanus*, *B. forskalii*, *B. tropicanus/truncatus*, and *B. reficulatus* group. *Bulinus globossus* and *Bulinus nasutus* species from *B. africanus* group are commonly found in East Africa (Christiensen and Madsen 1992).

The *S. haematobium* embroyonated eggs have a yellowish-brown transparent shell characterized by terminal spine and measuring 115-185 µm by 40-70 µm. These are discharged from urine of infected individuals. On entering a hypotonic water environment, there is a decrease in the osmotic pressure as water enters the egg and the activation of the enzyme leucine aminopeptidase, results in rupture of the eggshell. The egg, which is mechanically ruptured along

its long axis, releases a highly motile (2 mm/sec) ciliated miracidium, which measures approximately 150-180 mm in length. To increase the chance of the miracidia locating the host, it has a negatively geotactic and positively phototactic behavioral response that tends to place it in the general environment of the snail host of the *Bulinus* species. Chemical attractants from the snail such as mucus, long chain fatty acids and even amino acids attract the miracidia. It has also been discovered that amines such as dopamine are also highly attractive to the miracidia. (WHO, 2000).

Penetration of the miracidia is a combination of mechanical motion of the apical papillae and histolytic secretions released from the penetration glands. The cilia are lost after penetration is complete. The location of the next stage within the snail, the sporocyst, is dependent on the schistosome species. For *S. mansoni* and *S. haematobium* it's at the site of penetration, usually the foot. For *S. japonicum* there is a preference for cavity organs, viscera and heart.

The mother sporocyst undergoes sporogony/asexual multiplication and produces 300-600 daughter sporocyst after about 3 weeks. As a result of asexual multiplication within the mother and daughter sporocyst thousands of cercariae all of the same sex are produced from a single miracidium. The cercariae measure 300-400 μm in length including the tail (WHO, 2000).

The cercariae infect the definitive host by penetrating the skin aided by proteolytic enzymes secreted by the penetration glands. During penetration the cercarial glycocalyx is lost and replaced by a lipid double bilayer, it loses its tail and transforms into the next larvae stage the schistosomulae in the blood vessels.

The schistosomules of *S. mansoni* migrate into the lungs via the venous circulation, during this period they undergo essential development by acquiring surface antigens and they also become elongated (Coulson *et al.*, 1985). The schistosomules leave the lungs through the pulmonary capillaries and they are carried in blood through the left ventricle of the heart into systemic circulation, finally reaching the portal system. In the intrahepatic portal circulation, feeding begins and further growth occurs upon sexual maturation. Male worms are 10-15 mm in length by 0.8-1 mm in diameter. Females are slender (0.25 mm in diameter) and longer (up to 20 mm in length). Adult males have a ventral in folding from the ventral sucker to the posterior end forming the gynecophoric canal (Locker, 1990).

The adult female is held in the gynecophoric canal of the male and the paired worms migrate to the vesicle plexus (probably via anastomoses in the umbilical plexus (Sturrock, 1993). Adult

schistosomes live in pairs in the pelvic veins (especially in the venous plexus surrounding the bladder). The schistosomes live in copula for the whole of their life. Each female lay about 150-300 eggs per day. The adult worm may live for 20-30 years with a mean life span of 3-8 years (WHO, 1998). In humans, the prepatent period between penetration of cercariae and first appearance of eggs in urine is approximately 10 weeks in case of *S. haematobium* (Manson-Bahr, 1987).

The adult schistosomes residing in the venous system avoid the host's immune system mainly by undergoing continuous and rapid turnover of its surface lipid, progressively losing its own antigens and acquiring host's antigens on its surface. Thus it avoids humoral immune response by mimicking the host's antigens (Von Lichtenberg, 1987).

The miracidium develops inside the egg over a period of 6 days. Eggs passing the bladder each contain an embryo, which is usually visible and motile and ready to hatch when passed in the urine. However not all eggs that are produced are passed through the urine. Some eggs are carried back in the venous plexus blood and become lodged in the liver or other organs and here they can survive for 11-12 days before forming the granulomas. The worms do not multiply inside the host and the amount of cercariae one is exposed to determine the extent of pathology that is developed (WHO, 2000). Information regarding whether trickle infections with *S. haematobium* cercariae elicit a protective immunological reaction that eliminate subsequent infections and lowering the worm burden is lacking.

2.5 The Disease

Schistosomiasis ranks second behind malaria among parasitic diseases in terms of socio-economic global importance. The disease primarily results from lack of health education and public health facilities, appalling sanitary conditions found in rural agricultural and peri-urban areas of underdeveloped nations and hence genito-urinary schistosomiasis has been christened African schistosomiasis (Brooker *et al.*, 2003).

The pathology of schistosomiasis is as a result of a granulomatous reaction towards the soluble egg antigen (SEA), which is characterized by a delayed-type hypersensitivity (DTH) which is associated with Th-2 response rather than Th-1 response normally characteristic of a DTH reaction (Macdonald *et al.*, 2002). In the primary infection, the granuloma is composed of aggregation of mononuclear phagocytes, neutrophils, eosinophils, lymphocytes, plasma cells and

fibroblasts. The granulomas may vary in sizes and cellular components are dependent on the immune status of the host (Demeure *et al.*, 1993).

Each paired worms residing in the vesicle plexus produce about 200-300 eggs per day. The majority of eggs traverse the bladder tissue into the bladder lumen and are eventually passed out in urine, however some eggs are swept back in blood circulation and are trapped in the tissues inducing immunologically mediated granulomatous inflammation and fibrosis. The egg burden is directly related to the disease severity. Significant clinical manifestation results from the deposition of eggs and the host reaction followed by years of infection with varying degrees of the parasites (WHO, 1998).

The main organs affected by *S. haematobium* infections are the urinary bladder, ureter, kidneys and partly the liver. Bladder pathology as demonstrated by utrasonography reveals lesions that appear as irregular, focal and generalized thickening of the bladder wall. Advanced disease present, with masses and granulomatous pseudo-polyps protruding in the bladder lumen (Christie *et al.*, 1986). Bladder wall calcifications are considered to be almost pathognomic of urinary schistosomiasis.

Schistosomal infection is heaviest in lower third of the ureter and fades towards the kidneys; lesion in the liver and periportal fibrosis has been reported (Wynn, 1996). Lesions of the ureter wall and calcification around the egg deposits have been detected using pyelography (Lemmer and Fripp, 1999). As a result of obstruction of the ureter, congestive changes in the kidney have been reported (Christie *et al.*, 1986).

The clinical manifestation of urinary schistosomiasis is accompanied by recurrent painless haematuria resulting from ulcerations of the urinary bladder mucosa; a burning sensation on frequent micturation and suprapubic discomfort may follow. In severe cases hydronephrosis may result from obstruction of urinary tract, causing renal parenchyma dysfunction (El-Bolkainy *et al.*, 1981). Less commonly affected are the prostate, the testes and the epididymis; a relationship between the presence of eggs in seminal fluid and male infertility has not been demonstrated. Co-existence of bacteria may accelerate damage of the kidneys. *Escherichia coli* is the most common organism, although *Pseudomonas spp. Klebbsiela spp* and *Salmonella spp* are found in complicated cases (Jacques *et al.*, 1998).

The urine of patients infected with *S. haematobium* contain high levels of nitrosamine in association with nitrate-reducing bacteria which have been associated with endogenous

formation of carcinogenic N-nitroso compounds in the urine which leads to the cancer of the bladder (Lemmer and Fripp, 1999). *S. haematobium* eggs are not common in female genital organs as in male patients. In females however, lesions are found in the vulva, vagina and the cervix. The lesions in the genital mucosa are an important risk factor for transmission of human immune deficiency virus (HIV) (Macdonald *et al.*, 2002; WHO, 2000).

2.6 Immunity to Schistosome Infection

During *S. haematobium* infection the immune system responds to the soluble egg antigen (SEA) released by the deposited eggs. There is a Th-2 switch, whereby Th-2 principal cytokines (IL-3, IL-4, IL-5, IL-6, IL-10 and granulocyte-macrophage colony stimulating factor; GM-CSF) are elevated (Booth *et al.*, 2004). IL-3 is a pluripotent colony-stimulating factor; IL-4 induces switch to IgE in B cells promoting IgE and IgG1 production; IL-5 is an eosinophil colony stimulating and differentiation factor; IL-6 promotes B-cells function while IL-10 inhibits Th-1 functions (Sturrock, 1993).

These immune responses bring about accumulation of eosinophils and macrophages at the site of egg deposition and are responsible for the resultant granuloma. On the other hand, IgG antibodies participate in an antibody dependent, cell-mediated cytotoxicity (ADCC) response that is the main mechanism of killing the miracidium in egg shell, which is mediated by monocytes, eosinophils and platelets (Allen and Maizels, 1996).

Mice immunized with *S. mansoni* optimally irradiated cercariae develop IgG antibodies that passively transfer resistance to naive recipients, indicating that immunized animal's protective humoral immunity is induced (Wynn *et al.*, 1996). The enhanced protective response is associated with increases in IgG1, IgG2a and IgG2b antibody isotypes (Demeure *et al.*, 1993).

IL-12 a cytokine that shifts T cells from the Th-2 sub set to Th-1 has been shown to reduce granuloma formation and subsequent fibrosis (Cheever and Xu, 1992). However it should be noted that if down regulation of the granuloma is carried too far, leaking antigen from the egg might elicit toxic effects that could damage the surrounding cells (Bergquist, 1995).

A number of factors may influence both the development and the level of morbidity in an exposed population: The degree and the length of exposure, (Booth, *et al.*, 2004), intensity of infection (Kabatereine, *et al.*, 2003), co-infection (Booth, *et al.*, 2004), host and parasite genetics (King *et al.*, 2004) and the ability to modulate those reactions (Booth *et al.*, 2004).

Concomitant immunity defined as the resistance, partial or total, of an actively infected host to a subsequent challenge infection with the same type of organism has also been observed in schistosomiasis (Dean and Mangold, 1996).

In the present study the disease patterns of multiple and a single dose infection of *S. haematobium* in golden hamsters has been compared. Pathological, parasitological and immunological aspects have been the test indices.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3. 1 Rearing and Infection of Snails with Miracidia

Bulinus globossus snails were collected from a dam at Mazeras, along the Kenyan coast. The snails were exposed to an artificial strong light (100 watts for 2 hours) so as to detect the snails with cercarial infections from the field. This process was repeated for five weeks. After the fifth week of screening, the non-positive snails were acclimatized and housed in the Malacology laboratory at Fort Jesus (National Museums of Kenya) in Mombasa. Urine was collected from school children of Kajiwe primary school. For ethical reasons all the positive children were treated with praziquantel. Urine was filtered through a 50-µm sieve to recover *Schistosoma haematobium* eggs. The eggs were washed two times in phosphate buffered saline (PBS) pH 7.2. The eggs were left to sediment in a urine jar after which they were aspirated using a Pasteur pipette into a petri dish. The eggs were exposed to an artificial strong light (100 watts for 15 minutes) to induce hatching of miracidium.

The snails were placed individually in each well of a 24-culture plate. Each snail was infected with 3-6 miracidium. The miracidium were left for 30 minutes to penetrate. The infected snails were put in a glass aquarium tank containing 18 litres of de-chlorinated water (snail water), sterilized sand and pebbles with some aquatic plants. Three weeks post infection the snails were transferred in a dark room to prevent shedding of the cercariae (IPR SOPS, 2002).

3.2 Experimental Animal Models

Fifty mature golden hamsters (*Mesocricetus auratus*) were used. Twenty mature hamsters were used for single dose infections (SI) and twenty for multiple dose infections (MI). Ten hamsters were naive controls (NC).

3.3 Multiple Infections (MI) of Golden Hamsters

Positive snails were exposed to strong light to induce shedding of approximately 3000 cercariae. All Group (MI) hamsters were anaesthetized. The anesthesia mixture was Ketamine and Rompun in the ratio of 7:3, respectively. The anesthesia dose was 4.5 ml/10kg of the hamster body weight. One-milliliter syringe was used to administer the anesthesia

intraperitoneally. The anaesthetized hamsters were shaven at the abdominal region and placed on the infecting rack. A piece of wet cotton wool was used to dampen the shaven area to aid in the penetration of the cercariae. One-centimeter diameter metal ring was placed on the shaven area of each of the hamsters. 50 cercariae were administered to each hamster. The hamsters were left on the infecting rack for 30 minutes to allow penetration of the cercariae before being returned to the cages. Infection with the same dose of cercariae was repeated every week for a total of four weeks until each hamster received 200 cercariae (IPR SOPS, 2002).

3.4 Single Dose Infection of Hamsters (SI)

On the fourth week after the start of multiple infections (MI), all the SI hamsters were anaesthetized as described in 3.3. The anaesthetized hamsters were shaven at the abdominal region and placed on the infecting rack. A suspension of 200 cercariae was administered using the ring method technique as in described in section 3.3.

3.5 Antigen Preparation

3.5.1 18-Hour Soluble Schistosome Preparation (SSP)

The *S. haematobium* cercariae heads and tails were separated according to a revised method of Ramolho-pinto *et al.*, (1974). A discontinuous percoll gradient was made; 70% gradient was made by mixing 21 mls of percoll with 9 mls of RPMI 1640 media in 50 ml test tube. One drop of Hepes buffer was added to the mixture to maintain a constant pH of 7.4. The 45% gradient was made by mixing 9 mls of percoll to 11 mls of RPMI 1640. One drop of Hepes buffer was also added. The 45% gradient was carefully layered on top of the 70% gradient to make a discontinuous gradient. Cercariae were chilled at 4 ^{0}C for one hour in a refrigerator. 5% glucose solution was made in double distilled water.

The chilled cercariae were centrifuged in chilled glass tubes at 100g for 10 seconds. Using a Pasteur pipette, the supernatant was sucked out and the pellet suspended in 0.5 mls of 5% glucose. The heads and the tails of the cercariae were separated by vortexing for 90 seconds. Using a Pasteur pipette the heads and the tails were carefully layered on top of the percoll gradient and centrifuged at 450g for 10 minutes. The heads forming at the interface of the gradient were aspirated using a Pasteur pipette and washed four times in complete media.

containing RPMI 1640 media, 10% foetal calf serum (BDSL, Kilmarnock UK), 0.1% gentamycin and $5x\ 10^{-5}$ β-mercaptoethanol and 2g/l sodium bicarbonate.

The heads were re-suspended in complete media in a bijou tube and incubated at 37 ^{0}C, 5% carbon dioxide for 18 hours. The heads were washed three times in a sterile phosphate buffered saline, pH 7.2 (0.85% sodium chloride, 0.02% potassium hydrogen phosphate in a litre of distilled water).

The schistosomules were sonicated at 23 kHz, 16 µm amplitude for 10 minutes with intervals of 10 seconds, followed by centrifugation at 100, 000g for 1 hour at 4 ^{0}C in order to obtain soluble proteins in the supernatant. Protein concentration was assayed, based on a method described by Bradford (1976).

The proteins were aliquoted and sterilized by exposure to U.V light for 10 minutes, 5 cm from a 30 watt UV OSRAM bulb before *in vitro* assays. Aliquots were stored at –20 ^{0}C. Protein concentration was adjusted to 1mg/ml in RPMI/10 before use in *in vitro* Assays.

3.5.2 Preparation of Soluble Worm Antigen (SWAP)

Adult schistosome worms were obtained by perfusion of a chronically infected baboon. The worms were sonicated, centrifuged assayed for proteins, aliquoted, sterilized and concentration adjusted as described in section 3.5.1 for SSP.

3.5.3 Preparation of Soluble Egg Antigen (SEA)

Urine from positive children was obtained and transported in dark bottles to the laboratories to prevent hatching of the eggs. *S. haematobium* eggs were obtained by filtering the urine through a 50µm sieve. The eggs were washed three times in phosphate buffered saline. The eggs were left to sediment in a urine jar after which the supernatant was decanted and discarded. The eggs were sucked into 10 ml beaker. The eggs were sonicated, centrifuged assayed for proteins, aliquoted, sterilized and concentration adjusted as described in section 3.5.1 for SSP.

3.5.4 Concanavalin A (CON-A)

Commercially prepared Concanavalin A (Con-A; Sigma cell culture) was dissolved in sterile phosphate buffered saline to form a concentration of 1mg/ml. The solution was aliquoted and

UV sterilized as for SSP and stored at –20 ^{0}C. A concentration of 1µg/ml per well was used in the *in vitro* assays.

3.6 Dissection of Hamsters

At each time point the required hamsters were anesthetized as described in section 3.3. An incision was made on the skin and the skin peeled off. The thoracic cavity was opened by cutting the rib cage above the diaphragm avoiding the sternum. A 21-gauge needle with a 2 ml syringe was inserted in the left ventricle of the heart and blood was drawn in small jerks. After the collapse of the left ventricle, blood was again drawn from the right ventricle and stored in 15 ml tubes at 4 ^{0}C for sera preparation.

Auxiliary lymph nodes were located and incised from under the skin in the armpit region. Inguinal lymph nodes were identified at hind legs under the skin and were also incised and placed in a petri dish containing incomplete media. The abdomen was opened to expose the viscera. The spleen was obtained and transferred in to a petri dish containing incomplete media (complete media without 10 % foetal calf serum) (IPR SOPS, 2002).

3.7 Preparation of Spleen Cells

Each of the spleens of hamsters from each group had been placed individually in a petri dish containing incomplete medium. The spleens were then transferred to a sterile wire mesh in a petri dish containing 5 ml sterile incomplete media in the culture hood. A 10 ml syringe piston was used to squash the spleen and a Pasteur pipette used to disperse the cells. The cells were dispensed in a 15 ml tube and incomplete media added to 10 ml mark. The cell suspension was centrifuged at 450g, at room temperature (22 ^{0}C) for ten minutes. The supernatant was discarded and the pellet re-suspended in incomplete media and the cells washed twice. After the second wash the supernatant was discarded, and cells were re-suspended by adding 4 mls of complete media.

Lymphocyte viability was determined by tryphan blue exclusion test. The cells were diluted 1:10 tryphan blue (10µl of cells + 90 µl of tryphan blue). The haemocytometer was charged and cells were counted under the light microscope at x 40. The cell concentration was adjusted to 3.0 x 10^{6} cells/ml in complete media (IPR SOPS, 2002).

3.8 Preparation of Lymph Node Cells

Auxiliary and inguinal lymph nodes from each hamster had been placed in a petri dish containing incomplete medium. They were then transferred on a petri dish containing sterile incomplete medium in the culture hood. A sharp forceps was used to tease the lymph nodes. A Pasteur pipette was used to disperse the cells and the suspension dispensed in 15ml tubes. The cells were washed two times by centrifuging at 450g at room temperature (22 ^{0}C) for 10 minutes. The cells were re-suspended in 1ml of complete media. The lymphocyte viability was determined by the tryphan exclusion method and cell concentration adjusted to 3.0 x 10^{6} cells/ml in complete media (IPR SOPS, 2002).

3.9 Proliferation Assay

The spleen and lymph node cells were cultured in a 96-well microtitre plates (Nunclon, Denmark). Duplicate wells were set for each test antigen. Each well contained 3.0 x 10^{5} viable cells of either the spleen or the lymph node cells. Negative controls wells contained the medium and the cells only, while the positive controls contained cells and Con A (1µg/well). The test wells of each plate contained cells and 2µl/ well of SWAP or 1µg/ well of SSP or SEA. The total volume of culture media per well was 200µl.

The plates were incubated at 37 ^{0}C in 5% Carbon dioxide. After either 48 hr for Con A or 72 hr for other antigen setups, the cells were pulsed with 20µl of 18.5 MBq tritiated Thymidine (^{3}H-thymidine: Amersham Pharmacia, UK) and incubated again for 18 hours. After 18 hours the cells were harvested on microfibre filter using Filtermate Harvester (Packard Bioscience Co. USA). The incorporated label was measured by liquid scintillation counting. The results were expressed in differential counts per minute D (C.P.M). i.e. C.P.M of Con-A or antigen stimulated cultures – C.P.M of negative controls. The mean D.C.P.M for the five hamsters in each group was obtained and tabulated against the time points (IPR SOPS, 2002).

3. 10 IgG Antibody Enzyme Linked Immunosorbent Assay (ELISA)

3.10.1 Hamster IgG ELISA

At week 3, 6 and 9 post-SI, blood from five hamsters from each of the three groups was obtained using the heart puncture technique. Blood was centrifuged at 1000g for 10 minutes to make the sera. Immunolon 4 plates were coated with 50µl of 10µg/ml of SWAP, SEA or SSP

individually in PBS/0.05% tween-20 buffer (pH of 9.6). The coated plates were incubated at 4 ^{0}C overnight. The plates were then blocked with100µl of 3% bovine serum antigen (BSA) in PBS/0.05% Tween-20 (Fisher chemicals, USA) per well and incubated for one hour at 37 ^{0}C. The plates were then washed three times with PBS/0.05% Tween-20 using an automatic ELISA plate washer. A dilution of 1:100 of hamster's sera in 1% PBS/ 0.05% Tween-20 was prepared. 50µl of the diluted sera from each animal was dispensed in each well. The plates were incubated at 37 ^{0}C for two hours after which the plates were washed three times with PBS/0.05% Tween-20 using the automatic ELISA plate washer. Goat anti-hamsters IgG conjugated with horseradish peroxidase (Jackson's Immunoresearchs Laboratories INC) were diluted 1:2000 in 3% BSA. 50µl of the goat anti-hamster IgG conjugated with horseradish peroxidase was added to each well. The plates were incubated for one hour at 37 ^{0}C. The plates were then washed three times with PBS/0.05% Tween-20 using the automatic ELISA plate washer. 50µl of peroxidase substrate (TMB micro well peroxide substrate, Kirkegaard and Perry labs, USA) per well was added. After 15 min absorbance at 630 nm was determined using a Maxi Kinetic Micro plate Reader (Molecular Devices, Palo Alto, CA). The mean absorbance of the five animals in each group was obtained (Yole *et al.*, 1996).

3.10.2 Mouse IgG ELISA

Elisa was repeated as in 3.10.1 but instead of adding goat anti-hamster IgG conjugated to horseradish peroxidase, goat anti-mouse IgG conjugated to horseradish peroxidase (Jackson's immunoresearchs laboratories INC), diluted 1:2000 was added.

3. 11 Perfusion and Adult Worm Recovery

At week 12 post-SI, five hamsters from each group were perfused to recover adult worms using a modified method of Smither and Terry (1965). The hamsters were anaesthetized as described in 3.3. A transverse mid-ventral nick was made on the skin at the abdomen and the skin peeled off upwards and downwards. The thoracic cavity was opened by carefully cutting the rib cage until the heart was exposed. The abdominal wall was opened without cutting the viscera. The hepatic portal vein was located and incised. The perfusion fluid containing 0.85% sodium chloride and 1.5% sodium nitrate was used. The perfusion needle was inserted in the left ventricle of the heart and perfusion carried out until the liver, lower limb and mesenteries were

clear. The perfusate was collected in a beaker and then transferred in to a urine jar and left to settle. The worms were recovered, counted and the means of adults both males, females and stunted worm calculated according to a method described by Yole *et al.*, (1996).

3. 12 Gross Pathology and Histopathology

At weeks 6, 9 and 12 post-SI, gross pathology of the liver and the urinogenital system were determined. The observations considered in the liver included the colour, size and the presence or absence of granulomas. The urogenital system was observed for presence of granulomas. At each time point, the viscera, digestive system and urogenital system of all the five hamsters from each group were collected and stored in 10% buffered formalin for 2 weeks before histological examination.

Representative samples were cleared in toluene, infiltrated in hot paraffin and embedded on tissue-embedding paraffin wax (Sherwood Medical Co, USA). The tissues were sectioned serially at 6 µm using a rotary microtome. The thin tissue sections were stained with hematoxylin and eosin dyes (Farah *et al.*, 1997).

3. 13 Statistical Analysis

Worm burdens, ELISA data and histopathological (granuloma diameters) results and proliferation assays were compared using Student's two-tailed t test. $p < 0.05$ was regarded as significant.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4. 1 Immunological results

4.1.1 Lymphocytes proliferative responses

4.1.1.1 SSP stimulated lymph node cells

The proliferative responses of lymph node cells as stimulated by SSP at different time points post infection, from the two groups are shown in Figure 1.0. At week 3 post-SI, the SI group had higher responses than the MI group and the differences were significant at $p<0.05$. At week 6 post-SI, the MI group responses increased significantly while the SI responses only decreased slightly. However the difference between the SI and MI group was significant at $p<0.05$. At week 9 post-SI, the MI responses only slightly higher than the SI responses. The responses were not significantly different at $p>0.05$.

4.1.1.2 SWAP stimulated lymph node cells

Comparative results of proliferative responses by lymph node cells to SWAP at different sampling points post-infection of the two groups are shown in Figure 1.1. At week 3 post-SI, lymph node cells for both group showed little responses to SWAP. SI group had only marginally higher response than MI group. At week 6 post-SI, the MI group showed substantially increased sensitization with levels of SI remaining almost constant. The responses were significantly different at $p<0.05$. At week 9 post-SI, the MI group responses showed only slight increase while the SI group exhibited a drastic rise in sensitization, the difference between the two groups were statistically significant at $p<0.05$.

4.1.1.3 SEA stimulated lymph node cells

Proliferative responses of LN cells that were stimulated *in vitro* by SEA are shown in Figure 1.2. At week 3 post-SI, MI responses were higher than the SI group exhibiting a significant difference ($p<0.05$). At week 6 the MI responses increased substantially and was higher than the SI responses but the responses were not significantly different at $p>0.05$. At week 9, SI group had a drastic increase in lymph nodes sensitization with MI group recording a gradual increase

although its level remained lower than that of SI group. The differences in week 9 were significant at $p<0.05$.

4.1.1.4 CON A stimulated lymph node cells

Proliferative responses of lymph node cells stimulated *in vitro* by CON A (a plant mitogen) for the two groups at the different sampling points are shown in Figure 1.3. At week 3 post-SI, the singly infected group exhibited more proliferation responses than the MI group although the differences are not significant at $p>0.05$. At week 6 post-SI, however the MI responses increased only slightly with SI responses falling marginally. Week 9 post-SI, the MI levels remained almost stagnant while the levels of SI group increased significantly at $p<0.05$. The responses induced by CON A were much higher, about 20-50 fold compared to those by *Schistosoma haematobium* specific antigens.

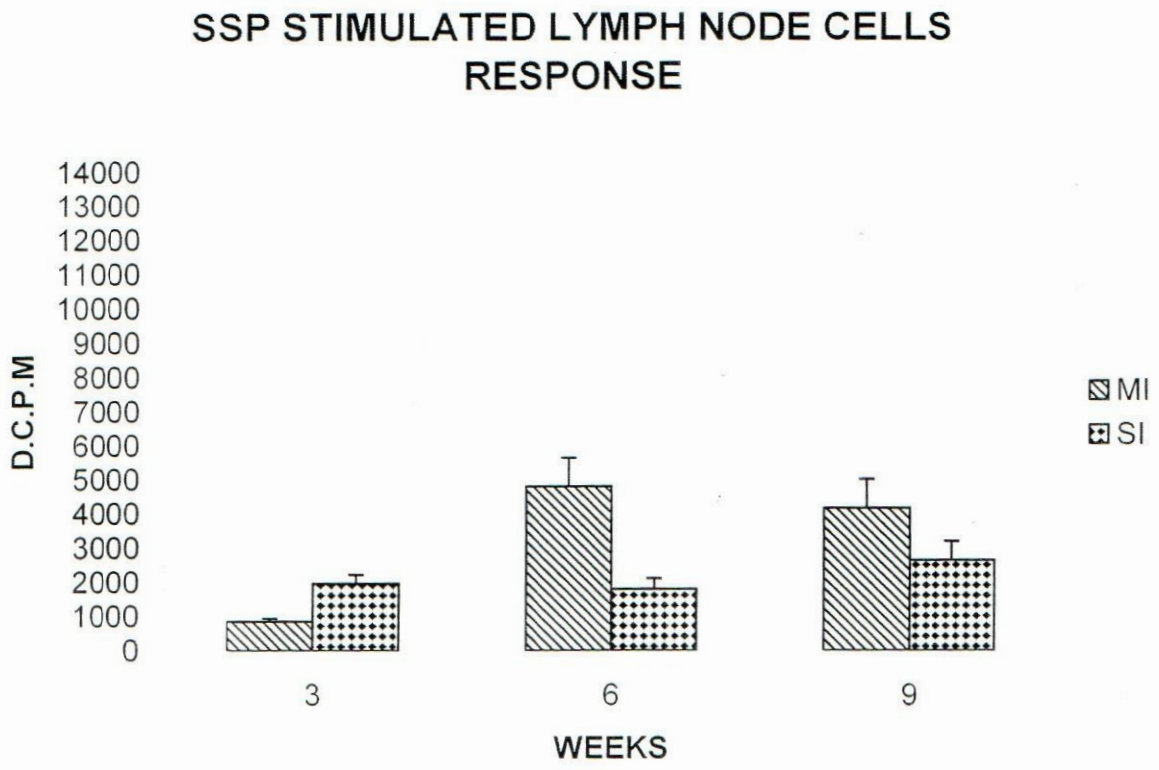

Figure 1.0

Graphical representation of lymph node cells stimulated by soluble schistosomula preparation (SSP) of *S. haematobium* in two groups of hamsters: multiply infected (MI) and singly infected (SI) at different time point's post-SI. The responses are in D.C.P.M-Differential counts per minute.

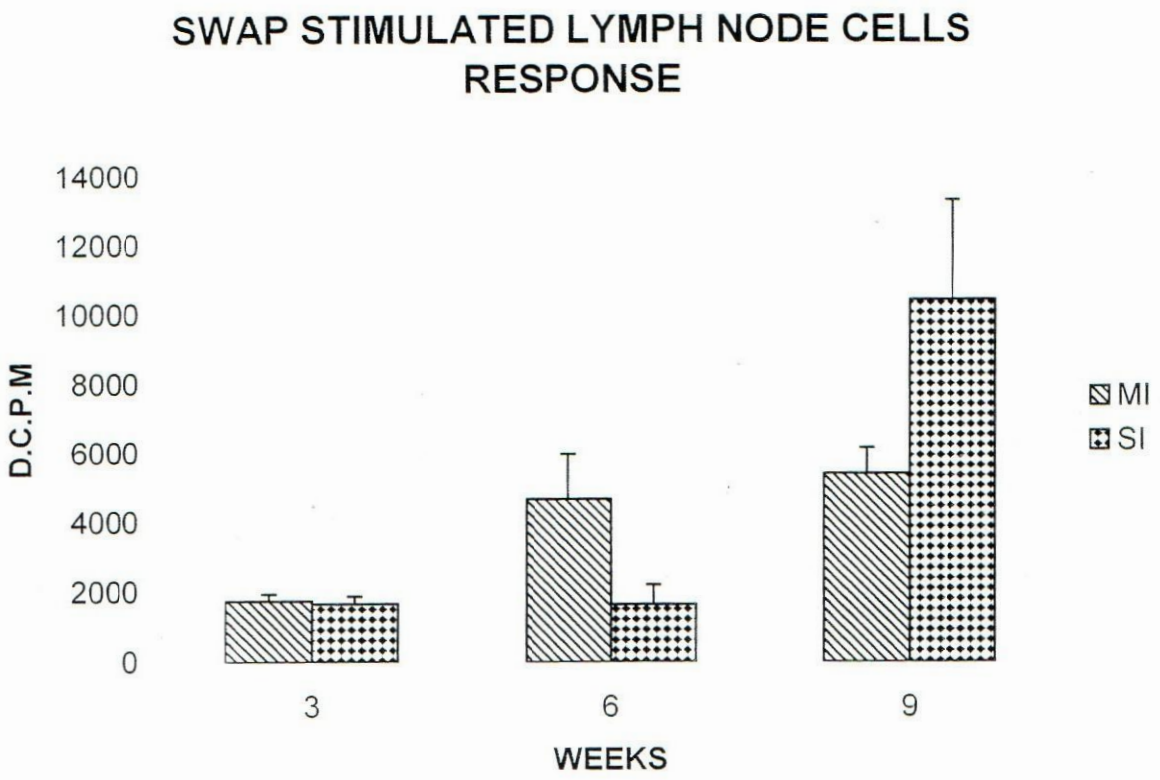

Figure 1.1

Graphical representation of lymph node cells stimulated by soluble worm antigen preparation (SWAP) of *S. haematobium* in two groups of hamsters; multiply infected (MI) and singly infected (SI) at different time points post-SI. The responses are in D.C.P.M-Differential counts per minute.

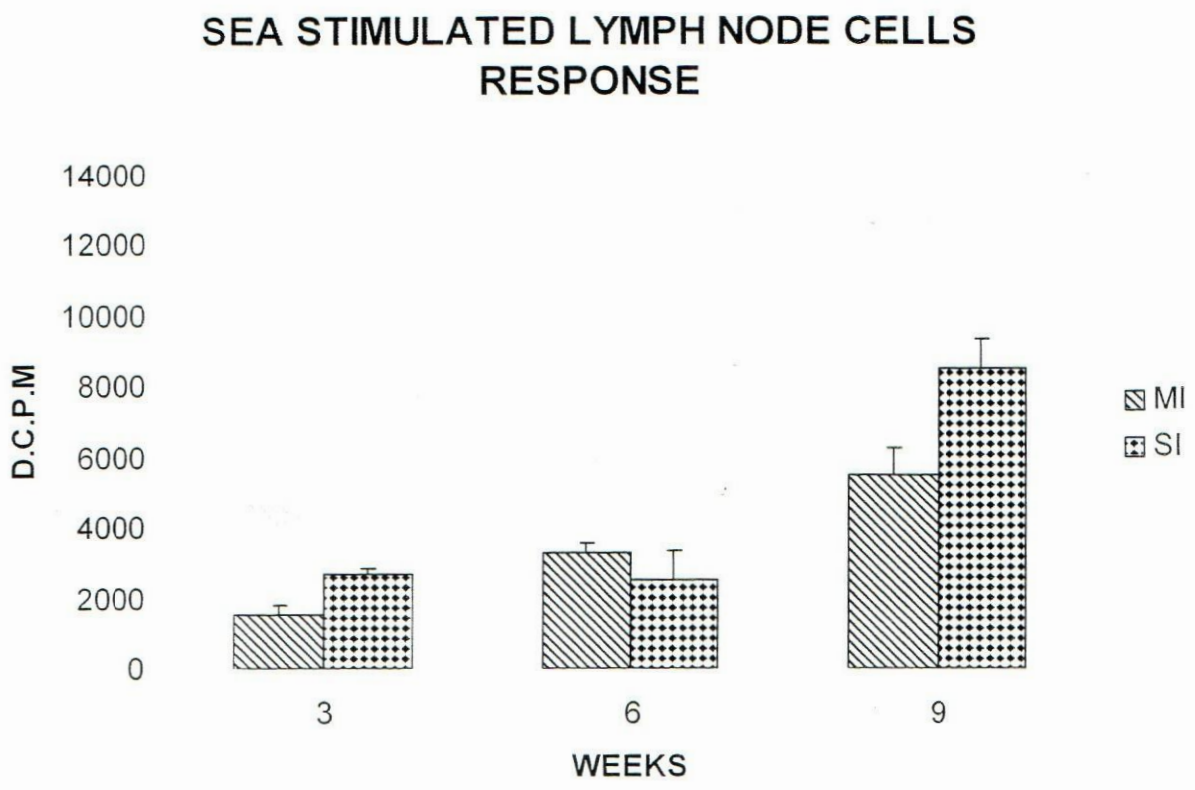

Figure 1.2

Graphical representation of lymph node cells stimulated by soluble egg antigen preparation (SEA) of *S. haematobium* in two groups of hamsters; multiply infected (MI) and singly infected (SI) at different time points post-SI. The responses are in D.C.P.M-Differential counts per minute.

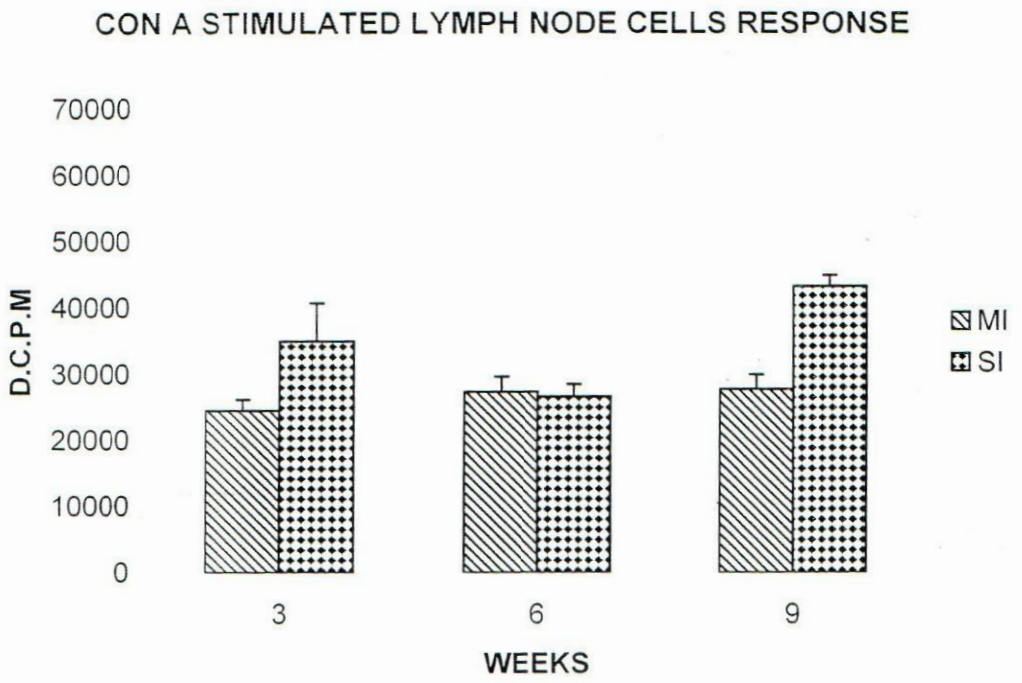

Figure 1.3

Graphical representation of lymph node cells stimulated by Concanavalin A (CON A) a plant mitogen in two groups of hamsters: multiply infected (MI) and singly infected (SI) at different time points post-SI. The responses are in D.C.P.M-Differential counts per minute.

4.1.2 Spleenocytes proliferative responses

4.1.2.1 Spleen cells stimulated by SSP

Proliferative responses of spleen cells stimulated *in vitro* by SSP are shown in Figure 2.0. Week 3 post-SI infection, MI group had significantly high responses as compared to SI group. At week 6 however, the MI and SI responses fell significantly although the MI responses remained marginally higher than SI stimulations with the two groups exhibiting a non-significant difference at $p<0.05$. At week 9 post-SI, both SI and MI stimulus to SSP increased only slightly with MI responses again higher than SI responses, although not statistically significant at $p<0.05$.

4.1.2.2 Spleen cells stimulated by SWAP

Spleenocytes proliferative responses to SWAP to are shown in Figure 2.1. At week 3 post-SI infections, the MI hamsters had a statistically significant high response than SI hamsters $p<0.05$. The responses for MI group fell drastically at week 6 post-SI, with SI group exhibiting a higher response with a significant difference $p<0.05$. At week 9 post-SI, both SI and MI responses to SWAP stimulus increased for both group with the SI group remaining higher than the MI group, and the differences were again statistically different at $p<0.05$.

4.1.2.3 Spleen cells stimulated by SEA

Spleenocytes proliferative responses to SEA are shown in Figure 2.2.Throughout the sampling points, week 3 post-SI recorded the highest responses. At Week 3 post-SI, the SI group posted slightly higher stimulation than the MI group though the differences were not significant at $p>0.05$. Week 6 post-SI, MI spleenocytes recorded a drastic fall in responses to SEA with a similar scenario for SI group, though SI responses exhibited a significantly higher response at $p<0.05$. Week 9 post-SI both groups recorded an increase in stimulation, with MI showing a higher response than SI. The difference was significant at $p<0.05$ in the two groups.

4.1.2.4 Spleen cells stimulated by CON A

Proliferative responses of spleen cells stimulated *in vitro* by CON A are shown in Figure 2.3. In both groups, stimulation by CON A was lowest at week 3 post-SI both increasing at week 6 and peak stimulation at week 9 for both groups.

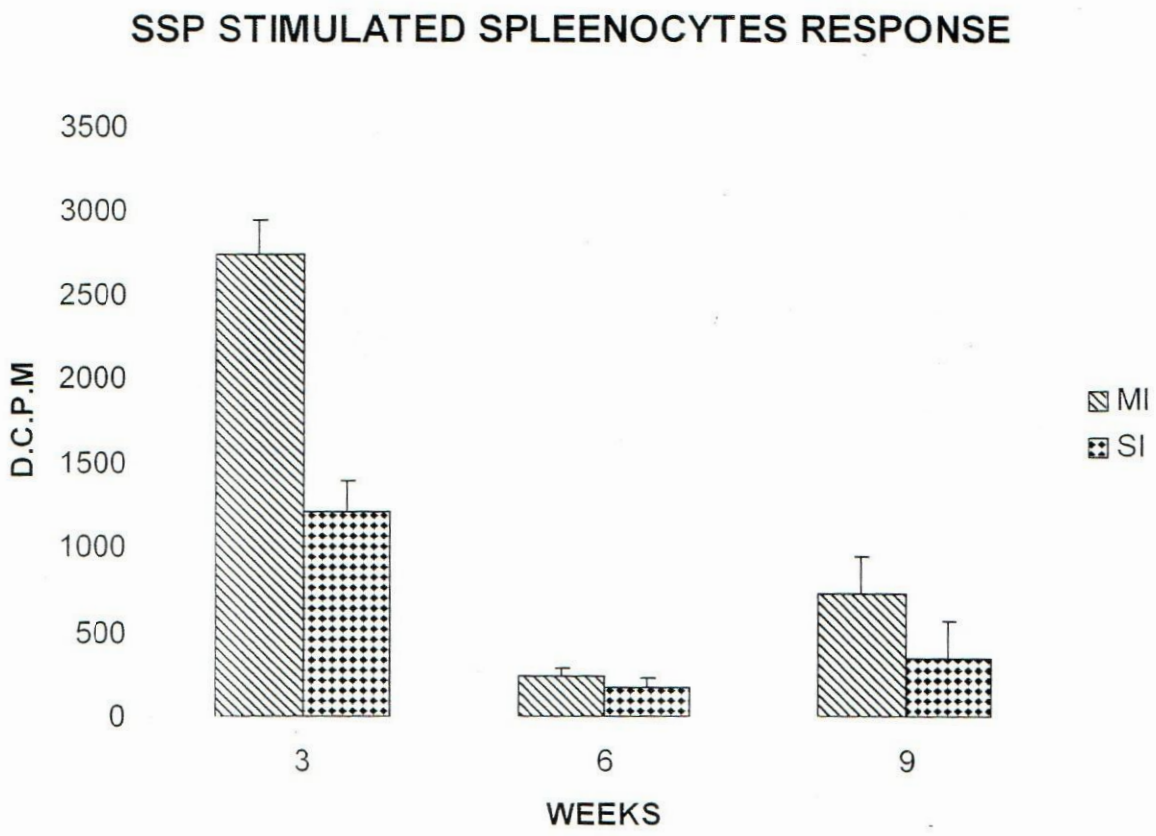

Figure 2.0

Graphical representation of spleenocytes stimulated by Schistosomula soluble preparation (SSP) of *S. haematobium* in two groups of hamsters: multiply infected (MI) and singly infected (SI) at different time points post-SI. The responses are in D.C.P.M-Differential counts per minute.

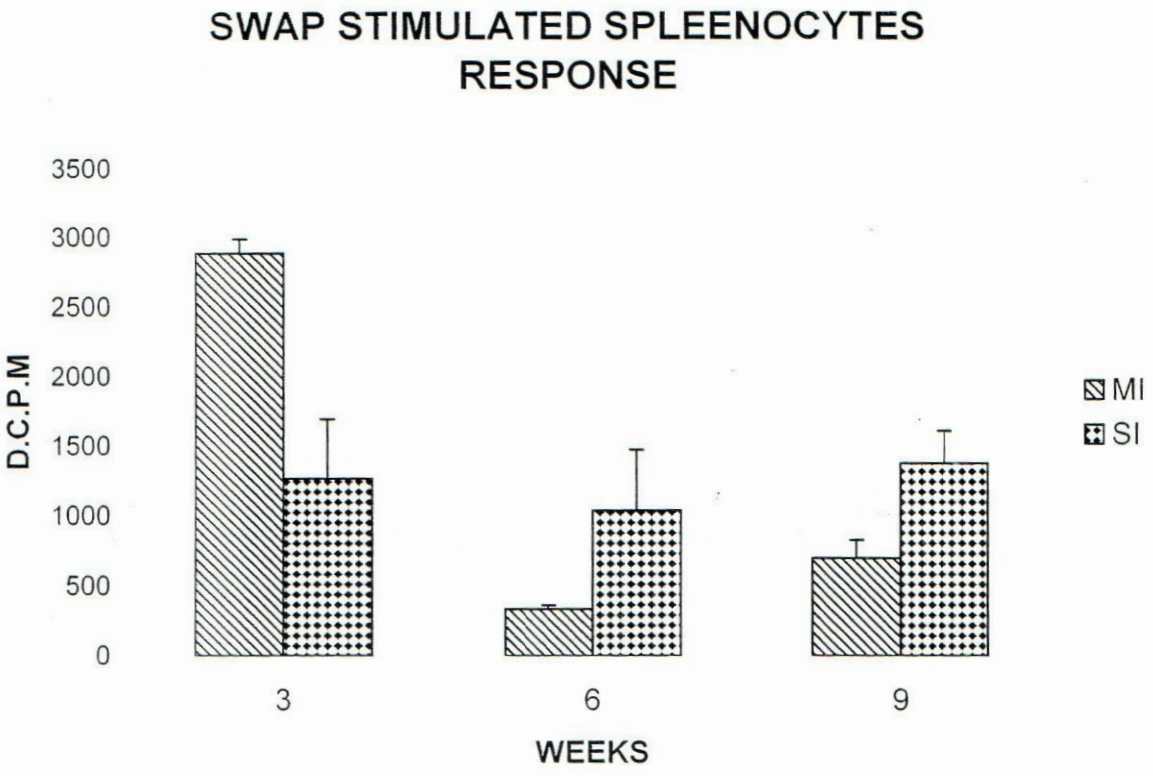

Figure 2.1

Graphical representation of spleenocytes stimulated by soluble worm antigen preparation (SWAP) of *S. haematobium* in two groups of hamsters; multiply infected (MI) and singly infected (SI) at different time points post-SI. The responses are in D.C.P.M-Differential counts per minute.

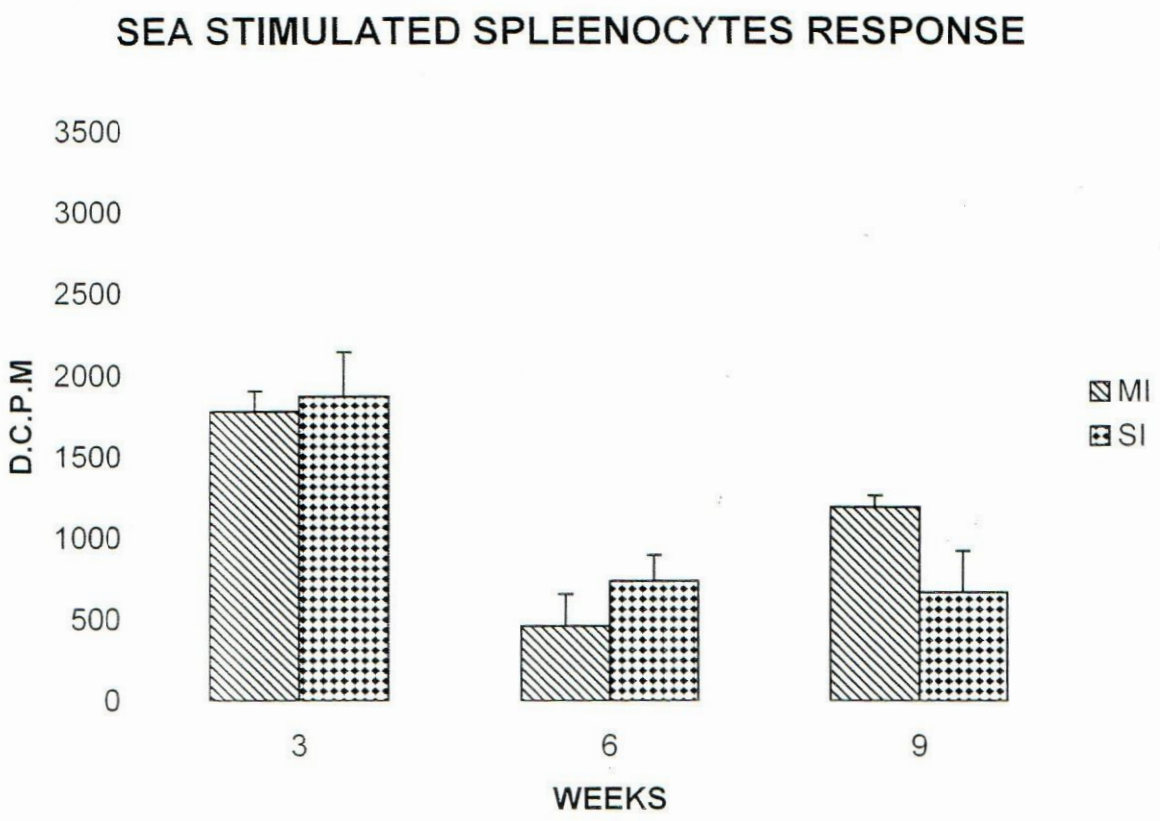

Figure 2.2

Graphical representation of spleenocytes stimulation by soluble egg antigen (SEA) in two groups of hamsters; multiply infected (MI) and singly infected (SI) at different time points post-SI. The responses are in D.C.P.M-Differential counts per minute.

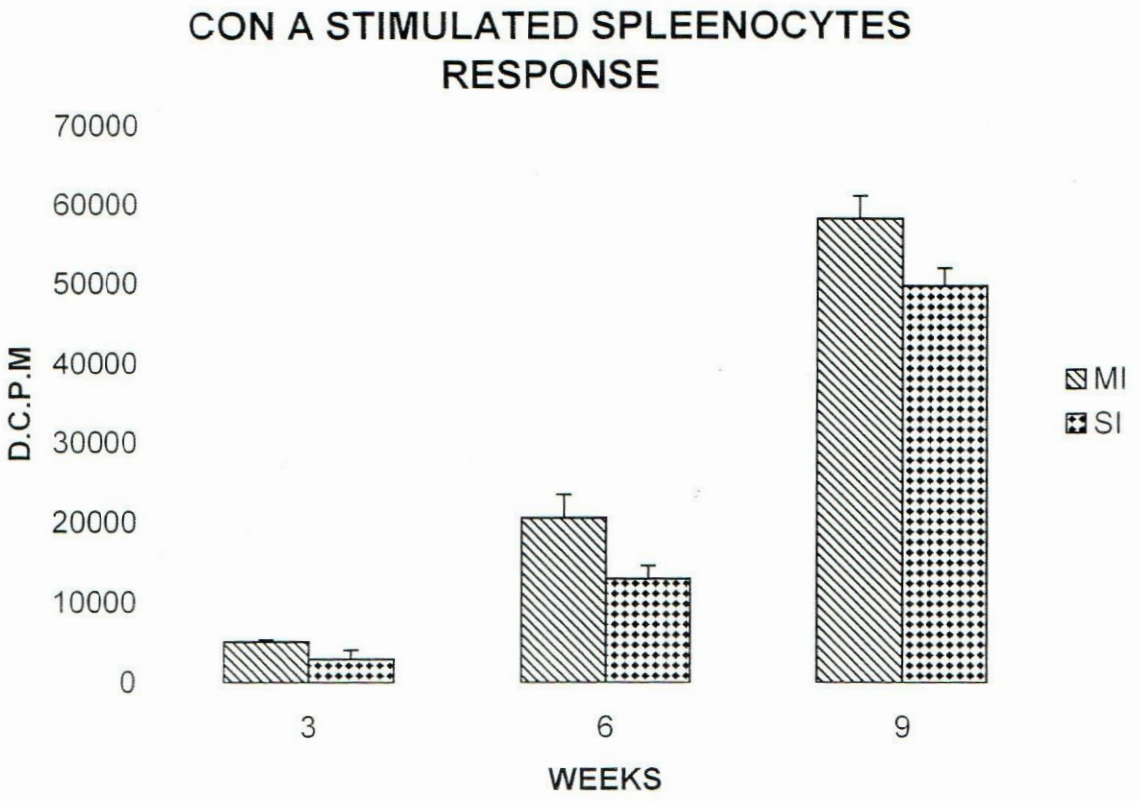

Figure 2.3

Graphical representation of spleenocytes stimulation by concanavalin A (CON A) in two groups of hamsters; multiply infected (MI) and singly infected (SI) at different time points post-SI. The responses are in D.C.P.M-Differential counts per minute.

4.1.2 IgG enzyme linked immunosorbent assays (ELISA)

4.1.2.1 IgG levels against SSP (anti-hamster)

SSP stimulated IgG levels for the two groups of hamsters, using goat anti-hamster IgG conjugated to horseradish peroxidase are shown in Figure 3.0. At week 3 post-SI infections, the MI group had lower levels of IgG than the SI group although the levels were not significantly different at $p<0.05$. At week 6 post-SI, the MI levels increased slightly while the SI levels fell, however the levels were not significantly different at $p<0.05$. At week 9 post-SI infections, both MI and SI levels recorded a slight decrease but the MI levels remained marginally higher than the SI levels and again the levels were not significantly different at $p<0.05$. Throughout the sampling points, the control group recorded the lowest IgG levels. The levels were significantly lower ($p>0.05$) than the levels recorded in the MI and the SI groups.

4.1.2.2 IgG levels against SWAP (anti-hamster)

SWAP stimulated IgG levels for the two groups of hamsters, using goat anti-hamster IgG conjugated to horseradish peroxidase are shown in Figure 3.1. SWAP stimulated IgG levels of MI group were almost equal to the SI levels at week 3 post-SI infection. MI levels increased marginally at week 6 post-SI infection with SI levels falling only slightly. The levels were however significantly different at $p<0.05$. At week 9 post-SI, there was no significant change in IgG levels in the two groups and the MI levels remained marginally higher than the SI group. Throughout the sampling points, the control group recorded the lowest IgG levels. The levels were significantly lower at $p<0.05$ than the levels recorded in the MI and the SI groups.

4.1.2.3 IgG levels against SEA (anti-hamster)

SEA stimulated IgG levels for the two groups of hamsters, using goat anti-hamster IgG conjugated to horseradish peroxidase are shown in Figure 3.2. At week 3 post-SI, SEA stimulated IgG levels of MI group were significantly higher than the SI group ($p<0.05$). At week 6 post-SI however, the SI group recorded a significant increase in the IgG levels and that of MI group remained almost unchanged. The levels of MI and SI were not significantly different ($p>0.05$) though the MI levels were marginally higher than SI levels. At week 9 post-SI the MI levels remained almost constant while the SI group recorded á marginal rise in IgG level. The levels were not significantly different ($p>0.05$), although the MI levels remained slightly higher

than the SI levels. Throughout the sampling points, the control group recorded the lowest IgG levels. The levels were significantly lower ($p>0.05$) than the levels recorded in the MI and the SI groups.

4.1.2.4 IgG levels against SSP (anti-mouse)

SSP stimulated IgG levels for the two groups of hamsters, using goat anti-mouse IgG conjugated to horseradish peroxidase, are shown in Figure 3.3. At week 3 post-SI, the MI group had significantly lower levels of IgG than the SI group at $p<0.05$. At week 6 post-SI, the SI group exhibited a significant fall in IgG levels specific to SSP while the MI group exhibited a marginal rise in IgG levels with the two levels being significantly different at $p<0.05$. At week 9 post-SI, IgG levels in the MI group remained almost constant while the SI group recorded only a marginal rise in the IgG levels. The levels were however statistically significant at $p<0.05$. These IgG trends were in line with the results recorded using the goat anti-hamster IgG conjugated to horseradish peroxidase. Throughout the sampling time points the IgG levels of the control group remained very low in comparison with the MI and the SI group with a significant difference at $p<0.05$, except for SI at week 6.

4.1.2.5 IgG levels against SWAP (anti-mouse)

SWAP stimulated IgG levels for the two groups ofhamsters, using goat anti-mouse IgG conjugated to horseradish peroxidase, are shown in Fgure 3.4. At week 3 post-SI, the MI group had a higher level of IgG than the SI group though the levels were no significantly different at $p>0.05$. MI levels had a significant rise in IgG levels at $p<0.05$ at week 6 post-SI, while the SI levels only increased marginally at this time point. The two groups had significantly different IgG levels at this time point at $p<0.05$. At week 9 post-SI infection the MI IgG levels had an insignificant fall at $p<0.05$ while the SI levels increased only slightly though the MI levels remained significantly higher than the SI levels at $p<0.05$. Again these trends were in line with the results obtained using the goat anti-hamster IgG conjugated to horseradish peroxidase, although more significant differences were recorded between the groups using mouse conjugate along the time points. Throughout the sampling time points the IgG levels of the control group

remained low in comparison with the MI and the SI group with a significant difference at $p<0.05$.

4.1.2.6 IgG levels against SEA (anti-mouse)

SEA stimulated IgG levels for the two groups of hamsters, using goat anti-mouse IgG conjugated to horseradish peroxidase, are shown in Figure 3.5. At week 3 post-SI, the MI group had higher IgG levels than the SI group. The levels were significantly different at $p<0.05$. At week 6 post-SI, the IgG levels of the MI group remained higher than those of SI group and again the levels were significantly different at $p<0.05$. At week 9 post-SI, the MI IgG levels remained higher than those of the SI group and again the difference was significant at $p<0.05$. Throughout the sampling time points the IgG levels of the control group remained low and constant in comparison with the MI and the SI group with a significant difference at $p<0.05$.

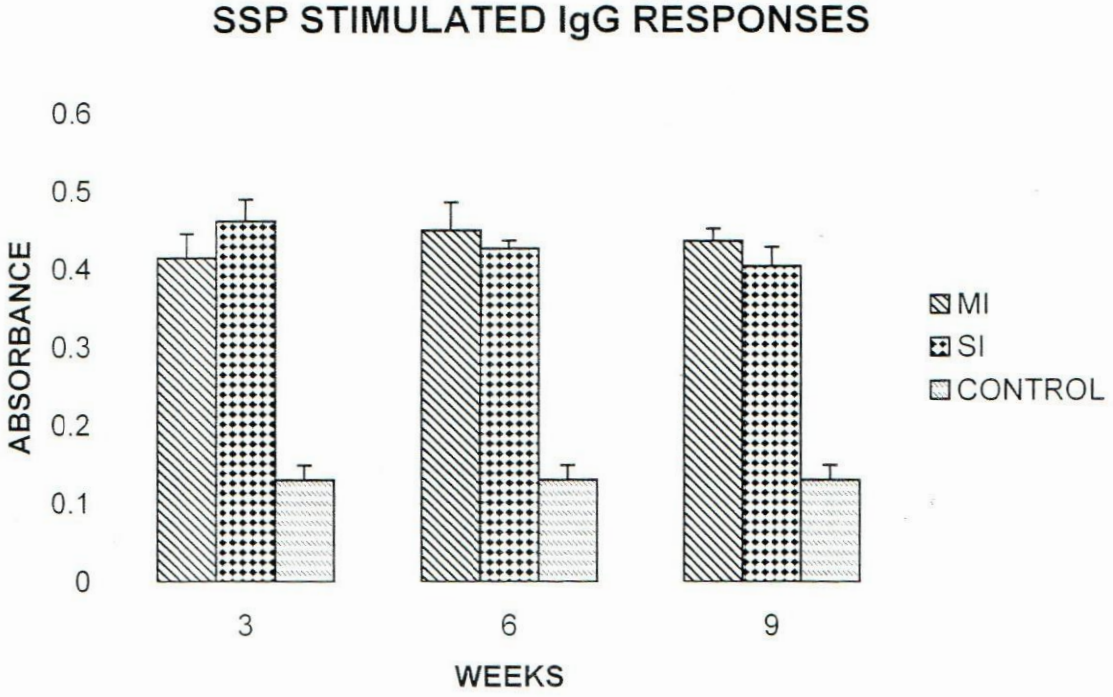

FIGURE 3.0

A comparative graphical representation of SSP specific IgG levels, in the multiply infected group (MI), singly infected group (SI) and the control group (NC). Using goat anti-hamster IgG conjugated to horseradish peroxidase at three point's post-SI.

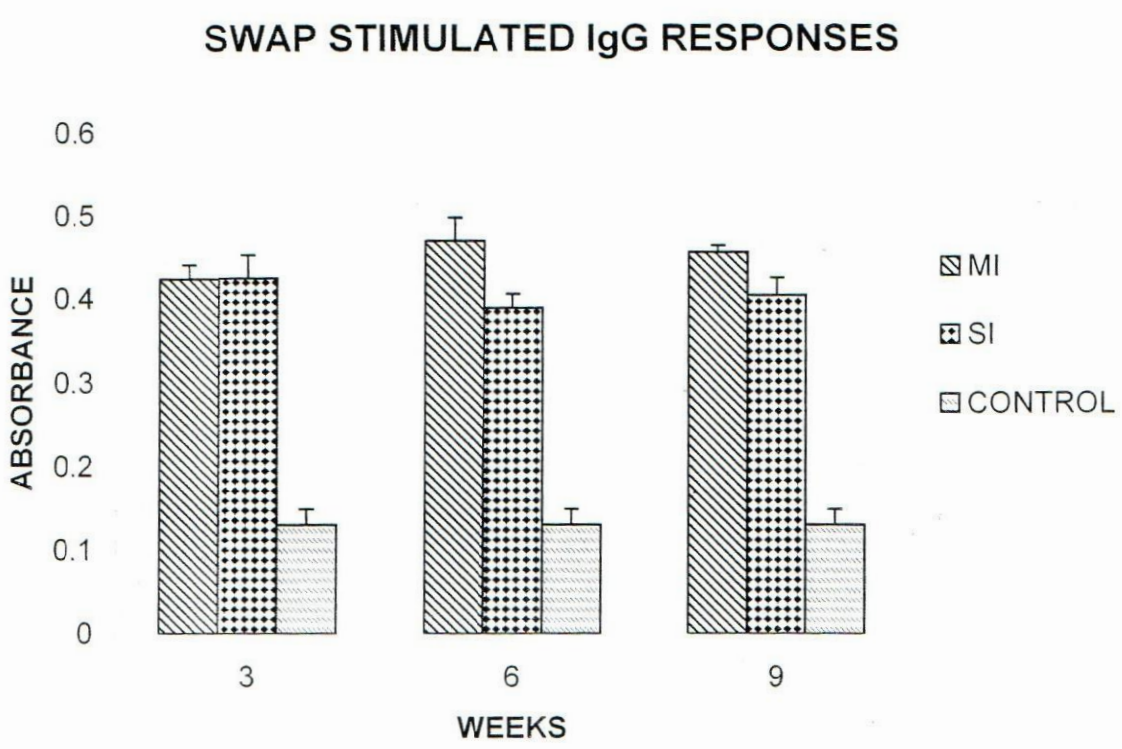

FIGURE 3.1

A comparative graphical representation of SWAP specific IgG levels, the multiply infected group (MI), singly infected group (SI) and the control group (NC) using goat anti-hamster IgG conjugated to horseradish peroxidase at three point's post-SI.

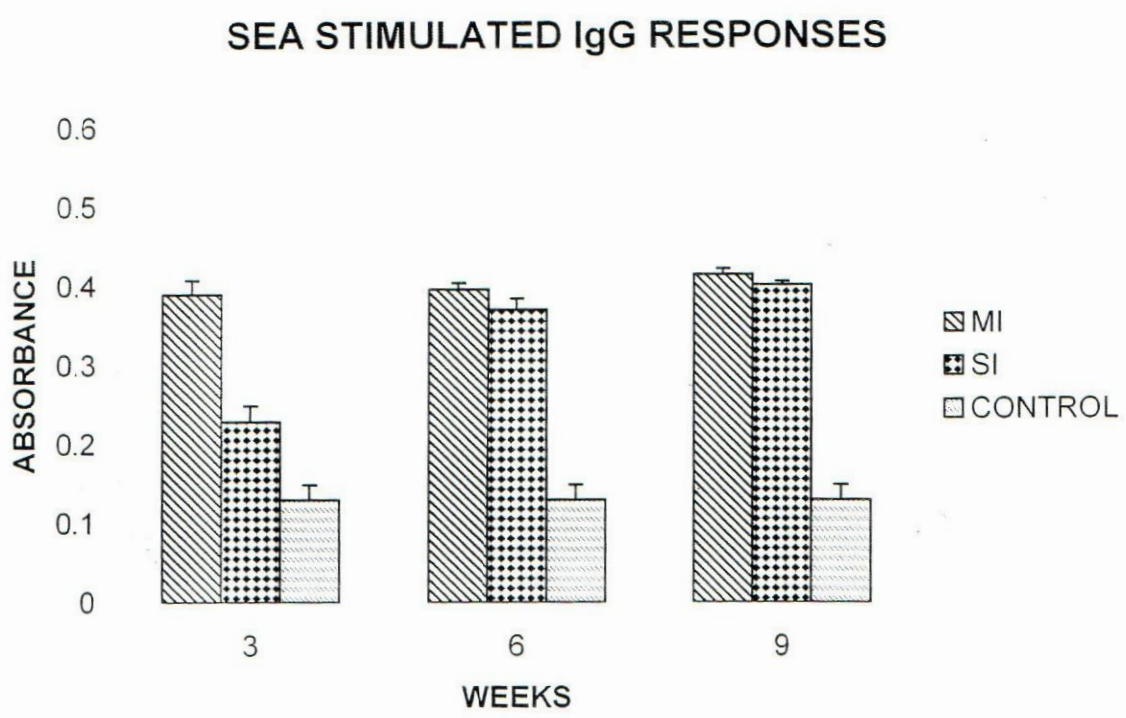

FIGURE 3.2

A comparative graphical representation of SEA specific IgG levels, in the multiply infected group (MI), singly infected group (SI) and the control group (NC) using goat anti-hamster IgG conjugated to horseradish peroxidase at three time point's post-SI.

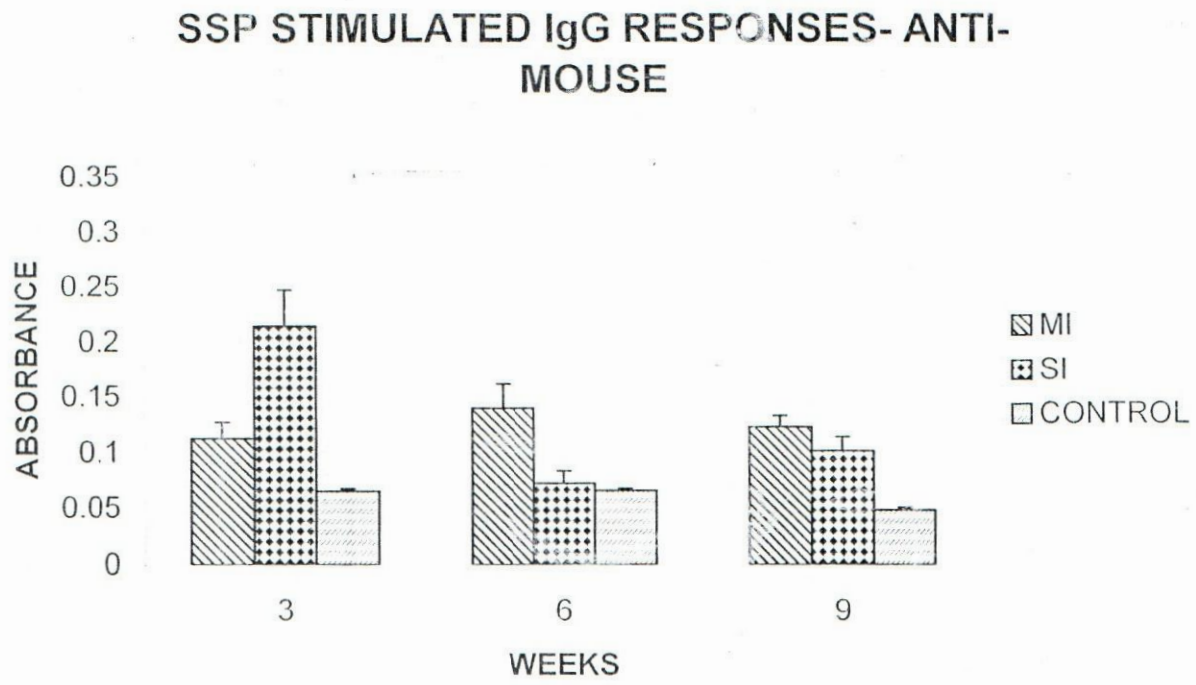

FIGURE 3.3

A comparative graphical representation of SSP specific IgG levels in the multiply infected group (MI), singly infected group (SI) and the control group (NC) using goat anti-mouse IgG conjugated to horseradish peroxidase at three time points post-SI.

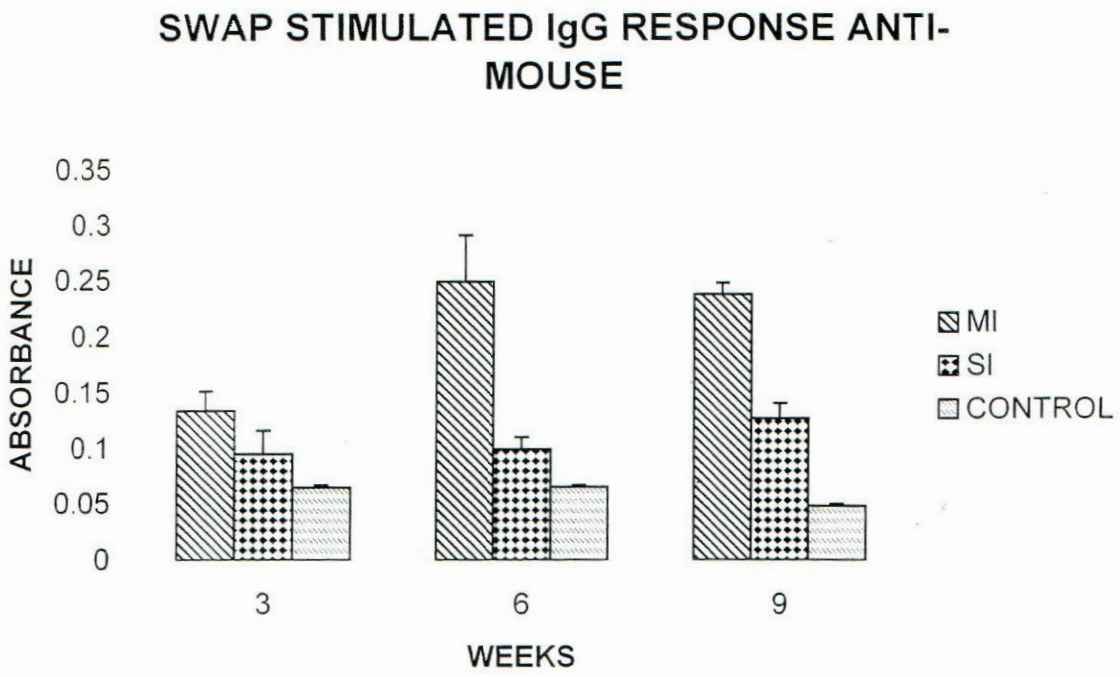

FIGURE 3.4

A comparative graphical representation of SWAP specific IgG levels in the multiply infected group (MI), singly infected group (SI) and the control group (NC). Using goat anti-mouse IgG conjugated to horseradish peroxidase at three-time point's post-SI.

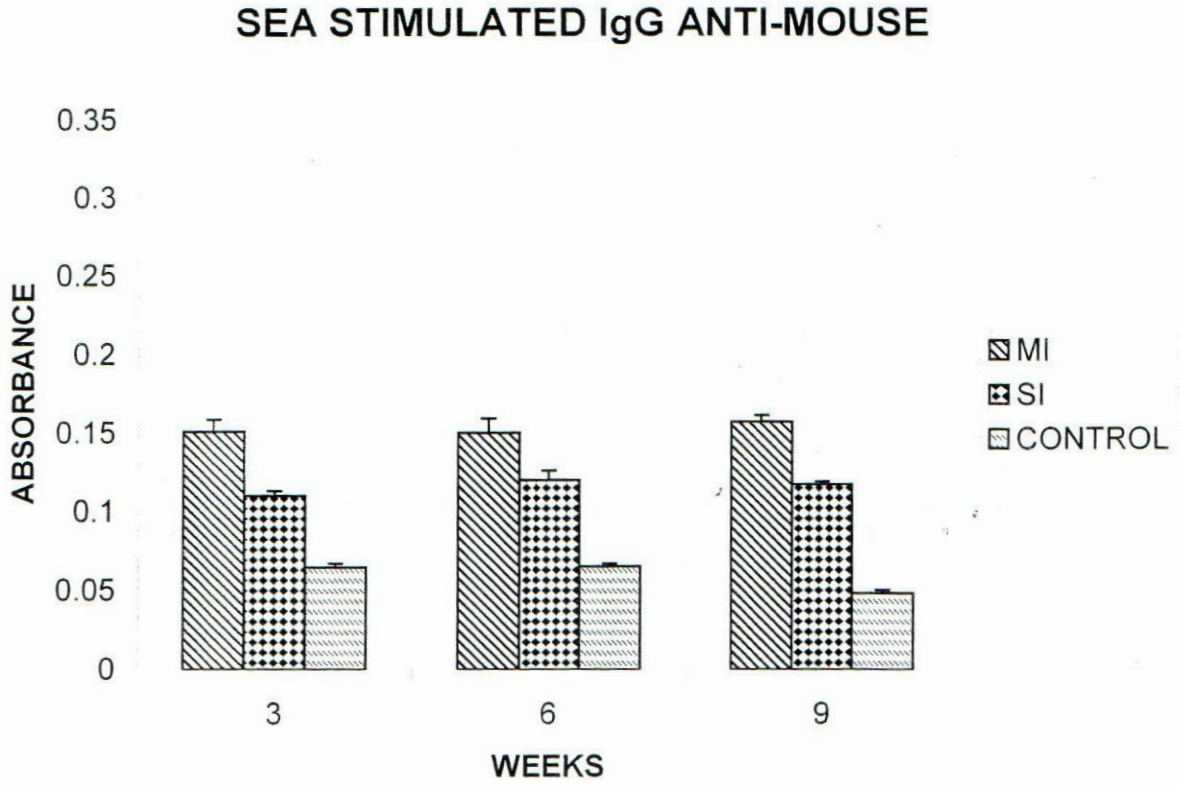

FIGURE 3.5

A comparative graphical representation of SEA specific IgG levels in the multiply infected group (MI), singly infected group (SI) and the control group (NC) using goat anti-mouse IgG conjugated to horseradish peroxidase at three time points post-SI.

4.2 Parasitological results

4.2.1 Worm burden

The mean number of *S. haematobium* males, females and the total number of worms recovered at week 12 post-SI infection in the two groups are shown in Figure 4. The MI group had a significantly higher number of males (28±1.92) than the SI group (14.8±2.41) at $p<0.05$. Similarly the MI group had significantly higher number of female worms (12.8±0.86) than the SI group (8.8±1.01). Consequently the total number of worms in the MI group was significantly higher (42.2±2.71) than the SI group (24.0±2.59) at $p<0.05$.

4.2.2 Worm maturation

Worm maturation in the two groups was calculated using the formula below.

$$\textbf{Worm maturation} = \frac{\textbf{Mean number of worms recovered}}{\textbf{Initial number of infecting cercariae}} \times 100\%$$

MI group had a mean burden of 42.2±2.71 and the infection dose was 200 cercariae, this gives a worm maturation of 21.1%. SI group had a mean burden of 24.0±2.59; this gives a worm maturation of 12.0%, a clear indication that MI group had a higher percentage in worm maturation than SI group.

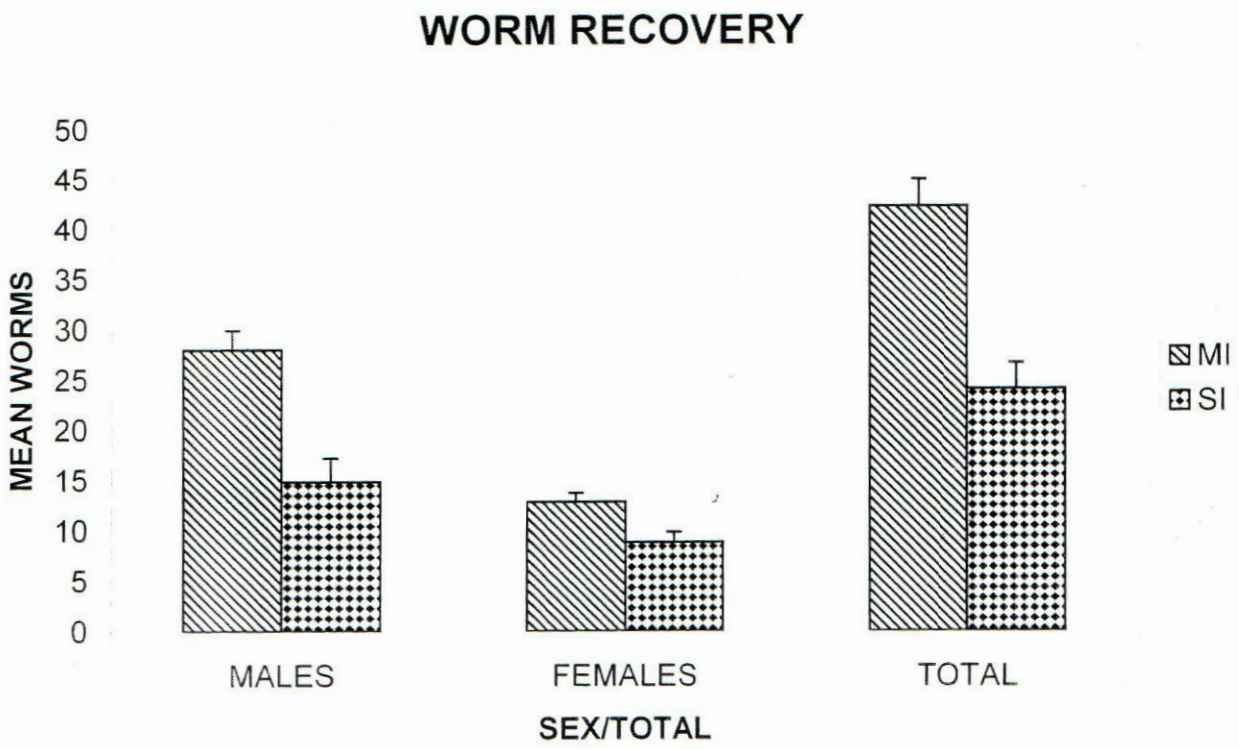

FIGURE 4.0

Bar chart, representation of adult worms recovered in relation to their sexes and their total number, at week 12-post-SI infection.

4.3 Pathological results

4.3.1 Gross pathology

Observations of Gross pathology of the liver and the urinary bladder were made. For the liver the indices of test were: colour, size, adhesions and presence or absence of granulomas. At week 3 post-SI, the MI hamsters exhibited normal colour and sized liver and no granulomas were observed. The SI animals also had normal color and sized livers and also no granulomas were found.

At week 6 post-SI, the MI hamsters had slightly swollen livers, whitish in colour but no visible granuloma. In the SI group the livers were only slightly swollen and also whitish in colour with few stomach adhesions but with no visible granuloma.

At week 9 post-SI, the MI hamsters liver were markedly swollen with rough surface, friable, more adhesions to the stomach and few granulomas were recorded. In the SI group the livers were normal colored, only slightly swollen and no granulomas were observed.

Week 12 post-SI, the MI animals had markedly swollen livers; more adhesions to the stomach, rough surface and had severe granulomas. At this time the SI group had swollen livers and exhibited moderate granulomas.

4.3.2 Histopathological examination

4.3.2.1 The liver

The normal liver tissue is shown in plate 1(a). At week 6 post-SI, the MI group had many small granulomas with an average diameter of 118.0 $\pm$12.3µm (Figure 5.0). The granulomas had a centrally placed egg (Plate 1 b) and only little infiltration was registered around the portal vein (Plate 1 c). At this time point the SI group had only a few granulomas with an average diameter of 91.0 $\pm$ 8.6 µm (Figure 5.0). The granulomas were very few and only a few infiltration cells were seen around the egg (Plate 1 d).

Week 9 post-SI, the MI group had a significant increase in granuloma diameter, as compared to week 6 post-SI, 214.4 $\pm$ 6.8 µm $p<0.05$ (Figure 5.0). The granulomas were conspicuous with centrally placed egg; fibrotic tissues forming around the egg were reported at this time point and characterized by massive infiltration (Plate 1e). The SI group, granuloma diameter had also increased significantly from week 6 post SI at $p<0.05$, diameter measurements being161.3 $\pm$21.6

μm (Figure 5.0). The granulomas had centrally placed eggs with mild infiltration around the egg (Plate 1f).

The peak granuloma size was observed at week 12 post-SI, in both MI and SI groups. The MI group had a mean diameter of 310.5 $\pm$13.3μm (Figure 5.0). A centrally placed disintegrating egg characterized the majority of granulomas; the granulomas also had concentric layers of fibrotic tissues surrounding the less conspicuous egg (Plate 1 g). Coalescing granulomas surrounding 2 or 3 eggs were also observed on the liver (Plate 1 i). At week 12 post-SI, the SI group had a significant increase in the granuloma diameter at $p<0.05$. Mean diameter of 203.0$\pm$1.33 μm was recorded. The eggs were conspicuous and had not started disintegrating (Plate 1 h); coalescing granulomas with concentric rings of fibrotic tissues were also reported at this time point (Plate 1 j).

Throughout the three time points the granulomas diameter of MI group remained significantly higher at $p<0.05$ than that of SI group (Figure 5.0).

At week 3 post- SI, moderate infiltration of lymphocytes around the portal triad was recorded in the MI group. At this time point infiltration was not evident in the SI group. At week 9 post-SI, the level of infiltration had increased significantly in the MI group while there was only mild infiltration in the SI group. 12 weeks post-SI, massive infiltration was observed in the MI group (Plate 1 k) while the SI group posted an increased level of infiltration (Plate 1 l).

4.3.2.2 Urinary bladder

Histopathological sections of the urinary bladder were prepared week 12 post-SI. Normal smooth muscles of the urinary bladder are shown in plate 2 (a). At week 12 post-SI, MI group showed mild epithelium congestion in the urinary bladder with massive cellular infiltration along the muscle layer bladder veins (Plate 2 c). The SI group only posted mild infiltration and congested epithelium (Plate 2 b).

At this time point few granulomas were observed in the MI group. The granulomas were of medium size and were all characterized by massive infiltration around the egg, Plate 2 (d). Unlike the liver granuloma were the egg was centrally placed and cell infiltration was around it, in the bladder cell infiltration seemed to run along the muscle. However no granulomas were observed in the SI group at this time point.

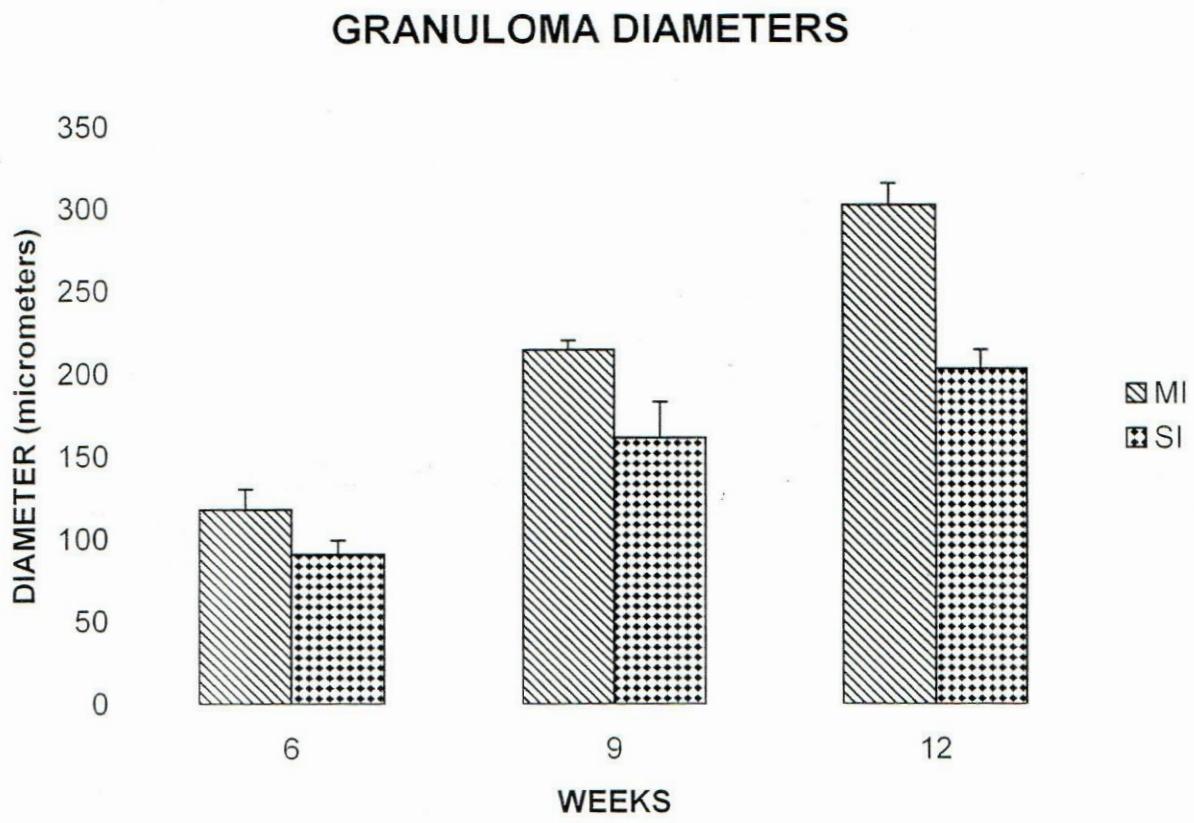

FIGURE 5.0.

A graphical representation of granuloma diameters in micrometers at three-time points post-SI, in the two groups of hamsters, multiply infected (MI) and singly infected (SI).

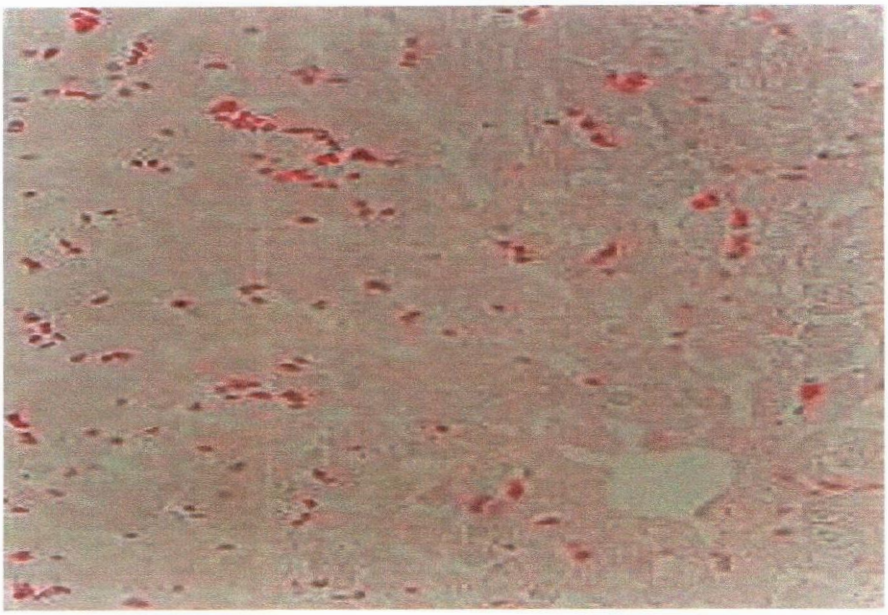

Plate 1 (a). Histological section showing the normal morphology of a hamster liver. Magnification at x 200.

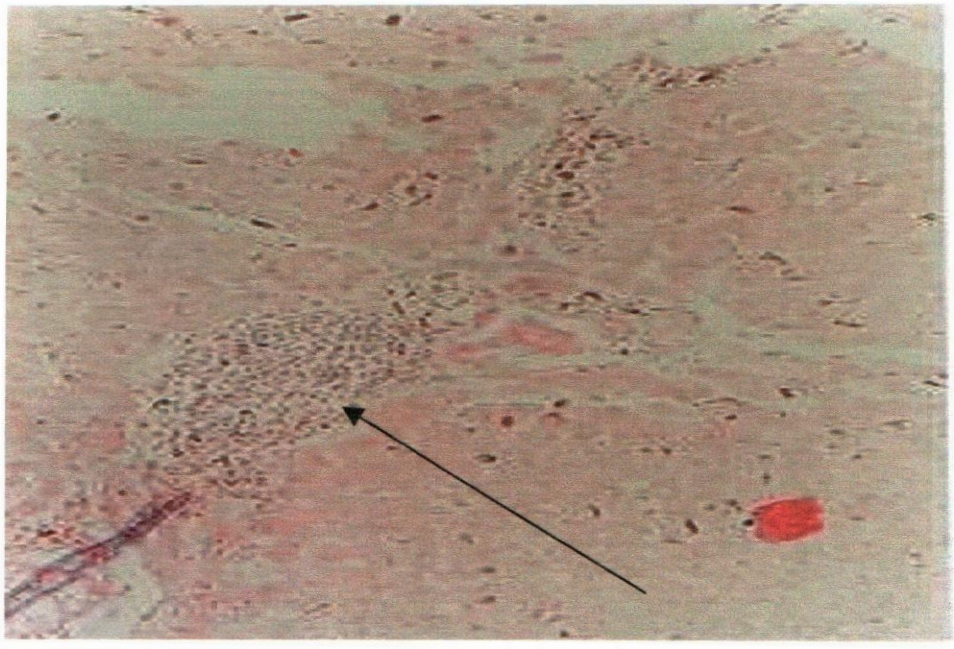

Plate 1 (b) Histological section of the liver showing a granuloma from the MI group at week 6 Post-SI. Magnification at x 200.

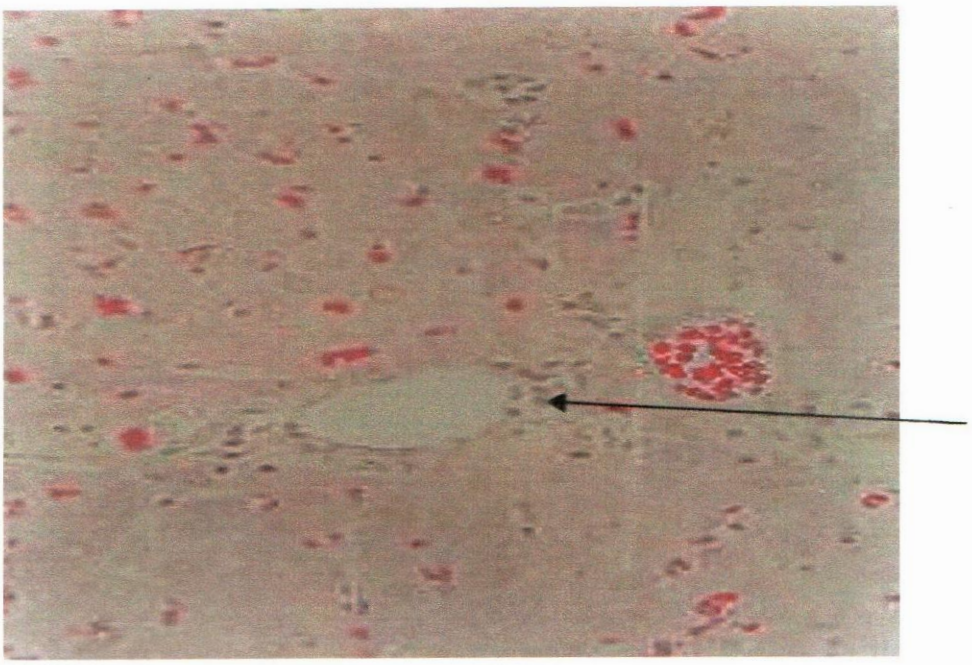

Plate 1 (c). Histological section showing slight cellular infiltration in MI group at week 6 post-SI. Magnification at x 200.

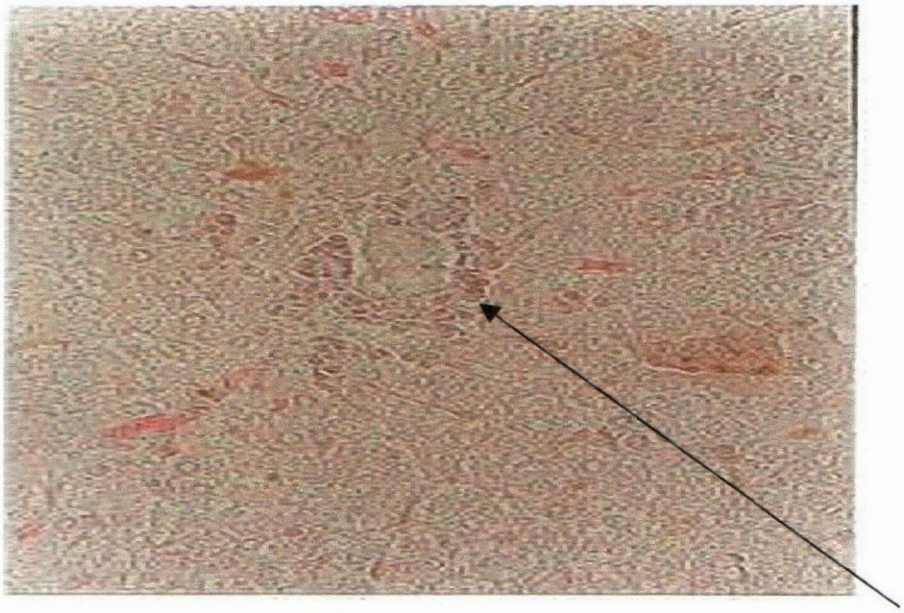

Plate 1 (d). Histological section of a liver, showing a small granuloma in the SI group at week 6 post-SI. Magnification at x 200.

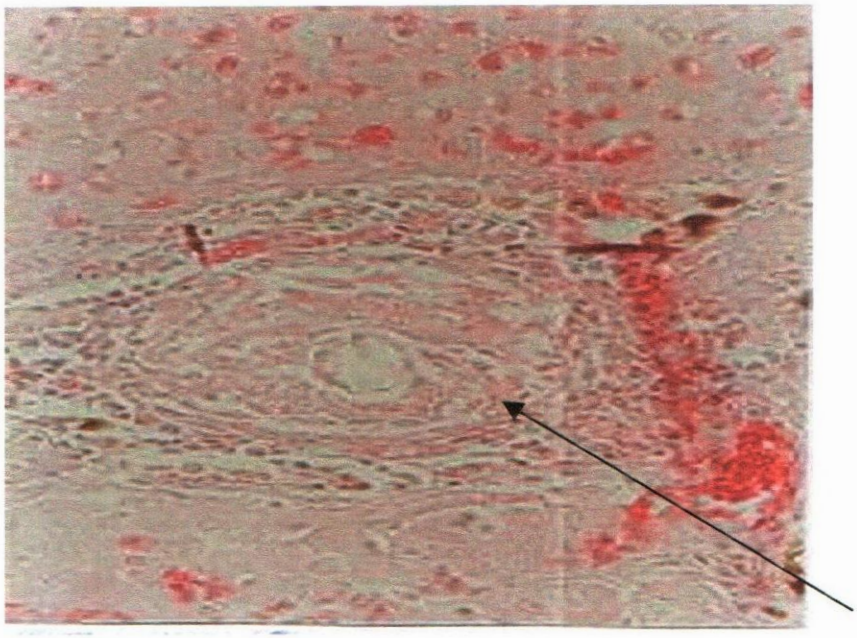

Plate 1 (e). Histological section of a liver, showing a large fibrotic granuloma in the MI group at week 9 post-SI. Magnification at x 200.

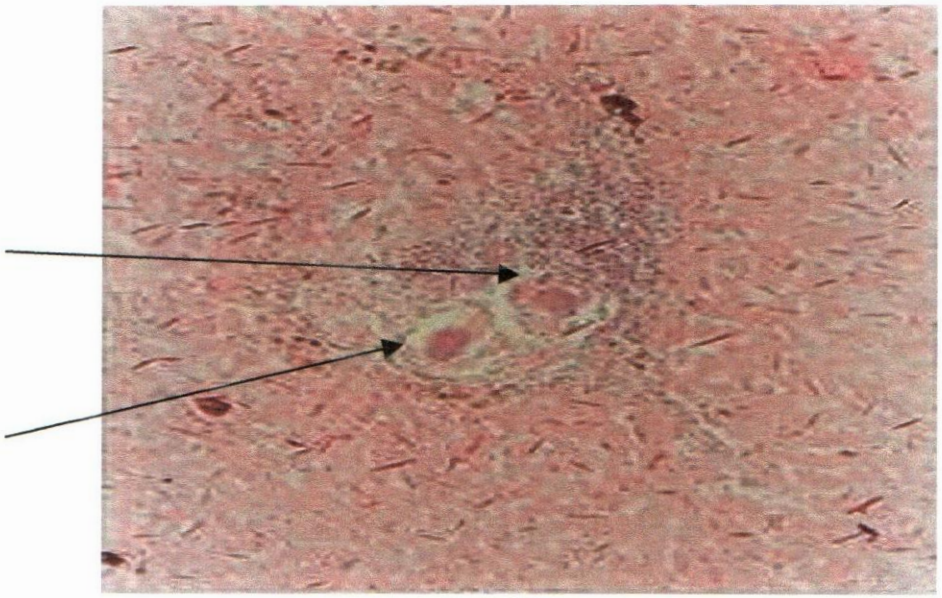

Plate 1 (f). Histological section of a liver showing, two eggs and a small granulomas from the SI group at week 9 post-SI. Magnification at x 200.

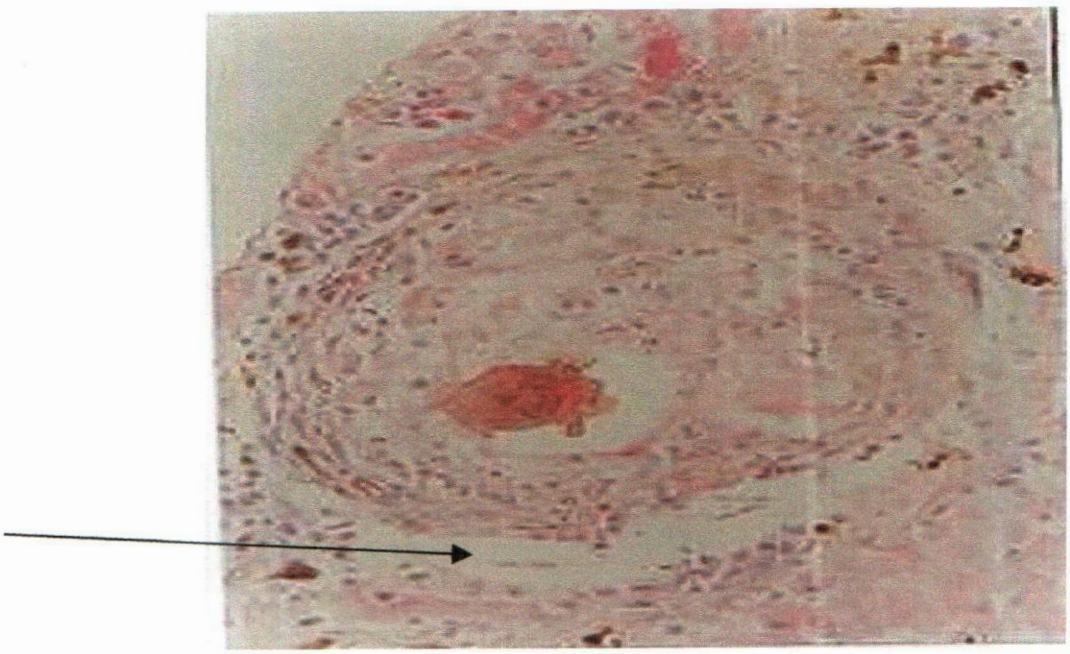

Plate 1 (g). Histological section of a liver, showing a resolving granuloma in the MI group at week 12 post-SI. Magnification at x 200.

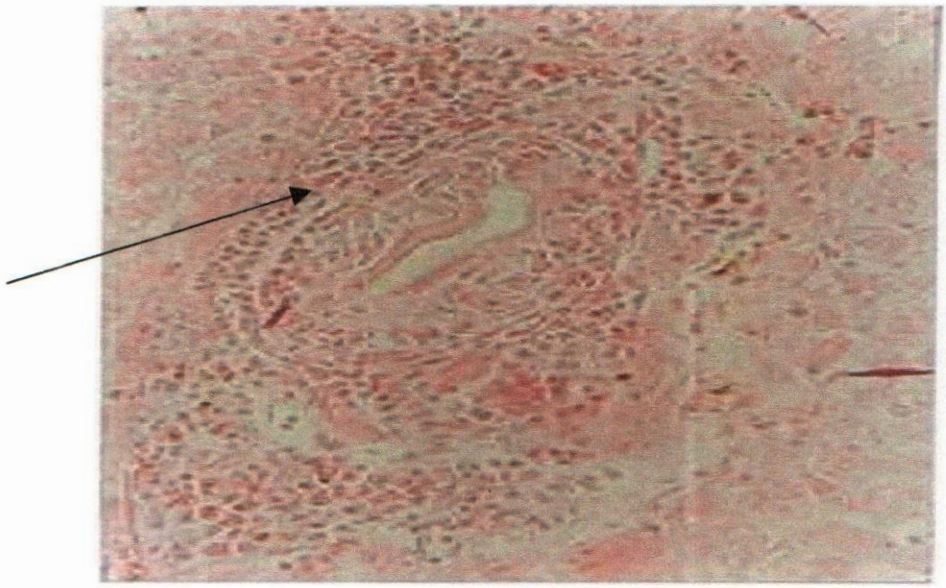

Plate 1 (h). Histological section of a liver, showing a normal granuloma from the SI group at week 12 post-SI. Magnification at x 200.

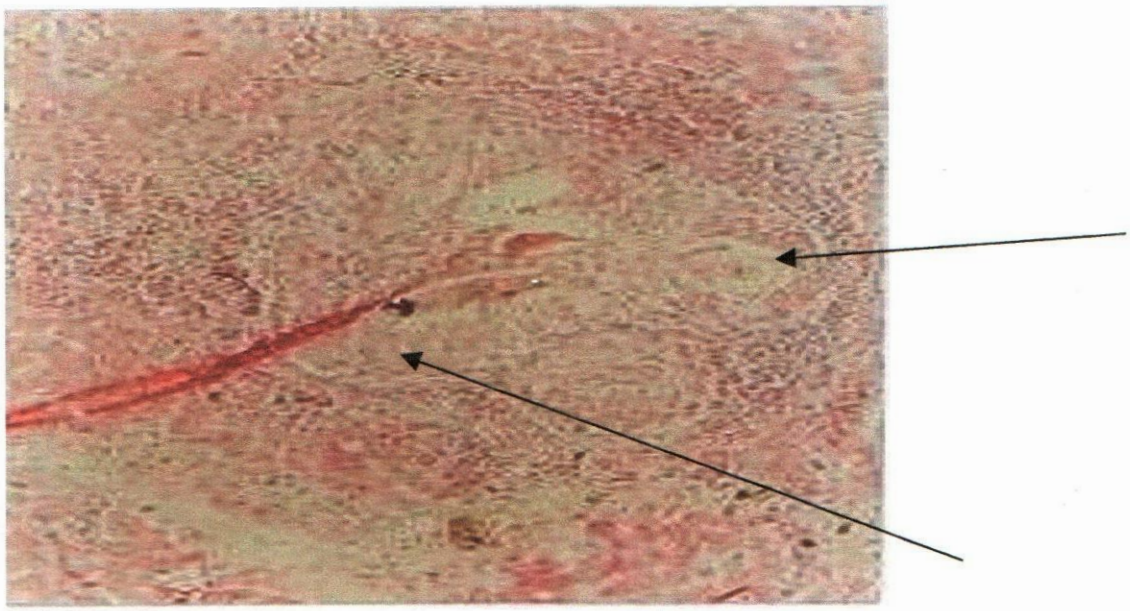

Plate 1 (i). Histological section of a liver, showing a coalenscing granuloma in the MI group at week 12 post-SI. Magnification at x 200.

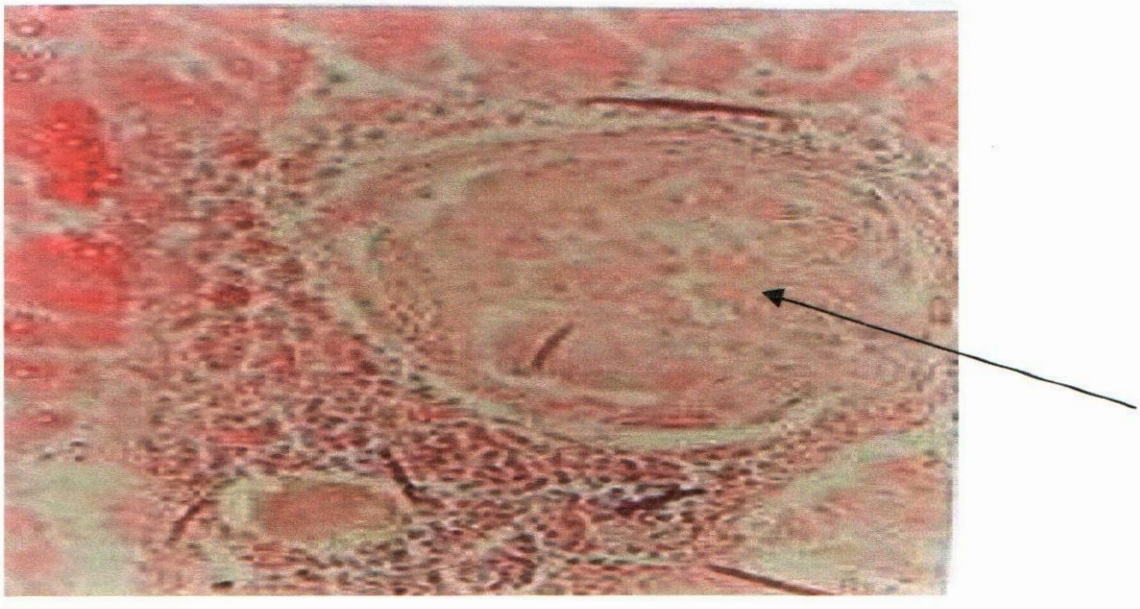

Plate 1 (j). Histological section of a liver, showing a small granuloma and a large resolving from the SI group at week 12 post-SI. Magnification at x 200.

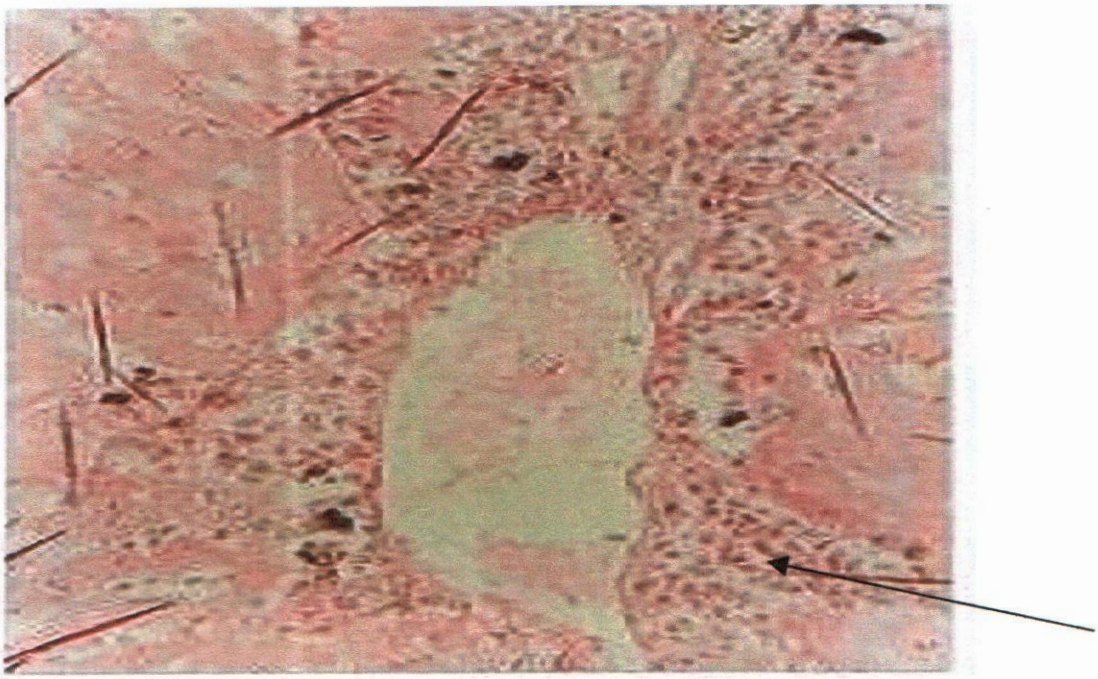

Plate 1 (k). Histological section of a liver, showing severe cellular infiltration around the portal triad, from the MI group at week 12 post-SI. Magnification at x 200.

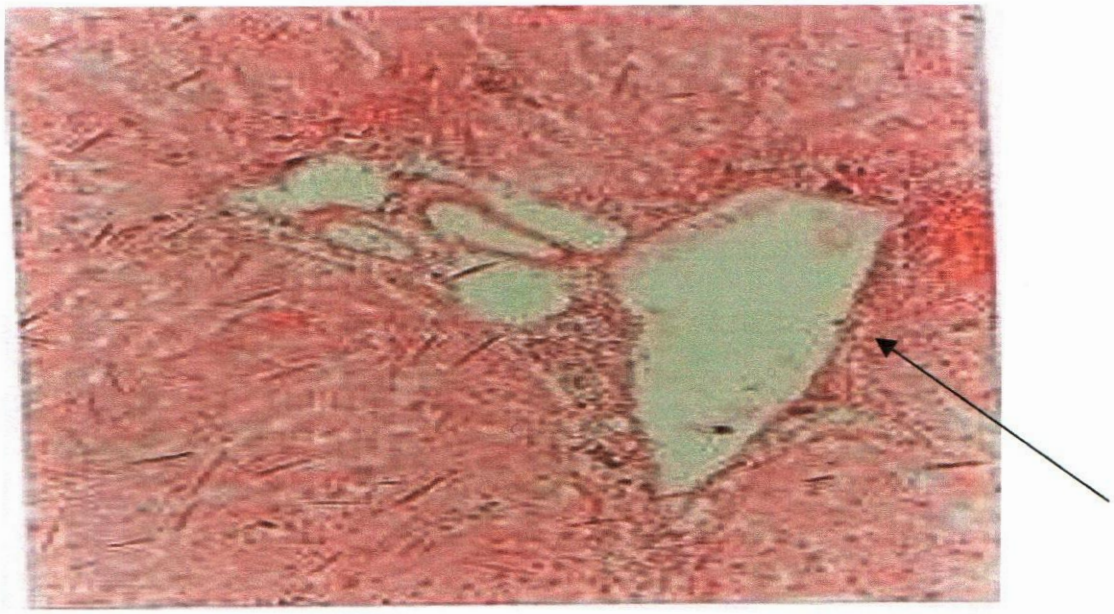

Plate 1 (l). Histological section of a liver, showing mild cellular infiltration in the SI group at week 12 post-SI. Magnification at x 200.

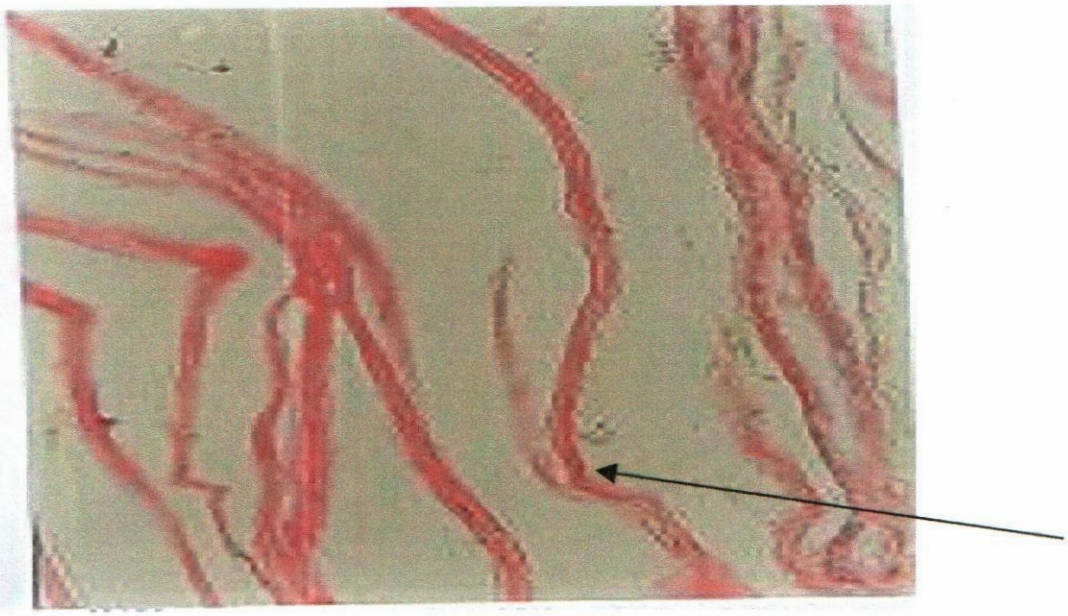

Plate 2 (a). Histological section of the urinary bladder showing the normal smooth muscles. Magnification at x 200.

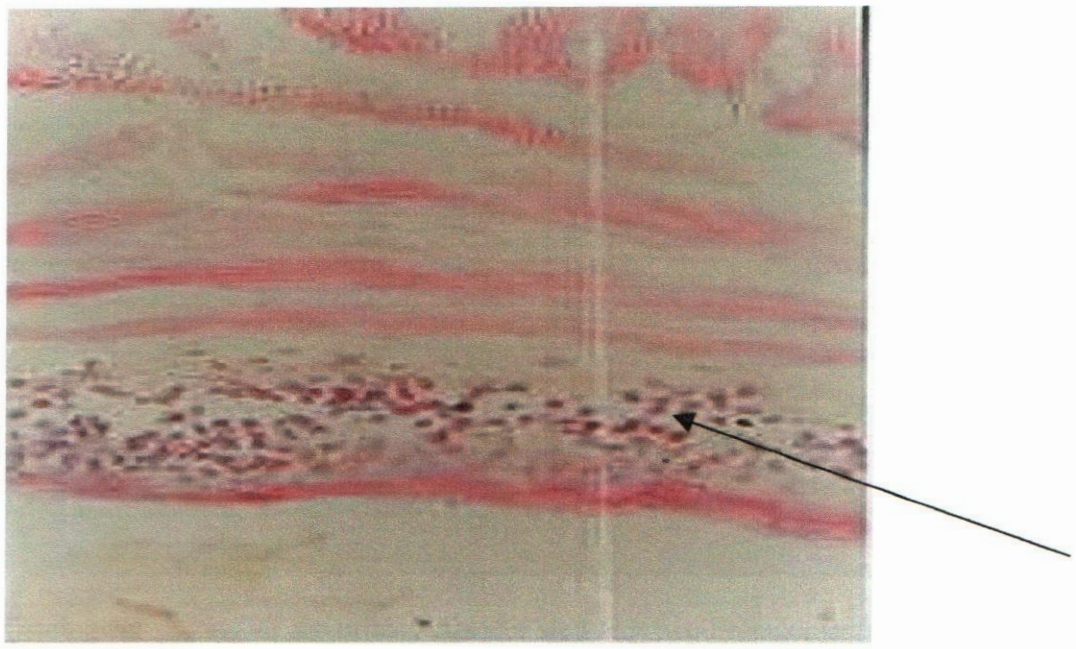

Plate 2 (b). Histological section of the urinary bladder showing mild infiltration in the SI group at week 12 post-SI. Magnification at x 200.

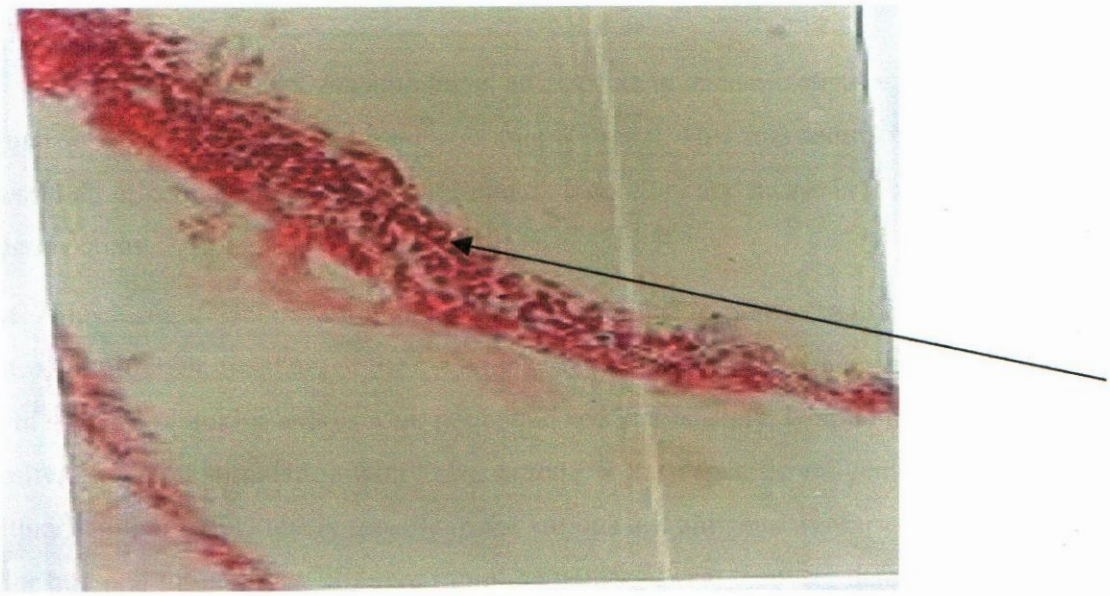

Plate 2 (c). Histological section of the urinary bladder showing severe infiltration in the MI group at week 12 post-SI. Magnification at x 200.

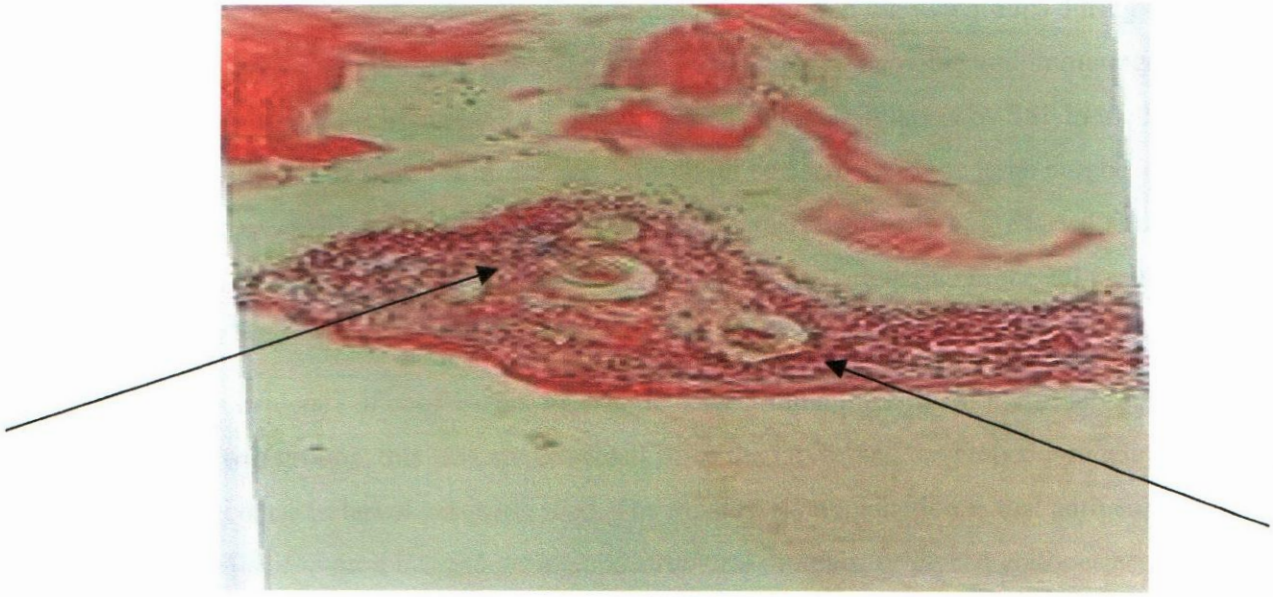

Plate 2 (d). Histological section of the urinary bladder showing two granulomas in MI group at week 12 post-SI. Magnification at x 200.

4.4 Discussion

In this study, a trickle infection of *S. haematobium*, as it occurs in endemic areas was studied in an experimental set up using *Mesocricetus auratus* animal model. This was compared with a single *S. haematobium* infection dose, a routine in research laboratory and a case in tourists visiting the endemic areas.

T lymphocytes play a significant and an important role in immune responses to infection with schistosomes through different members of the cytokine classes they produce (Cheever *et al.*, 1992). Though the specific cytokine assays were not conducted in this study, lymph node and spleen cells proliferation after stimulation with *S. haematobium* stage specific antigens was used to assay for cellular immunity. IgG levels specific to the various test antigens were used as indices of test for humoral immunity at different sampling points. Worm recovery demonstrated the parasitological patterns in the two groups while gross and histopathological assays elucidated the disease patterns in the two groups.

In both MI and SI groups, lymph node cells proliferation to CON A was about 20-50 folds higher than the *S. haematobium* antigen-induced proliferation in the two groups during the three time points. This was an indication that the lymph node cells were viable and immunogenic.

At week 3 post-SI, proliferation of lymph node cells after stimulation by SSP in the SI group was significantly higher than MI responses. This could be ascribed to the large number of *S. haematobium* larvae in the SI group and thus releasing a large quantity of the larval antigens being drained in the peripheral lymph nodes, while majority of cercariae in the MI group had started developing to adult worms thus reducing the larval antigenic load.

There was evidence of continued lymph node cells stimulation by SSP in the subsequent time points in the two groups; this was not expected since the larvae had developed into adult worms and thus a decrease in larval antigenic load. The most likely explanation is that antigenic epitopes, such as common house keeping and structural proteins, or glycan epitopes are shared between larvae, adult worms and eggs (Matthias *et al.*, 2001). These shared antigens could have led to extensive cross reactivity between schistosomula surface antigens and the worm tegumental antigens (Simpson, 1990). The slight decline in lymph node cells responses to SSP at the last time point could be as a result of lymphocytes anergy and/or unresponsiveness due to over stimulation by the protein antigens of the parasite (King et al., 2004).

The significantly high response of lymph node cells to SWAP in the SI group at week 9 post-SI was as a result of large number of worms maturing at the same time and hence consistently releasing large amount of worm antigens. In the MI group such high responses were not achieved as a result of the trickle maturation of worms. This could also be due to the fact that, continued stimulation of the lymph node cells by the schistosoma antigens lead to the unresponsive nature of lymph node cells characteristic of chronic infection (King *et al.*, 2004).

At week 9 post-SI the lymph node cells responses to SEA in the SI group was significantly higher than the MI group. This corresponds to the start of worm maturation and onset of oviposition in the SI group and thus higher egg antigenic load than the MI group.

Spleenocytes proliferation levels were lower than the lymph node cells. Although the spleen is the predominant organ in lymphocyte re-circulation surpassing all the lymph node put together (Pabst, 1988). Maximum responses of spleen cells was recorded at the 3rd week post-SI for all the antigens in the two groups, the responses waned at subsequent time points. This reduced response could be due to over stimulation of the spleenocytes with the schistosoma antigens which culminated into the unresponsive state of the spleenocytes (King *et al.*, 2004). Immunomodulation by the adult worms that render the host unresponsive to any further stimulation could also explain this phenomenon. (Mduluza *et al.*, 2001: Gazzinelli *et al.*, 1985). Suppressive factors in serum of individuals who had long durations of infection could not be ruled out (Mduluza *et al.*, 2001).

At week 3 post-SI, both groups recorded the peak levels of spleen cells stimulation by SSP. This could be due to presence of high quantity of larval antigens. However MI group had significantly higher levels of spleenocytes proliferation than SI, an indication of strong cross reactivity of the egg antigens and larval antigen as it was revealed by (Eberl *et al.*, 2001; Omer *et al.*, 1989).

At week 9 post-SI, SI spleenocytes responses to SWAP were higher than the MI responses; this is because of worm maturation at the same time in the SI group and hence high quantities of adult worm antigens. Despite the onset of oviposition at week 9 post-SI in the SI group, Spleenocytes responses to SEA were lower than the MI group. This could be due to over stimulation by cross-reacting larval antigens, as described above.

In the assay of IgG levels in the three groups of hamsters, it was observed that the control group had significantly lower levels than the SI and the MI group at all time points. This was an indication that the high levels of IgG were due to *S. haematobium* infection.

Using the goat anti-hamster IgG conjugated to hoarse radish peroxidase as the secondary antibody, very high IgG levels in both groups at all time points were recorded, the responses were however not significantly different. This was an indication that, high levels of IgG antibody peak early during *S. haematobium* infection in hamsters. It also underlines the need of using more dilute secondary antibodies or use of a less specific secondary antibody.

Using a less specific secondary antibody (goat anti-mouse conjugated to hoarse radish peroxidase), the differences in the two groups were more pronounced. The MI group had higher IgG levels against all the test antigens at all time points except for IgG responses to SSP at week 3 post-SI. This implies that IgG against the larvae antigen plays important role parasite elimination. IgG participates in the killing of the miracidium inside the eggshell which is mediated by monocytes, eosinophils, or platelets (Allen and Maizels, 1996). This explains the resultant large granulomas in the MI group throughout the time points and the subsequent disintegration of the eggs as was recorded in this group at week 12 post-SI.

The MI group had significantly higher number of females, males and the total number of worms than the SI group, an indication that trickle infection might not trigger a major immune response to eliminate subsequent *S. haematobium* infections in golden hamsters. Farah *et al.*, (1997) showed that by multiply infecting baboons with *S. mansoni* coupled with alternate chemotherapy with praziquantel, a resistance to re-infection was conferred which was evidenced by the low worm burden coupled to less severe pathology of the mesenteries and the liver. This phenomenon was not observed in this study, suggesting that a threshold level of antigens might be required to induce such a protection or that drugs against these parasites may also play a pivotal role in achieving such a resistance. A single dose of infection seems to have triggered a strong immune response leading to lower worm maturation in both sexes. The immunological responses in MI seems to have been subversive as evidenced by the higher worm maturation in this group.

At all time points the MI group had more severe gross pathology in terms of the liver morphology and granulomas as compared to the SI group. This was due the higher number of

maturing worms in the MI group, which translate to heavy oviposition and hence severe egg related pathology in the MI group.

The formation of the granuloma around the schistosome eggs in the liver and the urinary bladder is the major cause of pathology in *S. haematobium* infection. (Wynn, 1996). The live miracidia within each egg secretes antigenic materials through ultramicroscopic pores in the shell (Boros and Warren, 1970). Granulomas are thought to be host protective, as they wall off toxic egg products, such as hepatotoxic antigen omega-1 (Dunne *et al.*, 1991). The MI group had significantly larger granulomas than the SI group throughout the different time points. Cellular infiltration around the eggs and also the portal triad followed the same trend. Fibrosis was noted at weeks 9 and 12 in the MI group and it was only observed in the SI group at week 12 post-SI. This also confirms that the MI group had more severe histopathology than the SI group. This was an indication of a large subversive immunological response in the MI group, which lead to a worse disease pattern in terms of worm burden, gross and histopathology as compared to the SI group.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5. 1 Conclusions

There was evidence of differences in lymph node and spleen cells proliferative responses to the three stage specific antigens throughout the experiment, with variations in responses as the parasite matured and started producing eggs in the two groups. There was also cross-reactivity between the antigens specific to different parasite stages, with larval and egg antigens being the strongest.

The MI group had significantly higher levels of IgG against all test antigens than the SI group at all time points, except for SSP during the initial stages of infection. An indication that IgG was responsible for exacerbated pathology in the MI group. It seems that both humoral and cellular responses are involved in elimination of *S. haematobium* worms in golden hamsters.

The MI group posted a higher worm maturation and worm burden as compared to the SI group and consequently higher oviposition and hence the resultant worse gross pathology. MI group also had a large mean granuloma diameter throughout the entire period. The MI group had severe urinary bladder histopathology with granulomas and massive infiltration along the smooth muscles as compared to the SI group throughout the sampling points.

In this study it was found that infection of hamsters with a trickle infection of 50 normal cercariae of *S. haematobium* does not induce a strong enough immunological responses to eliminate subsequent infections while a single infection with 200 normal cercariae produced a strong immunological response that reduced worm numbers, hence reducing oviposition and consequently reducing gross pathology and histopathology.

5.2 Recommendations

It is recommended that; in drug efficacy and vaccine development tests, multiple exposure of the hamster model to the *S. haematobium* parasite should be adopted as a routine, since it reflects the real life situation, and it produces a different disease pattern from the single exposure which is a common routine in research laboratories.

REFERENCES

Allen J.E and Maizels R.M. (1996). Immunology of human helminthes infection. *International Archives of Allergy and Immunology*. **109**, 3-10.

Balloul J.M., Sonderrmeyer P., Dreyer D., Capron M., Capron A., Grzycn J.M., Lecoco J.P and Pierce R.J. (1987). Molecular cloning of protective antigen against schistosomiasis. *Nature*. **326**, 149-153.

Birgitte J.V and David W.D. (2004). Morbidity in schistosomiasis. *Current Options in Infectious Diseases*. **17**, 439-447.

Booth M., Mwatha J.K and Joseph F. (2004). Periportal fibrosis in human *Schistosoma mansoni* infection is associated with low IL-10 low IFN gamma and high TNF depending on gender. *Journal of Immunology*. **172**, 1295-1303.

Boros D.L and Warren K.S. (1970). Delayed hypersensitivity-type granuloma formation and dermal reaction induced and elicited by soluble factor isolated from *Schistosoma mansoni* egg. *Journal of Experimental Medicine*. **132**, 488-507.

Brooker S.L., De Vlas S.J and Van der Werf M.J. (2003). Quantification of clinical morbidity associated with schistosome infection in sub-Saharan Africa. *Journal of Immunology*. **86**, 125-139.

Bergquist N.R., Engers H.D and Modabber F. (1996). Progress on vaccines against parasites. *Developmental Biology Standards*. **87**, 73-84.

Bergquist N.R. (1995). Controlling schistosomiasis by vaccination: a realistic option? *Parasitology Today*. **11**, 191-194.

Bergquist N.R. (1995). Schistosomiasis vaccine development; approaches and prospects. *Journal of Immunology*. **90**, 221-227.

Bradford M.M. (1976). A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Analytical Biochemistry*. **72,** 248-254.

Capron A., Capron M., Dessaint J .P and Pierce R.J. (1992). Vaccine strategies against schistosomiasis. *American Journal of Immunobiology*. **184**, 282-294.

Capron A and Capron M. (1994). Immunoglobulin E and effector cells in schistosomiasis. *Journal of Science*. **264**,1876-1898.

Cheever A.W and Xu. (1992). The role of cytokines in pathogenesis of hepatic granulomatous disease in *Schistosoma mansoni* infected mice. *Memórias do Instituto Oswaldo Cruz.* **4.** 81-85.

Christie J.D., Pineda J and Crouse J.M. (1986). *Schistosoma haematobium* egg distribution in the lower urinary tract. *Journal of Tropical Medicine and Hygiene.* **35.** 743-751.

Christiensen N.O and Madsen H. (1992). Intermediate hosts of schistosomes. Ecology and Control. *Bulletin of the Society of Vector Ecology.* **17,** (1). 2-9.

Chu K.Y and Klump R.K. (1987). Focal molluscicding. An effective way to argument chemotherapy of schistosomiasis. *Parasitology Today.* **3.** 74-76.

Coili D., Pica-Mattoccia L and Archer S. (1996). Anti-schistosomal drugs: past, present and future? *Journal of Pharmacology.* **68.** 35-85.

Coulson P.S., Mountford A.P and Wilson R.A. (1985). Antigen localization and the induction of resistance in mice vaccinated with irradiated cercariae of *S. mansoni. Parasitology Today.* **97.** 11-25.

Dean D.A and Mangold B.L. (1986). Passive transfer with serum and Ig-G antibodies of irradiated cercariae-induced resistance against *Schistosoma mansoni* in mice. *Journal of Immunology.* **136.** 2644-2712.

Demeure C.E., Rihet P., Outtara M., Bourgeois A and Dessein A.J. (1993). Resistance to *Schistosoma mansoni* in humans: influence of the IgE/IgG balance and IgG2 in immunity to re-infection after chemotherapy. *Journal of Infectious Diseases.* **168.** 1000-1008.

Dunne D.W., Jones F.M and Doeonhoff M.J. (1991). The purification, characterization, serological activity and hepatotoxic properties of two cationic glycoproteins (Alpha-1 and omega-1). *Parasitology.* **103.** 25-236.

Eberl M., Langermans J.M., Vervene R.A., Nyame A.K., Cumming R.D., Thomas A.W., Coulson P.S and Wilson R.A. (2001). Antibodies to glycans dominate the host's response to schistosome larvae and eggs: is their role protective or subversive. *Journal of Infectious Diseases.* **183.** 1238-1247.

El-Bolkainy M.N., Mokhar N.M and Ghoneim M.A. (1981). The impact of schistosomiasis on pathology of bladder carcinoma. *Journal of Cancer.* **123.** 850-852.

Fallon P.G., Niang A.C and Sturrock R.F. (1995). Short report: diminished susceptibility to praziquantel in a Senegal isolate of *Schistosoma mansoni*. *Annual Journal of Tropical Medicine and Hygiene*. **53**, 61-72.

Farah I.O., Nyindo M., Suleman M.A., Nyaundi J., Kariuki T.M., Blanton R.E., Elson L.H and King C.L. (1997). *Schistosoma mansoni*: Development and the modulation of the granuloma after single or multiple exposures in the baboon (*Papio cynocephalus anubis*). *Experimental Parasitology*. **86**, 93-101.

Fenwick A., Saviola L and Engels D. (2003). Drugs for the control of parasitic diseases: Current status and development of schistosomiasis. *Trends in Parasitology*. **19**, 509-515.

Gazzinelli G., Lambertucci J.R., Katz N., Rocha R.S., Lima M.S and Colley D.G. (1985). Immune responses during human *Schistosoma mansoni*, XI. Immunological status of patients with acute infections and after treatment. *Journal of Immunology*. **135**, 2121-2127.

Goll P.H., Marshall T.F., Moore P.J and Wilkins H. (1996). Dynamics of *Schistosoma haematobium* in Gambian communities. The patterns of human infection in the study area. *Transactions of Royal Society of Tropical Medicine and Hygiene*. **78**, 216-221.

Grove D.I. (1990). A history of human helminthology. *Book first ltd.* Pp 287-289.

IPR SOPS. (2002). Institute of Primate Research Standard Operating Procedures. *Parasitology*. **9**, 5-24.

Jacques P., Maire C.M and Marroum M.D. (1998). Schistosomiasis of the bladder in an African immigrant to North California, *Southern Medical Journal*. **91** (6) 580-583.

Kabatereine N.B., Keminjumbi J.T and Ouma J.H. (2003). Epidemiology and morbidity of *Schistosoma mansoni* infection in a fishing community along Lake Albert in Uganda. *Trans Society Tropical Medical Hygiene*. **97**, 599-603.

King C.H., Blanton R.E and Muchiri E.M. (2004). Low heritable component of risk for infection intensity and infection associated disease in urinary schistosomiasis among Wandigo village population in coast province, Kenya. *American Journal of Tropical Medicine and Hygiene*. **70**, 57-62.

Lemmer L.B and Fripp P.J. (1999). Schistosomiasis and malignancy. *South Africa Medical Journal*. **84**, 211-215.

Locker E.S. (1990). A comparative study of life histories of mammalian schistosomes. *Journal of Parasitology*. **87**, 343-369.

Mac cullough F and Pointer J.P. (1989). Biological control of snail hosts of *Schistosoma mansoni* in the Caribbean area using Thiara spp. *Acta. Tropica.* **54**, 3-15.

MacDonald A.S., Pearce E. and Raujo M.I. (2002). Immunology of parasitic helminthes infections. *Journal of Infection and Immunology.* **70 (2)**, 427 – 433.

Madsen H. (1990). Biological method for the control of fresh water snails. *Parasitology Today,* **6**, 237-241.

Manson-Bahr. (1987). Mansons Tropical Disease. (*Rollinson.D and simpson A.J...eds*) 18th Edition. *Oxford Publishers.* Pp 823-876.

Matthias E., Langerhan A.M., Frost A.P., Vervenne R.A., Govert J.V., Deedler M.A., Thomas A.W., Coulson P.S and Wilson R.A. (2001). Cellular and humoral immune responses and protection against schistosomes induced by radiation attenuated vaccines in chimpanzees. *Infection and Immunity.* **69**, 5352-5362.

Mduluza T., Ndhlovu P.D., Midzi N., Mary C., Paris C.P., Turner C.M., Chandiwana S.K., Woolhouse M.E., Dessein A.J and Hagan P. (2001). T Cell Clones from *Schistosoma haematobium* infected and exposed individuals lacking distinct cytokine profiles for Th1/Th2 polarisation. *Memórias do Instituto Oswaldo Cruz.* **96**, 89-101.

Omer A., Mansour P.M., Woody J.N., Smither S.R and Simpson A.J. (1989). Antibodies to carbohydrates and polypeptides epitopes on the surface of schistosomes of *S. mansoni* in Egyptian patients with acute and chronic schistosomiasis. *Parasitology.* **98**, 417-424.

Pearce E., Hieny S and James S. (1986). Induction of protective immunity against *Schistosoma mansoni* by a non-living vaccine. *Journal of Immunology.* **136**, 3878-3883.

Pabst R. (1988). The spleen in lymphocyte migration. *Immunology Today.* **2**, 43-45.

Ramalho-Pinto F.J., Gazzinelli L.D., Homells R.E., Motasantos T.A and Fiquieiredo E.A. (1974). *Schistosoma mansoni*: A defined system for stepwise transformation of cercariae to schistosomulum *in vitro*. *Experimental Parasitology.* **6**, 360-372.

Simpson A.J. (1990). Schistosome surface antigens: Development, Expression and Immunological function. *Parasitology Today.* **6,** 456-582.

Smither S.R and Terry R.J. (1965). The infection of laboratory hosts with cercariae *Schistosoma mansoni* and the recovery of adult worms. *Parasitology Today.* **55**, 695-700.

Sturrock R.F. (1993). The parasites and their life cycles. *Oxford University Press.* Pp 465-468.

Topley and Wilson. (1998). Microbiology and microbial infections (Parasitology). *Oxford University Press.* Pp 480-500.

Utzinger J.L and Keiser J.M. (2004). Schistosomiasis and soil transmitted helminthiasis: common drugs for treatment and control. *Expert Opinion Pharmacology.* **5,** 263-285.

Von Lichtenberg F. (1987). In the biology of *Schistosoma. London Academic Press.* Pp185-232.

World Health Organization (WHO). (1993). Expert committee on the control of Schistosomiasis. Public health impact of schistosomiasis: Disease and mortality. WHO *Bulletin Oms.***71**, 657-662.

World Health Organization (WHO). (1994). The control of schistosomiasis. 2nd edition of WHO expert committee. *WHO Technical Report Series NO.* **830.** *Geneva.*

World Health Organization (WHO). (1998). Report of an informal consultation on research and biological control of snail intermediate hosts. *TDR\BCV-SCH\SIH* **84**, 3-4.

World Health Organization (WHO). (2000). The control of schistosomiasis. Report of WHO expert committee. *WHO Technical Report Series* NO. **830** Geneva.

Wynn T. A. (1996). Development of an anti-pathology vaccine for schistosomiasis. *Annual. New York Academy of Sciences.* **797**, 191-224.

Wynn T. A., Reynolds A., James S., Cheever W.A., Caspar P., Hieny S., Jankovic D., Strand M and Sher A. (1996). IL-12 enhances vaccine-induced immunity to schistosomes by augmenting both humoral and cell-mediated immune responses against the parasite. *Journal of Immunology.* **157,** 4068.

Yole D.S., Pemberton R., Reid G and Wilson R.A. (1996). Protective immunity to *Schistosoma mansoni* induced in the olive baboon, *Papio anubis,* by the irradiated cercariae vaccine. *Parasitology.* **112**, 37-46.