THE EFFECTS OF SCHISTOSOMIASIS AND MALARIA CO-INFECTION ON THE CLINICAL AND PATHOLOGICAL OUTCOME IN EXPERIMENTALLY INFECTED BABOONS (*Papio cynocephalus Anubis*)

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

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DEDICATION

This work is dedicated to my parents Mr. and Mrs. Isaac Kimenye. It has been such a long struggle and pursuit, but you stood the challenge. Through your wisdom, coupled with strength, determination and a strong desire to see your last born rise to the greatness of knowledge, many strides have been made, and this piece of work bears us witness. To my sisters and brothers, your dire support and encouragement remains highly appreciated. To the rest of the Kimenye family, your pivotal position in aligning my priorities carries a special acknowledgement.

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ABBREVIATIONS

ACT: Artemisin-derived Combination Therapy.

APIA: Alkaline Phosphatase Immunoassay.

CDC: Centre For disease Control.

CSA: Chondroitin Sulphate A.

CT: Computed Tomography.

COPT: Circumoval precipitation Test.

DAF: Decay Accelerating Factor.

ECF-A: Eosinophil Chemotactic Factor of Anaphylaxis.

ELISA: Enzyme Linked Immunosorbent Assay.

HA: Hyaluronic Acid.

HSS: Hepatosplenic Schistosomiasis.

ICAM-1: Intercellular Adhesion Molecule.

IFA: Indirect Immunofluoresence.

IFN-γ: Interferon Gamma.

IL: Interleukin.

IPR: Institute of Primate Research.

IRC: Institutional Research committee.

MAC: Membrane Attack Complex.

MRI: Magnetic Resonance Imaging.

PCR: Polymerase Chain Reaction.

*pf*EMP-1:	Plasmodium Falciparum Erythrocytic Membrane Protein.
RDT:	Rapid Diagnostic Tests.
SEA:	Soluble Egg Antigen.
SWA:	Soluble Adult Worm antigen.
Tc:	T cytotoxic cells.
TGF-β:	Transforming Growth Factor.
TNF-α:	Tumor Necrosis factor.
Ts:	T suppressor effector cells.
TSP:	Thrombospondin.
WHO:	World Health Organisation.
SE:	Standard Error of the Mean.
SOPs:	Standard Operating Procedures.

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ABSTRACT

Malaria and schistosomiasis are the two most prevalent parasitic infections in the tropics and subtropics. Both diseases share similar epidemiological distribution and therefore co-infections frequently occur. *Plasmodium falciparum* and *Schistosoma mansoni* are the major species that cause considerable morbidity and mortality in humans malaria and schistosomiasis, respectively. However, the impact of co-infection of the two diseases has not been elucidated fully. The objective of this study was to investigate the effects of schistosomiasis and malaria co-infection on the clinical and pathological disease outcomes, using the baboon as an animal model.

Twenty seven (27) baboons, that had been screened and confirmed free of both schistosomiasis and malaria were used in this study. Five hundred (500) schistosoma cercariae were percutaneously administered to induce schistosomiasis. Blood stage *P. knowlesi* parasites (1×10^4) were used to induce malaria. Four experimental groups were used in the entire study. Group A comprised three baboons that were infected with schistosomiasis alone. Group B consisted of eight baboons that were infected with schistosomiasis and then infected with malaria on the 19th week of the experiment and remained untreated for both diseases. Group C comprised eight baboons that were first infected with schistosomiasis, treated for the disease using praziquantel on the 14 and 15th week of the experiment, and then infected with malaria on the 19th week of the experiment (in the chronic phase of schistosomiasis). All eight animals in Group D were infected with malaria alone.

All animals were monitored daily for clinical signs associated with either of the disease and at the end of the experiment, post mortem and histopathology was done to assess disease severity.

The severity of malaria as a single infection or as a co-infection with schistosomiasis was determined by assessing the onset of disease, clinical manifestations and organ pathology at postmortem. Indicator clinical signs for the onset of malaria were lethargy, reduced physical activity, reduced feed intake and manifestation of hunched over position characterized by prolonged sitting. Clinical signs of severe malaria were hemoglobinuria, vomiting, anemia, respiratory distress and early deaths.

Severity of schistosomiasis either alone or as a co-infection with malaria, was indicated by bloody diarrhea, schistosoma worm burden and pathological lesions of schistosomiasis in the liver and the colon. The histopathological assessment of disease severity included counting the number of schistosomiasis egg-induced granulomas in the liver and colon, measuring the size of granuloma and counting the number of eosinophils within these granulomas.

Animals infected with schistosomiasis alone (Group A), developed the acute phase of the disease that was characterized by bloody diarrhea followed by an asymptomatic chronic phase. Animals in this group showed severe schistosomiasis egg-induced lesions characterized by significantly increased ($p<0.05$) number of hepatic granuloma and counts of eosinophils in these granulomas compared to either Group B or C. The number of schistosomal eggs lodged in colonic wall was also significantly high ($p<0.05$) compared to either Group B or C. There were no mortalities in

this group. Animals in Group B that were co-infected with schistosomiasis and malaria developed mild clinical signs of disease characterized by delayed onset, low mortalities and mild organ pathology. This Group also showed less severe schistosomiasis egg-induced hepatic lesions with significantly ($p<0.05$) fewer hepatic granuloma, low occurrence of eosinophils in those granuloma and fewer eggs lodged in colonic wall compared to Group A. Animals in Group C that was treated for schistosomiasis then infected with malaria developed moderate clinical signs of both malaria and schistosomiasis with low mortalities. Group D animals manifested severe malaria with high mortality.

From this study it was concluded that treatment for schistosomiasis aggravated clinical outcome of malaria. Induction of malaria to baboons with chronic schistosomiasis caused a reversal to the acute phase of the disease in two out of the eight schistosomiasis infected baboons. Results from this study indicate that schistosomiasis and malaria co-infection influenced the clinical and pathological outcome of either disease.

CHAPTER ONE

1.1 INTRODUCTION

Helminths are among the most common causes of chronic infections in the tropics and *Plasmodium* infections in humans produce a severe disease outcome. It is estimated that over a third of the world's population and especially individuals living in the tropics and sub-tropics are infected by parasitic helminths or by one or more of the species of malaria-causing *Plasmodium* (Snow *et al.*, 2005). *Schistosoma mansoni* and *Plasmodium falciparum* are the two major species of schistosomiasis and malaria, respectively causing high mordidity and mortalities in humans (Crompton D.W, 1999, Mendis *et al*, 2001).

These two groups of parasites have similar geographical distribution and co-infection is a common occurence. There is increased evidence that helminth infections may alter susceptibility to clinical malaria, eliciting a great interest in elucidating the consequences of schistosomiasis and malaria co-infection. So far the immunological interaction between the two parasites remains unclear and data from previous studies have yielded contrasting results.

Furthermore, a co-infection between helminths and malaria could either be synergistic or antagonistic in the overall disease outcome. Helminth infection may alter the host susceptibility to clinical malaria or malaria may influence the clinical consequences of helminth infection. Indeed published evidence has suggested that both synergism and antagonism occurs in plasmodium and helminth co-infections (Helmby *et al*, 1998; Yoshida *et al*, 2000; Cox, 2001).

This has however not been conclusive, and therefore the need for longitudinal studies to investigate these associations.

Most studies have investigated the effect of helminth infections on clinical malaria with only few studies in the converse. Moreover, most of these co-infection studies have been done on mice and rats that are lower in the phylogenetic rank hence the extrapolation of the results to humans is questionable.

The olive baboon (*Papio cynocephalus anubis)* has been used as an animal model in the study of both malaria and schistosomiasis. Baboons acquire natural schistosomal infections, develop disease and acquire protective immunity similar to that in man (Nyindo *et al*, 1999). On the other hand, *Plasmodium knowlesi* malaria infection has been experimentally induced in baboons (Ozwara *et al*, 2003).

This study was aimed at investigating the disease outcome in schistosomiasis-malaria co-infection using the baboon as an animal model to establish the interactions of these parasites.

1.2. OBJECTIVES

1.2.1. Overall objective

To determine the effects of schistosomiasis and malaria co-infection on clinical and pathological disease outcome in experimentally infected baboons.

1.2.2. Specific objectives

i. To determine the effects of a pre-existing schistosomiasis on clinical and pathological outcome of malaria infection in experimental baboons.

ii. To determine the effects of malaria infection on clinical and pathological outcome of a pre-existing schistosomiasis in experimental baboons.

iii. To determine the effects of schistosomiasis-malaria co-infection on schistosomiasis worm burden in experimental baboons.

1.3. Hypothesis

H_o: μ; Schistosomiasis and malaria co-infection doesn't affect the disease outcome.

H_a: μ; Schistosomiasis and malaria co-infection does affect the disease outcome.

1.4. Justification

Malaria and schistosomiasis are parasitic diseases that occur in Sub-Saharan Africa. They share common epidemiological and geographical factors and co-infections are often encountered. However, the effects of such co-infections to the host are not well known. So far, the different co-infection studies conducted in different animal models using different species of helminths

and protozoan have produced contrasting clinico-pathological results. Besides, retrospective studies in humans co-infected with helminths and protozoan parasites have also shown a similar trend. Knowledge of how these diseases interact, and their co-infection outcome will assist in planning of treatment and vaccination of these diseases in the endemic areas.

Most of the co-infection studies have investigated the effect of helminth infections on clinical outcome of malaria. Fewer studies have dwelt on the effects of malaria infection on an existing helminth infection. In addition, many of the co-infection studies have been done on mice and rats. These laboratory animals are much lower in the phylogenetic rank and may not give the appropriate scientific correlation to human infection due to the wide variation in the genetic makeup between the two. The strain of malaria that affects mice and rats are also non pathogenic to humans. The baboon is genetically closer to humans. It has been shown to acquire schistosomiasis infection naturally in the wild and it is an established animal model for malaria research since simian plasmodium, for instance *Plasmodium knowlesi* have a comparable phylogeny and host-parasite relationships to human plasmodium parasites. Baboons are not an endangered species in Kenya and are readily available.

CHAPTER TWO

2.1. LITERATURE REVIEW

Parasitic co-infections commonly occur in different regions worldwide. Malaria and schistosomiasis are both water-related vector-borne diseases that occur in common geographical and epidemiological areas in various tropical regions, especially on the African continent.

2.1.1. Schistosomiasis

Schistosomiasis, also known as bilharzia, bilharziosis or snail fever is a parasitic disease caused by several species of the Genus Schistosoma. Some of the documented species are *S.mansoni, S.haematobium, S.japonicum, S.mekongi and S.intercalatum.* Schistosomes are atypical trematodes with dioecious adult stages (have two sexes) occurring in mesenteric blood vessels of the definitive host. The life cycle of schistosomes includes several definitive hosts e.g. humans, non human primates, several species of livestock and rodents where the parasite undergoes sexual reproduction, and a single intermediate host, the snail, where there are a number of asexual reproductive stages.

2.1.1.1. Morphology of *schistosoma mansoni* adult worms

The male *S. mansoni* is approximately 0.6-1.4 cm long by 0.11 cm wide (Machado-Silva *et al*, 1995) and white in color. It has a tegument covered in numerous coarse tubercles, a gynacophoric canal in which the female worm is held and two suckers, a ventral and a larger funnel-shaped oral sucker with which it maintains its position in the mesenteric blood vessels. These suckers have small thorns in their inner part as well as in the buttons around them

compared to *S. haematobium*'s where the tubercles are much finer while in *S. japonicum* the tegument does not have tubercles. The worm's external part is composed of a double bilayer. This is continually renewed as the outer layer, also known as the membranocalyx, is shed continuously (Braschi *et al*, 2006). The genital apparatus are located dorsally and are composed of 6 to 9 testicular masses. From each testicle, there is one short vas deferent canal which is connected to a single deferent that dilates into the seminal vesicle, located at the beginning of the gynacophoric canal. Copulation occurs through the coaptation of the male and female genital orifices (Rey, 1991).

The female *S. mansoni* is longer and thinner compared to the male. It has a cylindrical body measuring 1.2-1.6 cm long by 0.016 cm wide. It is darker in appearance and this is due to the presence of hemozoin pigment in its digestive tube derived from the digested blood. Its ovary is elongated, slightly lobulated and located at the anterior half of the body. A short oviduct connects to the ootype which continues with the uterine tube. The uterine tube normally has 1 to 2 eggs, but only 1 egg is observed in the ootype at any one time. The genital opening is located ventrally. The posterior two-thirds of the body contains the vittelogenic glands which unite with the oviduct just before it reaches the ootype.

The digestive tube begins at the anterior extremity of the worm, at the bottom of the oral sucker and is composed of an esophagus which divides into a left and a right branch. These branches reunite into a single cecum at the anterior half of the body. The intestines end blindly, and therefore there is no anal opening.

2.1.1.2. Distribution of *schistosoma mansoni*

S.mansoni is the most widespread of the human-infecting schistosomes and is present in 54 countries infecting about 83.31 million people (Crompton, 1999). These countries are predominantly in South America and the Caribbean, Africa including Madagascar and the Middle East.

2.1.1.3. Life cycle of *schistosoma mansoni*

2.1.1.3.1. Eggs

Adult *S. mansoni* pairs (male and female) residing in the mesenteric blood vessels produce up to 300 eggs per day during their reproductive lives. These eggs are approximately 140 x 60 μm in size and bear a lateral spine. They are laid one at a time by the female parasite. Many of the eggs pass through the walls of the blood vessels, and through the intestinal wall to be passed out of the body in feces and into water masses. Eggs that find their way to body tissues get trapped and elicit inflammatory reactions (Mathew and Boros, 1986, Boros, 1989).

2.1.1.3.2. Miracidium

The ripe miracidium hatches out of the egg in response to temperatures within a range of 20^0 C to 30^0 C (McGarvey *et al*, 1992). This process is also enhanced by presence of light and dilution of feces with water. The miracidium is 150 - 180 μm in length by 70 - 80 μm in width. It comprises of four outer layers of epidermal plates which are covered in numerous cilia. These cilia facilitate movement of the miracidium through the water. It has no developed gut hence it is unable to feed untill it has penetrated its snail intermediate host. Its energy requirements are

however met by glycogen stores within the larvae. This stored glycogen gets depleted after hatching and this means that the infectivity of the miracidium decreases over time, becoming non-infective after around 4 - 6 hours after hatching.

Its excretory system comprises of a pair of anterior and posterior flame cells. At the apex of the miracidium, there are a number of gland cells, apical papilla and a terebratorium with ciliated sensory organelles. The terebratorium helps the miracidium in attaching itself to the snail host before penetration. The nervous system of the miracidium connects to a variety of sensory organs. These allow the larval parasite to respond to its environment and locate its snail intermediate host. For instance, it has photoreceptors, and with a positive phototrophy behavior, it tends to swim towards light. However, it is negatively geotrophic and so swims upwards away from gravity. These two adaptations help keep the larvae near the water surface where their intermediate hosts are located. They also have chemoreceptors that detect fatty acids excreted by the snails and certain amines secreted from the snail's foot.

The miracidia are already sexually differentiated and both male and female miracidia give rise to the respective forms. This may be of importance experimentally in cases where single sex infections are required.

Once the miracidium is in contact with its snail host (*Biomphalaria glabrata*, *Biomphalaria pffeifeiri*, *Biomphalaria straminea*, *Biomphalaria tenagophila* or *Biomphalaria sudanica* (Gatlin *et al*, 2009), it undergoes exploratory movements over the surface of the snail until it reaches its

preferred site of penetration, for *S. mansoni* usually the anterior portion of the lateral edge of the foot. Here, it is fixed to the snail by the terebratorium, which acts like a sucker. Penetration is aided by secretions from the apical gland cells and is carried out by boring movements of the miracidium.

On penetration, the ciliated epidermal plates are shed and the muscle layers disappear as the miracidium develops into the primary or mother sporocyst in the snail. The germinal cells of the primary sporocyst replicate and bud off as secondary daughter sporocysts that migrate to the snail's liver to mature. This phase of asexual reproduction which usually takes about 3 to 5 weeks gives rise to hundreds of cercariae in the snail. These fork-tailed larval forms of the parasite will eventually exit the snail and actively swim in search of a definitive host (Garcia *et al*, 1999).

2.1.1.3.3. Cercarie

Cecariae exit the snail during day light, usually 25 to 30 days post miracidium infection of the snail and actively swim in search of a host using their long tails that are bifurcated at the end. Their tegument is covered with a trilaminate plasma membrane, a glycocalyx on the outer surface, spines and sensory papillae. They are already sexually differentiated into male and female forms. Cercarie don't feed, instead they survive on glycogen stored in their tails and body, and hence failure to find a susceptible host within 48 to 72 hours leads to their death (Garcia *et al*, 1999).

The cercariae exhibit a number of behavioral features that enable them to locate their definitive host. These include waves of upwards swimming to bring them to the surface of the water, followed by periods of passive sinking. The cercariae are also affected by other stimuli such as shadows on the water, turbulence and chemicals secreted by the host's skin. If a susceptible vertebrate host is encountered, penetration occurs in three stages as described by Haas and Schmidt (1982).

There is an initial attachment to the skin by means of oral sucker followed by the cercaria migrating through the skin searching for a suitable penetration site, often a hair follicle. During this dermal migration, they shed their forked tails and glycocalyx and form a bilayered tegument that protects them from the host's immune system. Penetration of the skin into the epidermis is aided by secretions of mainly elastinolytic protease from the cercarial post-acetabular and pre-acetabular glands. Once they have penetrated, the head of the cercaria transforms into an endoparasitic larva called the schistosomule. Each schistosomule spends a few days in the skin and then enters the circulation at the dermal lymphatics and venules. Here they feed on blood, regurgitating the haem as haemozoin (Oliveira *et al*, 2000).

2.1.1.3.4. The Lung Stage Schistosomulum

The schistosomule migrates to the lungs 5-7 days post-penetration. It undergoes a large increase in its surface area but not in its overall body mass, therefore becoming much longer and slenderer. It then moves via circulation, through the left side of the heart to the hepatoportal circulation.

2.1.1.3.5. The Liver stage Schistosomulum and immature adult

Once in the liver, the parasite undergoes true growth, with completion of the development of the gut, and maturation of the gonads. Sexually mature adult male and female parasites pair up and migrate against the flow of blood back to the mesenteric vessels along the hepatic portal vein (CDC, 2007).

2.1.1.3.6. Adult schistosome worms

The pairing of the adult worms is monogamous (Beltran and Boissier, 2008). Male schistosomes undergo normal maturation and morphological development in the presence or absence of a female although behavioral, physiological and antigenic differences between males from single-sex as opposed to bisex infections may occur. On the other hand, female schistosomes do not mature without a male. Female schistosomes from single-sex infections are underdeveloped and exhibit an immature reproductive system. Although the maturation of the female worm seems to be dependent on the presence of the mature male, the stimuli for female growth and for reproductive development seem to be independent from each other. The adult female worm resides within the adult male worm's gynaecophoric canal, which is a modification of the ventral surface of the male forming a groove.

The paired worms move against the flow of blood to their final destination in the mesenteric circulation where they begin egg production. The *S. mansoni* parasites are found predominantly in the small inferior mesenteric blood vessels surrounding the large intestine and cecal region of the host. Each female lays approximately 300 eggs a day at a rate of one egg every 5 minutes.

These are deposited on the endothelial lining of the venous capillary walls (Loverde and Chen, 1991). Most of the body mass of female schistosomes is devoted to the reproductive system. The eggs move across the endothelium into the lumen of the host's intestines and are released into the environment with the faces.

2.1.1.3.6.1. Adult schistosome worms survival in the host

Adult worms are in constant contact with the host's blood circulation and must therefore evade the host's immunity in order to survive. The tegument coating the worm acts as a physical barrier to host antibodies and complement. Adult worms produce superoxide dismutases, antioxidant proteins that block the effect of superoxides produced by the host. This protects the worm from the host's membrane attack complex (MAC) (Wilson and Coulson, 2009). Schistosomes are also able to coat themselves with the host's specific proteins to avoid recognition, for instance, immunocytochemistry techniques have revealed decay accelerating factor (DAF) protein on the tegument of the worm. This protein is found on host cells and protects host cells by blocking formation of MAC. It has also been found that the schistosome genome consists of human CD59 homologs which also inhibit MAC.

2.1.1.4. Pathogenesis and pathology of *S.mansoni* infection

In humans, pathology is dependent on the various stages of infection. During cercarial penetration, immediate-type hypersensitivity dermatitis due to cercarial antigens occurs and is mediated by mast cells and IgE (Capron and Capron, 1994). This is also called the 'swimmer's' itch and is characterized by a skin rash, whose severity may be dependent on a previous

exposure. A transient allergic pneumonitis due to the migration of schistosomule in the lungs may also be observed. However, acute toxaemic schistosomiasis, also known as katayama fever is the most important sequel associated with migration of schistosomule and adult worms and initial deposition of eggs. Katayama fever is characterized by lymphadenopathy, hepatosplenomegally and peripheral eosinophilia.

The laid eggs enzymatically digest their way through the intestinal mucosa and provoke an inflammatory response. Only mature eggs are capable of crossing into the digestive tract possibly through the release of proteolytic enzymes, but also as a function of host immune response which fosters local tissue ulceration. A local CD4+ T-helper (Th) lymphocyte-mediated inflammatory response facilitates the passage of eggs into the intestinal lumen. Movement of eggs through the intestinal wall is associated with gut dysfunction leading to malnutrition (King *et al*, 2003). A number of these migrating eggs remain lodged in the wall of the colon and die (Cheever, 1969). Grossly, the intestinal mucosal surface appears hyperemic, granular, and friable. Petechiations, as well as small and shallow ulcerations may be present. In later stages of the disease, papillomas and polyps may develop in the rectum and sigmoid (Strickland, 2000).

Up to half of the eggs released are trapped in the mesenteric veins, and lodge in the intestinal wall, or are washed into the liver via the portal circulation where they lodge in the small portal vein tributaries. As such, a hepato-intestinal disease will manifest (Cheever and Andrade, 1967). Ectopic eggs sometimes occur in the lungs, spinal cord and other tissues.

Trapped eggs normally mature and begin to secret antigens that elicit a vigorous immune response. Interaction between host immune system and the trapped eggs leads to a granulomatous inflammatory reaction (Andrade, 1987) which is the major cause of morbidity in schistosomiasis. In the liver, these granulomas impair blood flow and consequently induce portal hypertension; a contributory factor to portal hypertension is Symmers' fibrosis which develops around branches of the portal veins (Cheever, 1972). With time, collateral circulation is formed and the eggs may disseminate into the lungs where they cause more granulomas, pulmonary arteritis and subsequent cor pulmonale. Such patients may show hepatomegally and periportal or portal fibrosis (Symmers, 1904, Bindseil *et al*, 2004) leading to portal hypertension and formation of varices around rectum, stomach and oesophagus (Von Lichtenberg, 1987).

Microscopic features of chronic schistosomiasis include portal fibrosis with partial or complete destruction of the main branches of the portal vein, but the arterial and ductal structures are preserved (Hancock *et al*, 1997), and this explains why the hepatic signs remain subclinical due to the increased circulation to the organ through arterial compensation.

2.1.1.4.1. Clinical manifestations of schistosomiasis

Schistosomiasis causes several syndromes that include cercarial dermatitis, larval pneumonitis, acute schistosomiasis (Katayama fever) and chronic schistosomiasis which may be urogenital, cardiopulmonary, ectopic, intestinal, or hepato-splenic (El-Garem, 1998).

2.1.1.4.1.1. ***Acute schistosomiasis (Katayama fever)***

This occurs at the onset of egg laying, approximately 5weeks post- infection. At this time granuloma formation occurs around eggs that are trapped in the liver and intestinal wall. It is an acute immune complex syndrome characterized by hepatosplenomegaly, eosinophilia, fever, lethargy, nausea, headache, rash, bronchospasm and in extreme cases presence of bloody mucoid diarrhea containing necrotic material. In some instances, such acute signs may not manifest (Leshem *et al*, 2008).

2.1.1.4.1.2. ***Chronic Intestinal Schistosomiasis***

Schistosomiasis eggs that lodge in colonic wall elicit a granulomatous inflammation with possible subsequent fibrosis. The large intestine is more frequently affected and the lesions are more severe in the colon where they give rise to multiple polyps that often bleed (Cheever, 1978, Auriault *et al*, 1990). Grossly, the large intestines develop mucosal edema, foci of granulomatous inflammation, pinpoint hemorrhages and shallow ulcers. The affected animal often develops mucoid diarrhea. Microscopically, the granulomatous response in the gut wall is characterized by deposition of collagenous matrix with infiltration by macrophages, eosinophilic granulocytes and lymphocytes (Jacobs et al, 1998, Weinstock, 1992).

2.1.1.4.1.3. ***Chronic Hepato-Splenic Schistosomiasis (HSS)***

HSS is the most important complication of *S. mansoni* and *S. japonicum* infections.This results from an initial deposition of eggs in the small and anastomosing portal vasculature causing vascular obstruction. These eggs induce granulamatous reactions that cause portal inflammation.

Transition from granulama to fibrous tissue deposition causes fibro-obstructive lesions with subsequent presinusoidal block to liver blood flow and an accompanying high portal pressure. Development of portal hypertension ensues with congestive splenomegally and development of porto-systemic collateral circulation. The spleen responds with hyperplasia of reticular tissue with diffuse fibrosis. The disease is mainly mesenchymal and not parenchymal and therefore the liver parenchyma is normal and lobular architecture is not disturbed (El-Garem, 1998). It is manifested by hepatomegaly and hepatic fibrosis as the disease progresses (Rojkind and Dunn, 1979).

Portal hypertension occurs with eventual development of portosystemic collateral blood flow. As the infection progresses, splenomegaly, esophago-gastric varices and hemorrhoids develop. Bleeding from esophageal varices leads to hematemesis while gastrointestinal bleeding causes melena (Kamel *et al*, 1978). A characteristic feature of HSS is the maintenance of liver perfusion through an increase in hepatic arterial flow. This in turn allows for the preservation of hepatocytes function. This is the compensated stage of HSS (Warren *et al*, 1965). Liver function tests show normal transaminases and mildly elevated alkaline phosphatase. There is elevated IgG, leukopenia, mild hemolytic anemia and thrombocytopenia (Manoukian and borges, 1984). The decompensated phase of HSS is characterized by a shrunken liver, splenomegaly, esophageal varices, hepatic encephalopathy, massive ascites, hypoalbuminemia, and muscle wasting (Ruppel *et al*, 1990).

Histologically, portal inflammation, kupffer cell hyperplasia, mild concentric fibrosis originating from the portal triads and variation in size and number of parenchymal cells has been documented (Hutt, 1971).

2.1.1.5. Pathogenesis and pathology of *S.mansoni* infection in Baboon Model

As in humans, both acute and chronic phases of schistosomiasis have been documented in experimentally infected baboons (Damian *et al*, 1992, Farah *et al*, 1997). In acute phase there is diarrhea, occasional blood stained feces, abdominal pain, anorexia and weight loss. This phase is also associated with hepatic and intestinal granulomas. Chronic phase shows spontaneous decline in the granulomatous reaction (Boros *et al*, 1975) and the clinical signs of hepato-intestinal disease. Baboons have also been shown to develop peri-portal fibrosis from natural infection or following experimental infection with *S. mansoni* (Njenga *et al*, 1998). Intestinal schistosomiasis in the baboon is characterized by granulomatous inflammation of the mucosae of ileum and colon, villous atrophy, hypertrophy of *Muscularis mucosa* and hyperplasia of the goblet cells (Farah and Nyindo, 1996).

2.1.1.6. Pathogenesis of Schistosomiasis egg granuloma

Schistosomiasis egg-induced granuloma occurs as a Delayed Type Hypersensitivity (Type IV) reaction that is aimed at protecting the host from toxic components of Soluble Egg Antigen (SEA) secreted by the entrapped egg (Boros, 1989, Mathew and Boros, 1986). The granuloma consists of cellular infiltrates including lymphocytes, macrophages, giant cells, epitheloid cells,

mast cells, plasma cells, fibroblasts and eosinophils. These cells interact directly and through a broad spectrum of cytokines (Wynn and Cheever, 1995).

Macrophages are involved in phagocytosis of the entrapped egg. This is followed by antigen presentation by these macrophages leading to an expansion and activation of populations of antigen specific major histocompatibility class II-expressing cells and secretion of lymphokines that attracts other immune cells to the growing granuloma. The CD4+ve cells of T lymphocytes get activated, and in turn stimulate specific antibody responses to SEA. They possess cells exhibiting Th1 and Th2 responses.

It has been shown in *S.mansoni* infected mice that Th1 cytokines, namely interferon gamma (IFN-γ) and Interleukin-2 (IL-2), predominate prior to egg laying. This is then followed by an increase in Th2 cytokines which include Interleukins 4, 5 and 10 (IL-4, IL-5 and IL-10). Therefore *S. mansoni* egg antigen (SEA) stimulates a predominantly Th2 response while *S. mansoni* worm antigen (SWA) stimulates a nonpolarized Th1 response (Williams *et al*, 1994). Such findings have been confirmed in schistosome antigen-specific T-cell clones derived from *S. mansoni*-infected individuals. These cells are predominantly interleukin-4 (IL-4) secreting $CD4^+$ T cells (Couissinier-Paris and Dessein, 1995).

Another group of T lymphocytes, the CD8+ve cells are involved in the modulation of granuloma formation. They comprise of the T suppressor effector cells (Ts) and cytotoxic cells (Tc). The Ts cells appear to down regulate the activity of the CD4+ve cells.

The granulomas formed differ in size and composition, based on the prevailing Th1 or Th2 responses and this determines the pathological outcome. Increased granulomata size has been attributed to Th2 responses while the Th1 cytokines are associated with a reduction in the size (Jankovic *et al*, 1997). Indeed, recent studies have shown that granuloma size is positively correlated to the levels of IL-13 (Mentink-Kane *et al*, 2004).

Similarly, Th2 responses determine the cellular responses. Iinterleukin 5 (IL-5), a Th2 cytokine, has been shown to enhance eosinophil recruitment into the schistosoma egg granuloma (Sher *et al*, 1990) while interleukin-4, another Th2 cytokine, stimulates IgE responses, and this causes eosinophilia through triggering the release of eosinophil chemotactic factor of anaphylaxis (ECF-A) by mast cells (Gleich and Adolphson, 1986).

On the other hand, fibrosis occurs when there is replacement of other cellular infiltrates with fibroblasts. Fibrogenesis has been shown to be influenced by cytokines (Booth *et al*, 2004). Interleukin-13 is involved in fibrosis since it promotes collagen production (Ribeiro de Jesus *et al*, 2004). Collagen deposition in the granuloma leads to fibrosis and eventually hepatosplenic disease (Andrade, 1987). Transforming growth factor beta (TGF-ß) in association with IL-10 down regulates the process of fibrosis (Hesse *et al*, 2004, Kitani *et al*, 2003).

2.1.1.7. Diagnosis of *S. mansoni*

There are several approaches to the diagnosis of *S. mansoni* infection;

Detection of eggs: - Eggs in stool are identified using the Kato-Katz technique (Katz *et al*, 1972). Eggs of *Schistosoma mansoni* are approximately 140 by 60 µm in size and bear a lateral spine.

Those in tissues are detected using techniques such as colonoscopy and endoscopy to examine the intestines and to take liver biopsy for histological examination. Ultrasound, chest X-rays, Computed Tomography (CT), Magnetic Resonance Imaging (MRI) and echocardiograms may also be used to determine the extent of the infection in other organs.

Immunodiagnosis using enzyme linked immunosorbent assay (ELISA), circumoval precipitation test (COPT) and alkaline phosphatase immunoassay (APIA) to detect *S.mansoni*.

2.1.1.8. Treatment of *S.mansoni*

Chemotherapy: - Praziquantel (PZQ) and Oxamniquine are the drugs of choice.

Praziquantel is given orally at a dose rate of 60 mg/kg of body weight and Oxamniquine at 15 mg/kg body weight orally. Praziquantel acts by increasing the permeability of the membranes of schistosome cells towards calcium ions. The drug thereby induces contraction of the parasites, resulting in paralysis in the contracted state. The dying parasites are dislodged from their site of action in the host organism and may enter systemic circulation, a situation that may cause embolism or may be destroyed by host immune reaction through phagocytosis.

Chronic schistosomiasis with hepatosplenic involvement may require symptomatic treatment.

2.1.1.9. Prevention of the disease

Prevention of schistosomiasis is through elimination of snails, the intermediate hosts coupled with personal protection by avoiding getting into contact with snail infested river or pond water in endemic areas.

2.1.2. Malaria

2.1.2.1. Epidemiology and transmission of Malaria

Malaria is commonly found in tropical and subtropical regions where it causes deaths of up to three million people. Majority of the deaths occur in young children in Sub-Saharan Africa (Snow *et al*, 2005).

Five species of the causative plasmodium parasite can infect humans and the most serious disease is caused by *Plasmodium falciparum*. Malaria due to either *P. vivax*, *P. ovale* or *P. Malariae* is a milder disease. *P. knowlesi* is a zoonotic parasite of macaques and it is capable of infecting humans (Fong *et al*, 1971, Singh *et al*, 2004).

2.1.2.2. Life cycle of malaria plasmodium parasite

Female mosquitoes of the Genus *Anopheles* are the primary hosts and transmission vectors, while humans and other vertebrates are secondary hosts of malaria parasite. Mosquitoes get infected by taking a blood meal from an infected person. Once ingested, the parasite gametocytes differentiate into male or female gametes and then fuse to produce an ookinete in the mosquito gut. The ookinete then penetrate the gut lining to produce an oocysts in the gut wall. Upon

rupture the oocyst releases sporozoites that migrate through the mosquito's body to the salivary glands where they remain until the next bite when they are then released to the next bitten host. This type of transmission is referred to as anterior station transfer (Talman *et al*, 2004).

Malaria in humans is mainly caused by *Plasmodium falciparum* (Mendis *et al*, 2001) and develops in two phases: an exoerythrocytic phase with hepatic system as the target organ and an erythrocytic phase where erythrocytes are infected. Following a mosquito bite, sporozoites in the mosquito's saliva enter the bloodstream and migrate to the liver and infect the hepatocytes. In the hepatocytes, these sporozoites multiply asexually and asymptomatically for up to two weeks. The organisms undergo schizogony in the liver. This involves only nuclear division but no cytoplasmic division. They then bud off to produce merozoites which, following rupture of the hepatocytes, escape into circulation and infect red blood cells to initiate the erythrocytic phase (Bledsoe, 2005).

The parasite evades the host's immune surveillance when leaving the liver by wrapping itself in the cell membrane of the parasitized hepatocytes (Sturm *et al*, 2006). Merozoites invade erythrocytes, in which they develop through early trophozoites (ring forms) to late trophozoites and eventually to schizonts. Trophozoites are feeding stages while the schizonts are a reproduction stage. The schizonts undergo differentiation (schizogony) to produce merozoites which periodically burst the hosts' red blood cells and invade new ones. Such episodes manifest as waves of fever.

Some merozoites turn into male and female gametocytes. If picked by a feeding mosquito, fertilization and sexual recombination occurs in the mosquito's gut, hence the mosquito becomes the definitive host of the disease. Sporogony occurs in the mosquito and the new sporozoites travel to the mosquito's salivary gland, completing the cycle.

Some *P. vivax* and *P. ovale* sporozoites do not immediately undergo asexual replication, but enter a dormant phase known as the hypnozoite. This hypnozoite can reactivate and undergo schizogony at a later time resulting in a relapse. Hypnozoites are responsible for long incubation and late relapses in these two species of malaria (Cogswell, 1992). Relapse is the reactivation of the infection via hypnozoites while recrudescence is used to describe a situation in which parasitemia falls below detectable levels and then later increases to a patent parasitemia.

The parasite spends much of its human life cycle residing within the liver and red blood cells being invisible to immune surveillance and so relatively protected from the body's immune system (Sturm *et al*, 2006). Circulating parasitized erythrocytes are usually destroyed in the spleen. However, to avoid this sequel, the *P. falciparum* parasite displays adhesive proteins on the surface of the infected blood cells causing the blood cells to stick to the walls of small blood vessels thereby sequestering the parasite. This inturn ensures the parasites don't reach the spleen through the general circulation (Chen Q *et al*, 2000). This cytoadherence can lead to blockage of high endothelial venules with subsequent severe symptoms such as observed in placental and cerebral malaria (Aikiwa *et al*, 1990).

2.1.2.3. Pathogenesis and pathology of malaria infection

Malaria infection can clinically present as a mild infection, severe or cerebral malaria. The manifestations of severe malaria differ depending on the age and previous exposure (Newton and Krishna, 1998). In children of less than two years, severe anemia is a common manifestation. In older children seizures and cerebral malaria predominate, while in adults, acute renal failure, acute pulmonary oedema, liver dysfunction, and cerebral malaria may all occur. Metabolic acidosis mainly a lactic acidosis type is common at all ages (Day *et al*, 2000). Severe malaria is a multisystemic disease and the outcome often depends on the degree of dysfunction of vital organ.

2.1.2.3.1. Red blood cells

The growing parasite consumes and degrades hemoglobin in the red blood cell. This interferes with the integrity of the red cell membrane. The cell losses its deformability and transport properties and becomes more spherical (Dondorp *et al*, 2000). The parasitized cell forms knobs. These 'knobs' are expressed during the trophozoite and schizont stages and are formed as a result of parasite proteins exported to the erythrocyte membrane. They express strain specific, adhesive variant proteins that mediate red cell attachment to receptors on venular and capillary endothelium and syncytiotrophoblasts causing cytoadherence.

Cytoadherence involves a receptor-ligand association where proteins expressed on the surface of the infected erythrocyte (ligand) bind to proteins expressed on the surface of the endothelial cells (receptor). *plasmodium falciparum* erythrocyte membrane protein (*Pf*EMP-1) is a parasite

protein which has been implicated as the cytoadherence ligand (Magowan *et al*, 1988). Other surface proteins believed to be involved in cytoadherence are rifin (Kyes *et al*, 1999) and sequestrin (Ockenhouse *et al*, 1991). *P. falciparum* infected red cells also adheres to uninfected red cells to form rosettes. However, not all parasitized erythrocytes form rosettes (Udomsangmetch *et al*, 1989).

Cytoadherence and rosetting are central to the pathogenesis of *P. falciparum* malaria. They lead to the formation of red cell aggregates and intravascular sequestration of red cells in high endothelial venules and capillaries in vital organs like the brain, kidney and the heart. This interferes with local perfusion and metabolism while favoring parasite development away from the spleen. This therefore means that in *P. falciparum* malaria, only younger forms of the parasite are found in the peripheral circulation while the mature forms are rare and when found indicate severe infection (Silamut and White, 1993). On the other hand peripheral parasitemia comprising of young forms of the parasite is usually an underestimate of the true parasite load.

Expression of variant surface neoantigens by parasitized erythrocytes also stimulates the reticuloendothelial system leading to intravascular hemolysis and this causes anemia, tissue hypoxia and cytokine production. Cytokines such as interleukins, interferons and tumor necrosis factor released from macrophages or other cells at the time of schizont rupture are thought to be the cause of fever, paroxysms, headache and other pains associated with malaria (Kano and Aikawa, 1999).

2.1.2.3.2. Cytoadherence and sequestration of parasitized red blood cells in blood vessels

As discussed in section 2.2.2.3.1, cytoadherence leads to sequestration. The sequestration of red cells containing mature forms of the parasite (trophozoites and merozoites) in the microvasculature is thought to cause the major complications of falciparum malaria, particularly cerebral malaria (White and HO, 1992). In addition, intravascular accumulation of platelets is believed to aid in cytoadherence (Grau *et al*, 2003). When it occurs in the relatively hypoxic venous beds, it allows for optimal parasite growth and prevents the parasitized red blood cells from being destroyed by the spleen (Marsh *et al*, 1988). It is the sequestered parasites that cause pathology in severe malaria and prognosis is related to the sequestered biomass.

In vitro studies have identified several cell-surface molecules as potential receptors for parasitized red blood cells binding. This include CD36 which occurs in most vascular beds except in brain and chondroitin sulphate A (CSA) which is the main receptor in the placenta (Cooke *et al*, 2000).The intercellular adhesion molecule 1 (ICAM-1) is found in the brain vascular endothelium (Turner *et al*, 1994) and is influenced by the TNF-α. Other factors are thrombospondin (TSP), vascular cell adhesion molecule, E-selectin, CD31 and hyaluronic acid (HA).

2.1.2.3.3. Anemia in malaria

Anemia is one of the most common complications of *P. falciparum* malaria leading to increased mortality especially in cases with brain and lung complications (Marsh *et al,* 1995). It is due to bone marrow depression and hemolysis.

2.1.2.3.3.1. Bone marrow suppression in malaria infection

Studies done in both animals and humans have shown erythroid hypoplasia especially in acute malarial infection (Srichaikul *et al,* 1967). Further, Colony Forming Unit of Erythroid Lineage (CFU-E) and the Burst forming Unit erythroid (BFU-E) in bone marrow of malaria patients have been shown to be decreased (Jootar *et al,* 1995) together with suppression of the serum erythropoietin response to anemia (Burgmann *et al,* 1996). Ineffective erythropoiesis has also been observed in chronic malaria (Wickramasinghe *et al,* 1989) due to dyserythropoiesis (Abdella and Wickramasinghe, 1998).

In animal models, TNF-α has been shown to suppress bone marrow by inducing dyserythropoiesis and reducing erythroid proliferation (Clark and Chaudhri, 1988).

2.1.2.3.3.2. Extravascular hemolysis

Hemolysis of both parasitized and non-parasitized red blood cells occurs in malaria infection (Looareesuwan *et al,* 1991). Parasitized red blood cells are destroyed during 'pitting' of the parasites from the red blood cell by macrophages in the spleen (Schnitzer *et al,* 1973). After the 'pitting' process the now non-parasitised red blood cells become defective by losing their membrane deformability and are destroyed in the spleen. The non-parasitized cells are destroyed through increased phagocytosis (Mohan *et al,* 1995) and immune mediated destruction (Woodruff *et al,* 1979).

2.1.2.3.3.3. Intravascular hemolysis

The intravascular hemolysis can be due to non-immune destruction of parasitized red cells in case of high parasitemia or due to immune mediated destruction of both parasitized and non-parasitized red cells. The changes in the red cell antigen structure brought about by the parasitic invasion stimulate the production of antibodies against the red cell. This triggers the immune mediated red cell lysis (Woodruff *et al,* 1979).

Sensitivity to anti-malaria drugs such as quinine may play a role in some patients who have been treated with quinine earlier. Patients with deficiency of glucose 6-phosphate dehydrogenase enzyme may develop hemolysis when treated with oxidant drugs like primaquine (Reeve *et al,* 1992, Sarkar *et al,* 1993).

Anemia in malaria is dependent on the degree of parasitemia, duration of the acute illness and the number of febrile paroxysms. The anemia is usually normocytic hypochromic due to reduced hemoglobin broken down by the parasite and iron sequestration. Later on there is an increase in the number of reticulocytes and polychromatophils indicating regeneration.

2.1.2.3.3.4. Leukocytic changes and cytokines profiles

Leukocyte count is usually low to normal in most cases of malaria (Taylor *et al,* 2008) and when increased it indicates either a severe infection or secondary bacterial infection. Reduction in the leukocyte count is attributed to hypersplenism or sequestration in the spleen.

Other cellular responses observed especially with chronicity include relative lymphocytosis, monocytosis, eosinopenia and presence of stab neutrophils. Adherence of monocytes in cerebral vessels and neutrophils in lung vessels has been observed in murine malaria (Senaldi-G *et al,* 1994).

The proliferation and activation of macrophages and T lymphocytes leads to production of cytokines involved in the pathogenesis of malaria. These include the pro-inflammatory cytokines TNF-α, interleukin1, interleukin 2, interleukin 6 and IFNγ that are recruited to fight the malaria parasite (MacPherson *et al,* 1985). Interleukin 1 in synergy with TNF-α induces production of nitric oxide, causes hypoglycemia and lactic acidosis in malaria infection (Rockett *et al,* 1994).

2.1.2.3.4. Platelet involvement in malaria

2.1.2.3.4.1. Thrombocytopenia

This occurs especially in *P. falciparum* infection. It is due to peripheral destruction and consumption of platelets. Reduction in platelet lifespan during malaria infection (Kreil *et al,* 2000) has been attributed to immune complexes comprising of malaria antigens and IgG and IgM antibodies that cause sequestration of platelets by macrophages in the spleen rather than splenic or hepatic clearance (Karanikas *et al,* 2004). Increased activity of macrophages causes more destruction of the platelets.

Pro-inflammatory cytokines especially TNF-α (Tacchini-Cottier *et al,* 1998) and IL-10 (Sosman *et al,* 2000) activate platelets. In turn activated platelets lose sialic acid from their membrane

resulting in intravascular lysis and accompyaning thrombocytopenia (Essien, 1989). Consumption of platelets during disseminated intravascular coagulation seen in malaria infection leads to thrombocytopenia (Boonpucknavig *et al,* 1984). Infected erythrocytes have been shown to adhere to platelets, forming clumps and this causes thrombocytopenia since these clumps are subsequently destroyed in the spleen (Pain *et al*, 2001).

2.1.2.3.4.2. Platelet dysfunction

Following malaria infection platelets become activated by immune complexes, contact with parasitized red blood cells and due to damage to endothelial cells a phenomenon called platelet hyperactivity. Once stimulated these platelets lose sialic acid from their membranes and become hyperactive followed by intravascular lysis (Essien, 1989). This lysis together with hyperaggregation of platelets and increased release of substances from platelet granules lead to formation of thrombi and disseminated intravascular coagulation.

The hyperactive platelets release substances from their granules resulting in degranulated circulating platelets. These degranulated platelets become exhausted and lose their function, hence leading to platelet hypoactivity. They become hypoaggregated to various stimulators. This leads to bleeding disorders.

2.1.2.4. Complications of malaria

2.1.2.4.1. Jaundice and hepatic dysfunction

Intravascular hemolysis causes jaundice and this result in elevation of unconjugated bilirubin levels and conjugated bilirubin as well (Murthy *et al,* 1998). Hemolysis can also increase the levels of hepatic enzymes e.g aspartate aminotransferase (SGOT). Hepatic dysfunction seen in cases of severe falciparum malaria is due to hepatocellular damage caused by impaired local microcirculation and it leads to conjugated hyperbilirubinemia, marked elevations of aspartate aminotransferase and alanine aminotransferase and prolongation of prothrombin time (Joshi *et al*, 1986, Anand *et al*, 1992, Premaratna *et al*, 2001).

2.1.2.4.2. Hemolysis, hemoglobinuria and black water fever

Hemoglobinuria is as a result of massive intravascular hemolysis which can be due to non-immune destruction of parasitized red cells in case of high parasitemia or due to immune mediated destruction of parasitized as well as non-parasitized red cells. The change in the red cell antigen structure brought about by the parasitic invasion stimulates the reticuloendothelial system to mount antibodies against the red cell. This triggers the immune mediated red cell lysis. The increased release of hemoglobin into the circulation results in hemoglobinuria and the urine appears dark brown or black hence the name black water fever. There is methemoglobinuria and heavy albuminuria. Renal functions are also affected with subsequent rise in the levels of urea and creatinine (Trang *et al*, 1992).

2.1.2.4.3. Hypoglycemia

Hypoglycemia in malaria infection is due to increased consumption of glucose by the host and the growing parasites, failure of hepatic gluconeogenesis and glycogenolysis due to hepatic dysfunction, acidemia and hyperinsulinemia and stimulation of pancreatic insulin secretion by anti-malarial drugs for instance, quinine. Hypoglycemia is also associated with increased pro-inflammatory cytokines (Grau *et al*, 1990, Kwiatkowski, 1990, Huynh *et al*, 2006).

2.1.2.4.4. Hyperparasitemia

Parasite counts above 5% is usually considered as hyperparasitemia and is a form of severe falciparum malaria. High parasitemia and presence of schizonts of *P. falciparum* in the peripheral blood are associated with a higher mortality (Nacher *et al*, 2002, Ikekpeazu *et al*, 2010).

2.1.2.4.5. Hyperpyrexia

Malaria parasites break down hemoglobin in the infected erythrocytes and one of the products is the malaria pigment hemozoin. These metabolites are believed to be pyrexic. Increase in body temperature may also be due to increased muscle activity associated with convulsions. High temperature may cause permanent severe neurological sequelae while in pregnancy it could result in fetal distress or fetal loss (Marsh, 1992).

2.1.2.4.6. Disseminated Intravascular coagulation (DIC)

DIC occurs mainly in non-immune hyperparasitemic patients infected with *P. falciparum* and with severe systemic complications (Punyagupta *et al,* 1974). It is due to activation of the clotting system resulting in thrombin generation and intravascular coagulation (Pukrittayakama et *al,* 1989). This is brought about by the alterations occuring in the cell mebrane of parasitized red blood cells (Maquir *et al,* 1991) and the intravascular lysis of red blood cells and platelets (Essien, 1989). Endothelial damage causes the release of tissue factor by activated monocytes (Pernod *et al,* 1992) causing activation of the complement and together with the increased levels of cytokines e.g. the TNF-α lead to intravascular coagulation. DIC is also brought about as a result of microcirculatory stasis caused by vascular damage.The proliferation of leukocytes causes a hyperimmune reaction with increased production of cytokines.This causes endothelial cell damage and activation of thrombin generation leading to DIC.

2.1.2.4.7. Fluid, electrolyte and acid-base disturbances

Metabolic acidosis may develop in severe cases with shock, hypoglycemia, hyperparasitemia or renal failure. Lactic acidosis is very common. Hyponatremia, though clinically insignificant is caused by inappropriate aldosterone (ADH) secretion, hypervolemia and replacement of fluids with only glucose containing IV solutions. Hypokalemia is associated with hyperventilation due to hyperpyrexia and leads to respiratory alkalosis. Hyperkalemia occurs in cases with marked intravascular hemolysis and/or well-established acute tubular necrosis (Ikekpeazu *et al*, 2010 Trang *et al*, 1992, WHO, 1990).

2.1.2.5. Specific organ pathology in malaria

2.1.2.5.1. Liver

The liver is usually enlarged, firm and may be tender (Booth *et al,* 2004, Britta *et al,* 2005). It is oedematous, brown, grey or even black due to deposition of malaria pigment (hemozoin). Histologically, there is gross congestion of the sinusoids and centilobular veins by parasitized erythrocytes. Hepatic sinusoids are dilated. In these sinusoids are hypertrophied Kupffer cells (Walters and McGregor, 1960) containing parasitized and non parasitized red cells, remnants of plasmodium parasites and granules and clusters of hemozoin (Chen *et al,* 2001), with hemosiderin occurring inconsistently. The parenchymal cells may contain hemosiderin but not hemozoin. Areas of centrilobular necrosis may be seen in severe cases and these may be due to shock or disseminated intravascular coagulation.

Chronic infection may be associated with stromal induration and diffuse proliferation of fibrous connective tissue, without cirrhosis. Involvement of hepatocytes may result in malarial hepatitis characterized by hyperbilirubinemia, increased levels of transaminases and alkaline phosphatase. It may also be associated with renal failure, anemia or other complications of falciparum malaria. Liver involvement in severe falciparum malaria is due to impairment of local microcirculation associated with hepatocellular damage.

2.1.2.5.2. Spleen

Splenomegally seen early in the infection is due to engorgement, oedema of the pulp and later due to lymphoid and reticulo-endothelial hyperplasia with an increased hemolytic and

phagocytic function of the organ (Brinkmann *et al,* 1984). When rapid and severe such enlargement may cause splenic rupture, which is a serious complication of malaria (De Aguirre *et al,* 1998). It is however more common in primary attacks of malaria. Frequent relapses and re-infections may also lead to pulp sclerosis and dilated sinuses. Due to fibrosis and perisplenitis, rupture is less likely in case of chronic splenomegaly (Mashaal, 1986).

A study performed on human patients who had died of falciparum malaria also showed some changes in the architecture and distribution of leukocytes. In this study the white pulp showed a marked architectural disorganization (Britta *et al*, 2005). There was dissolution of the marginal zones with relative depletion of B cells and alterations in splenic leukocytes. Macrophages in the red pulp were loaded with parasitized erythrocytes, ghost erythrocytes and hemozoin. Many of these parasitized erythrocytes contained trophozoites or mature schizonts and were knob positive, indicating that there was expression of variant surface antigens mediating cytoadherence (Britta *et al*, 2005).

2.1.2.5.3. Lungs

Pulmonary involvement is secondary to the changes in red blood cells and changes in local microcirculation (Boulos *et al,* 1993). Acute pulmonary oedema caused by capillary endothelial lesions, reduced vascular osmotic pressure due to reduced plasma proteins and perivascular oedema is an infrequent but nearly fatal complication of P. falciparum malaria (Aursudkij *et al,* 1998). Pulmonary capillaries and venules contain inflammatory cells and parasitized red cells. Oedema of the vascular endothelium causes narrowing of the lumen and this raises the

intravascular circulatory pressure which may lead to diapedesis. Interstitial oedema and hyaline-membrane formation are also seen. Focal or lobar pneumonia and bronchopneumonia can also complicate Malaria (Tong *et al,* 1972).

Due to anemia and tissue hypoxia caused by erythrocyte destruction and sequestration there is increased ventilation. Labored breathing is also encountered and is due to the pulmonary edema. The smaller vessels are packed with parasitized erythrocytes and hemorrhages maybe witnessed. Alveoli are congested and contain pigment containing macrophages, plasma cells, neutrophils and parasitized erythrocytes. The alveolar septa may also be thickened with areas of hemorrhage (Tatke and Malik, 1990).

2.1.2.5.4. Gastro-intestinal tract (GIT)

Malaria infection is often accompanied by nausea and vomiting, symptoms that are mainly mediated centrally (Romero *et al,* 1993). In the acute phase patients may present with anorexia, abdominal distention, and pain in the epigastrium. There is also watery diarrhoea which may be confused with gastro-enteritis or cholera. Acute colitis may be associated with malaria.

Infections of the GIT, for instance bacillary dysentery and amoebiasis may complicate malaria. When the splanchnic microcirculation is affected there is a resulting ischaemia of the gut, mucosal oedema, necrosis and ulceration and hemorhages which hamper absorption hence fluidy evacuations (Prasad and Virk, 1993). There is evidence of sequestration and cytoadherene especially in the capillaries of lamina propia of both small and large intestines. Some of these

gastrointestinal changes, for instance ulceration and necrosis may lead to absorption of toxins causing septic shock.

2.1.2.5.5. Malarial nephropathy

Malaria affects kidneys leading to both tubule-interstitial damage and glomerulonephritis. The most common renal lesion of malaria is acute renal failure due to acute tubular necrosis (Mahakur *et al*, 1983). Sequestration and cytoadherence interferes with renal microcirculation resulting in ischemia of the peritubular vessels in the kidney (Kyes *et al,* 2001). This in turn causes ischemic acute tubular necrosis. The loss of deformability of parasitized erythrocytes exacerbates ischaemia by causing sluggishness of blood flow with associated increase in viscosity, which is also contributed to by an increase in acute phase proteins such as fibrinogen (Barsoum, 2000).

Malaria infection leads to change in blood volume. Hypovolemia is frequently encountered due to excessive fluid loss attributed to hyperpyrexia, vomiting, increased capillary permeability hence fluid loss to the interstitium (Sitprija, 1998). Reduced blood pressure causes hypoperfusion of the kidney and this leads to local hypoxia with subsequent necrosis of the glomeruli and renal tubules.

On the other hand, disseminated intravascular coagulation together with hemolysis of erythrocytes attracts immune complexes that are deposited in the kidney causing an immune complex mediated nephropathy (Quartan Malaria Nephropathy) characterized by albuminuria,

oedema and hypertension (Barsoum and Sitprija, 1996). Under light microscopy, there is widening of mesangium and endothelial cell proliferation. Pigment laden macrophages and parasitized erythrocytes may be seen. Platelet fibrin thrombi and patchy necrosis of tubules may be associated (Boonpucknavig and Soontornniyomkij, 2003, Nguansangiam *et al,* 2007). The glomerulonephritis usually resolves with proper treatment of the malarial episode.

2.1.2.5.6. Central nervous system

The histopathological hallmark of cerebral malaria is engorgement of cerebral capillaries and venules with parasitised and non parasitized red blood cells (MacPherson *et al*, 1985).This is due to sequestration of red cells containing mature forms of the parasite (trophozoites and merozoites) in the microvasculature of the brain especially in the greymatter. Decreased deformability of erythrocytes, increased cytoadherence, rosetting and thrombosis with subsequent occlusion of the microcirculation results in cerebral anoxia, development of malaria granulomas and punctate haemorrhages leading to malarial encephalitis and meningoencephalitis.

Studies conducted in African children have shown a mild increase in the permeability of the blood brain barrier with disruption of the endothelial intercellular tight junctions (Brown *et al*, 1999). This is however not seen in adults (Davis *et al*, 1992). Some of the gross pathology findings of the brain include oedema (Walker *et al*, 1992), which is more common in children than adults (Looareesuwan *et al*, 1995), congestion of small blood vessels with parasitized red cells, meningeal congestion. In larger vessels, the parasites form a layer along the endothelium a

phenomenan called 'margination'. The endothelium shows pseudopodial projections, which may be in close apposition to the 'knobs' on the surface of the parasitized red cells. Petechiations are common in the white matter especially near the areas of obstruction of the arteriole, a feature rarely seen in the grey matter due to its numerous capillaries and anastomotic channels. Dürck's granulomata, which are small collections of microglial cells surrounding an area of demyelination, may be seen at these sites of haemorrhages (Claire et al, 2004).

Clinically, cerebral malaria is a diffuse encephalopathy in which focal neurological signs are relatively unusual. It presents with febrility and unconsciousness with divergent gaze and variable tone (Kennedy *et al*, 1987). The seizures may be caused by intracranial sequestration of metabolically active parasites or toxic metabolites produced by the parasites (Gordeuk *et al*, 1992). The febrile paroxysms are usually accompanied by headaches, vomiting, delirium, anxiety and restlessness and disappear with normalization of body temperature. Seizures may also be attributed to the side effects of anti-malarial drugs such as chloroquinine (Luijckx *et al*, 1992). Severe neurological outcomes may be seen and these are associated with protracted seizures, prolonged and deep coma, hypoglycaemia (Bondi, 1992 and Brewster *et al*, 1990) severe anemia and severe intracranial hypertension (Newton *et al*, 1997).

Terminal stage of cerebral malaria is marked by coma. Some studies have suggested a relationship between pro-inflammatory cytokines and cerebral malaria hence implicating them in the pathogenesis of coma. Clark *et al,* 1992 suggested that TNF-α induces the release of nitric oxide (NO), which interferes with synaptic transmission, causing coma. However, other studies

did not agree (Day *et al*, 1999) and it is still unknown of the mechanisms involved in cerebral malaria, but it is agreed they are multifactorial.

2.1.2.6. Pathogenesis and pathology of malaria in a baboon model

Primates have a similar immune system to humans and the baboon has been characterized as a model for understanding host response to *P. knowlesi.* Baboons usually develop either severe *P. knowlesi* malaria with multiple organ dysfunction and cerebral involvement, or a controlled parasitaemia resulting in a mild infection (Ozwara *et al*, 2003).

2.1.2.7. Diagnosis of malaria

Clinical diagnosis of malaria is based on the patient's signs and symptoms and other findings on examination. Specifically severe malaria is characterized by respiratory distress, hypoglycemia, renal failure, hyperbilirubinemia, and acid-base disturbances (Redd *et al*, 2006).

Microscopically, a giemsa stained blood smear will show presence of malaria parasites in their different stages of growth in the infected erythrocytes (Warhurst and Williams, 1996).

Molecular diagnosis involves detection of the parasite nucleic acids using polymerase chain reaction (PCR) (Mens *et al*, 2006). Antibodies against malaria parasites can be detected on serology using either indirect immunofluorescence (IFA) or enzyme-linked immunosorbent assay (ELISA). For instance the Rapid Diagnostic Tests (RDTs) is an ELISA-based test (Pattanasin *et al*, 2003).

2.1.2.8. Treatment of malaria

Mild malaria is treated orally using artemisinin-derived combination therapy (ACTs) (WHO, 2001a, Ogbonna and Uneke, 2008). Severe malaria may require intravenous (IV) drug treatment and fluid therapy, glucose supplement and anticonvulsants.

2.1.2.9. Prevention of malaria

Prevention of the spread of malaria and especially in areas where malaria is endemic has been achieved through the use of prophylactic drugs, mosquito eradication, and the prevention of mosquito bites (WHO, 2001b, UK Health Protection Agency, 2007).

2.1.3. Protozoan and helminth co-infections

Studies in different animal models using various species of helminths and hemoprotozoans have yielded varying results on the outcome of clinical manifestation in co-infection experiments.

An early study on concurrent infections of *Schistosoma mansoni* and *Plasmodium yoelii* in Swiss TO mice concluded that there was no difference in parasitaemia or the development of anaemia due to malaria (Lewinsohn, 1975). However, a more recent study in Swiss albino mice super infected with a lethal strain of *P. berghei* (ANKA) seven weeks after infection with *S. mansoni,* showed an increased parasitaemia and mortality, and delayed reduction and/or clearance in parasitaemia. Likewise, mice that were co-infected with *S. mansoni* and *P. chabaudi* developed an increased parasitemias (Helmby *et al*, 1998) and a delayed parasite clearance of *P. berghei*

after treatment with chloroquine (Legesse *et al*, 2004) compared with those mice that were infected with plasmodium species alone.

When similar studies were carried out in a different mouse strain the results of co-infection were not the same. The A/J mice co-infected with *S. mansoni* and *P. chabaudi* did not die and showed a concomitant enhancement of IFN-γ production to malaria antigens, compared to mice infected with *P. chabaudi* only (Yoshida *et al*, 2000).

The strain of malaria parasite used also determines the outcome of a co-infection. CBA mice co-infected with *S. mansoni*, and *P. chabaudi*, developed a lower parasitaemia compared with mice not infected with *S. mansoni* (Lwin *et al*, 1982). In this model it was also shown that unisexual infection with male *S. mansoni* worms had little inhibitory effect on malaria, indicating that egg deposition by the female schistosomiasis worms was also an important factor in the moderation of the responses to malaria. Similarly, mice that had pre-existing *Brugia pahangi* infection were found to have some protection against cerebral malaria (Yan *et al*, 1997).

Rats co-infected with *Strongyloides ratti* and *Trypanosoma brucei,* were shown to have survived longer than those infected with *T. brucei* infection alone (Onah *et al*, 2004).

Studies conducted in humans have also given contrasting results. One study conducted in Senegal revealed that the risk of clinical malaria was reduced in helminth-free children compared to children positive for Ascaris, Ancylostoma or Trichuris (Spiegel *et al*, 2003). Another study

conducted in northern Senegal showed that the prevalence of malaria attacks was higher in children positive for infection with *S. mansoni*, especially in subjects with the highest helminth loads (Sokhna *et al*, 2004).

In contrast, two recent studies have reported a protective effect of infection with *S.haematobium* on *P. falciparum* infection. A study conducted in Senegal showed that light infections of *S. haematobium* (1–9 eggs/10 mL of urine) had a protective effect by decreasing *P. falciparum* densities as compared to helminth-free children (Briand *et al*, 2005). In another study carried out in Mali, children with light infections of S. *haematobium* demonstrated a delayed time to first signs of clinical malaria, fewer malaria episodes and lower parasite densities (Lyke *et al*, 2005). In Thailand, it was shown that infection with *Ascaris lumbricoides* protected malaria patients from acute renal failure (Nacher *et al*, 2001) and from cerebral malaria (Nacher *et al*, 2000). In this study it was also noted that helminth-infected individuals had less mature and a lower frequency of circulating schizonts than patients without helminths (Nacher *et al*, 2001).

Increasing evidence now suggests that an interaction occurs between helminth and malaria infections (Helmby *et al*, 1998; Yoshida *et al*, 2000; Cox, 2001) but it is not clear if this interaction harms or protects the host.

Splenomegally and hepatomegally are significant lesions occurring in humans with co-infections of malaria and schistosomiasis (Whittle *et al*, 1969, Wilson *et al*, 2008). A recent cross-sectional study has also demonstrated that chronic exposure to malaria is associated with

hepatosplenomegaly even in the absence of *S. mansoni* infection (Wilson *et al*, 2007). It has also been suggested that there is a possible immunological mechanism for childhood hepatomegaly in areas where both *S. mansoni* and *P. falciparum* exists (Wilson *et al*, 2008, Mwatha *et al*, 2003). The two causes of hepatosplenomegaly (malaria and schistosomiasis) were originally thought to have a confounding relationship (Ongom and Bradley, 1972 and Smith *et al*, 1979). However, there is now evidence that there is an additive or synergistic effect and that chronic co-exposure to the two parasites can result in both greater prevalence of hepatosplenomegaly (Fulford *et al*, 1991, Whittle *et al*, 1969) and a greater extent of organ enlargement (Booth *et al*, 2004, Wilson *et al*, 2007). This has been attributed to augmentation of common underlying immune mechanisms (Diallo *et al*, 2004).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Experimental animals

Sub-adult olive baboons (*Papio anubis*) of both sexes, weighing between 7-13 kilograms were used in this study.

These baboons were trapped from a non-schistosomiasis-endemic region of Kenya (Aberdares area) and transported to the Institute of Primate Research. They were quarantined for 90 days in the baboon facility, at the Institute of Primate Research, during which time they were allowed time to acclimatize and were screened for zoonotic bacterial, viral and parasitic diseases and for tuberculosis, according to Standard Operating Procedures at the Institute of Primate Research. To rule out existing schistosomiasis infection, 1) fecal samples were collected from each animal within a 24 hour window period and ova examination done using the Kato technique (Katz *et al*, 1972). 2) immunodiagnosis of schistosomiasis was done since significantly low schistosoma egg counts may not be picked by the Kato technique. Serum obtained from each animal was immunoassayed for schistosomiasis specific IgG antibody against a soluble adult worm antigen (Nyindo *et al*, 1999). From these two tests, animals that were Kato test negative and, with a schistosoma antibody concentrations of at most 22 Units/mililitre ± 3 standard deviations were assigned to this study. This animals were also treated for other intestinal helminthes.

Since this was a co-infection study between schistosomiasis and malaria, animals confirmed free of the former were again screened for the latter. By use of microscopy, polymerase chain

reaction (PCR) and antibody response against *P. knowlesi* parasites, animals free of malaria hemoparasites, or prior exposure were selected. The selected cohort of baboons was then randomly divided into three groups, each comprising of eight animals and a fourth group of three animals. Equitable distribution of sexes was observed. Animals were individually caged to ensure that correct fecal samples were easily collected and that animals were subjected to minimal stress during experimental manipulation and monitoring. These cages were fitted with squeeze mechanism that ensured fast and safe sedation of animals without undue excitement and stress. Commercial monkey cubes were fed daily on these animals. Baboons are known to easily develop vitamin C deficiency, hence fresh fruits and vegetables were supplemented thrice a week. Water was provided *ad libitum.*

3.2. Ethical review

This study was approved by the Institute of Primate Research Review Committee (IRC) which evaluates scientific protocols and animal welfare and ethics.

3.3. Experimental Protocol

This study had four experimental groups. Group A was infected with schistosomiasis only (SO) and not treated. Group B was infected with schistosomiasis and later super-infected with malaria (SM) and not treated for either of the diseases. Group C was infected with schistosomiasis then treated for the disease before being super-infected with malaria (STM). Group D was infected with malaria only (MO) (Table 1).

A summary of the experimental protocol is shown in table 1.

Table 1. Experimental protocol

Group	Week				
	0	14	15	19	23
A (n=3)	S.m				
B (n=8)	S.m			P.k	P.k
C (n=8)	S.m	1st Rx	2nd Rx	P.k	P.k
D (n=8)				P.k	P.k

S.m: Infection with *S.mansoni.*
1st Rx: First treatment for *S.mansoni* with Praziquantel.
2nd Rx: Second treatment for *S.mansoni* with Praziquantel
P.k: Infection with *P.knowlesi*. Groups B, C and D had each four animals infected on the 19th and four on the 23rd week.
Group A: Schistosomiasis only, Group B: Schistosomiasis +Malaria,
Group C: Schistosomiasis (treated) +Malaria, Group D: Malaria.

3.4. Experimental infections

3.4.1. Infection of baboons with schistosomiasis

3.4.1.1. Source of infecting parasite

Schistosomiasis-infected baboons, kept in single cages at the Institute of Primate Research were used as donors for *S. mansoni* eggs. Fecal collecting trays were fitted overnight underneath the cages housing these baboons and the fecal samples collected in the morning. *S. mansoni* eggs were isolated from these fecal samples, by diluting the samples with neutral buffered saline and the solution allowed to stand for two hours, after which the supernatant was discarded. The sediment was centrifuged repeatedly, each time the supernatant was discarded untill the remaining sediment was clean. The eggs were then collected through a sieve.

These eggs were then placed in plates containing water and left for 20 to 30 minutes to hatch into miracidia, which were used to infect snails. The naive freshwater snails, *Biomphalaria pffeifeiri* are kept at the institute. Each snail was placed in a plate containing water. Using a Pasteur pipette, approximately ten miracidia were suctioned from the plates containing hatching eggs and transferred to the plates containing the snails. The snails were left in these plates for about an hour during which they were periodically exposed to light to facilitate shedding of cercarie. The collected cercariae were quantified and used to infect the experimental baboons as described in section 3.4.1.2.

3.4.1.2.Animal inoculation

Infection of baboons with *S. mansoni* cercariae was done under anesthesia. Animals were anesthetized using a mixture of 10% xyalazine (Rompun®) and 10% ketamine (Rotex medica GMBH Tritau-Germany) mixed in the ratio of 10cc of ketamine to 0.5cc of xyalazine. The mixture was then given at 0.1cc/kg body weight intramuscularly. The inguinal region of each animal was shaved clean to expose the skin. Animals belonging to Groups A, B and C were each exposed to 500 cercariae percutaneously by the "pouch method" (Sturrock *et al*, 1976). A pouch was made in the inguinal region of each anaesthetized baboons by folding the surrounding skin with pegs. A suspension containing the isolated cercariae was then poured into the pouch and left standing for 30 minutes to enhance active penetration of the cercariae through the skin. After the infection, animals were returned back to their cages. They were monitored daily for infection by fecal examination for the presence of *S. mansoni* eggs.

On the 14th week post schistosomiasis infection, animals in Group C were given the first treatment against the disease. The anti-schistosomal drug praziquantel® was given at 60mg/kg body weight orally. The equivalent tablets were crashed, mixed with fruit juice and given through a stomach tube to the anesthetized animals. The animals were monitored in the first 3 hours post-drug administration for any signs of vomiting, a side effect of praziquantel. The second treatment against schistosomiasis was administered one week later.

3.4.2. Infection of baboons with malaria

3.4.2.1. Source of infecting parasite

Plasmodium knowlesi H strain, Pk1 (A+) clone (Barnwell *et al,* 1983) blood stage parasites were used to induce malaria infection in the experimental animals. This parasite was originally isolated by Chin *et al,* 1965. Barnwell *et al,* 1983 later on succeeded in cloning this parasite by micro-manipulation and passaging in rhesus monkey. The clone is used because it produces gametocytes and its genome has been fully sequenced (Pain *et al,* 2008).

In this study, *Plasmodium knowlesi* H strain blood stage parasites were retrieved from liquid nitrogen and cultured overnight. During the overnight culture, the cryopreserved cultures of *P. knowlesi* were quickly thawed at 37°C in a water bath and transferred into 50ml centrifuge tubes. The parasites were washed twice in 3.5% sodium chloride and twice with RPMI 1640 (Rosewel Park Memorial Institute Media) with 10% baboon serum at 1200 rpm (Hettich Zentrifugen Rotanta 460R; Germany) for 10 minutes. The parasite pellet was then transferred to

an incubator and kept at 37°C for about 18 hours. The cultured parasites were adjusted to $2x10^5$ cells/ml in RPMI 1640 for optimal infection of the animals.

Inoculation of experimental animals with *P. knowlesi* was done in two phases. Four animals from Groups B, C and D each were infected with malaria on the 19th week and the remaining four on the 23rd week of the experiment. Each animal was challenged with 1×10^4 blood stage *P. knowlesi* parasites suspended in 1cc volume of phosphate buffered saline. The inoculum was given intravenously using the saphenous vein.

3.5. Animal monitoring

Animals were monitored daily from the beginning of the experiment for signs associated with either schistosomiasis or malaria. Animals with acute schistosomiasis had mucoid diarrhea, which was periodically bloody. Animals with protracted acute signs were put on supportive therapy mainly fluids and electrolytes and multivitamins. In this study, the severity of schistosomiasis as a single infection or co-infected with malaria was determined by monitoring clinical signs before and after introduction of malaria and by recording the gross and histopathological changes in the liver and colon at post mortem and recovery and quantification of adult worm at post mortem.

Animals infected with malaria were monitored daily for signs of the disease. General observation involved assessment of clinical signs that included demeanor, appetite (measurement of leftover

food), urine color, stool composition and consistency, hair coat, gait, posture, neurological signs and vomiting.

Physical examination involved monitoring respiratory rate, pulse or heart rate, state of visible mucous membranes and state of visible lymph nodes. The severity of malaria as a single infection or co-infected with schistosomiasis was determined by assessing the onset of disease and clinical manifestations among the infected animals and gross pathology at postmortem. Onset of malaria disease was associated with signs such as lethargy, reduced physical activity, reduced feed intake and prolonged sitting position.

Humane endpoints were established as criteria to end experimental studies in individual animals in order to avoid or terminate unrelieved pain and/or distress. In this study, humane endpoints were categorized as acute or sub-acute. Humane end-points occurring between days 0-10 post malaria infection were classified as acute while those occurring between days 11-20 post malaria infection were classified as sub-acute. Animals reaching humane endpoints were euthanized.

3.6. Experimental end point

The experimental end-point was set at the 30th day post malaria infection. This was the point at which all procedures were terminated based on the experimental protocol.

3.6.1. Humane end points

Humane end points were permitted based on the clinical assessment and presentation of an animal and the level of stress observed, indicating the inability of an animal to continue with the experiment. The assessment included high parasitaemia, severe anorexia, respiratory distress, anemia, hemoglobinuria, prostration and vomiting. In this study, animals reaching humane endpoints were euthanized.

As an ethical rule, and in accordance with animal welfare principles, Pentobarbitone sodium (Euthatol® May and Baker ltd, England) was administered to the animals while in an unconscious state. Animals were therefore sedated with a mixture of 10% xyalazine (Rompun®) and 10% ketamine (Rotex medica GMBH Tritau-Germany) mixed in the ratio of 10cc of ketamine to 0.5cc of xyalazine. The mixture was given at 0.1cc/kg body weight intramuscularly. Euthanasia was done through intravenous administration of Pentobarbitone sodium 200mg/ml (Euthatol®) May and Baker ltd, England) at a dose rate of 1ml/3-5 kg body weight. Each animal was confirmed dead upon stoppage of heart beat.

3.7. Post mortem examination and tissue collection

For ease of manipulation during post mortem, the four limbs were each extended and tied to the four corners of the post mortem table in a supine position.

A thorough post mortem examination was done on each animal. All organ systems were examined. However, (for the purposes of this study) five organs, namely the lungs, liver, spleen,

the colon and the brain were targeted for gross pathology. The lungs, liver and spleen are target organs for both schistosomiasis and malaria. Changes in the brain indicate cerebral involvement in malaria, while the colon is a good indicator of intestinal schistosomiasis.

Schistosomiasis infected animals were perfused as described in section 3.7.1 to recover adult worms before tissue sampling. Thereafter tissue sampling and fixation was done.

3.7.1. Perfusion to collect schistosomiasis adult worms.

Perfusion was done as described by Smithers and Terry, 1965. A midline incision was made from the neck to the groin region of each animal to be perfused and the skin undermined to expose the intercostal and abdominal muscles. The abdomen was opened by separating the muscles through an incision along the linear alba while the thoracic cavity was exposed by incising through the costochondral junctions. The abdominal aorta and the posterior venacava were clamped using self-locking atraumatic tissue forceps. The omentum was removed since it may entangle some of the perfused worms.

The abdominal aorta was clamped just before the bifurcation to the iliac arteries while the posterior venacava was clamped just before entering the heart. A small incision was then made into the abdominal aorta just after leaving the heart and a canula, connected to a perfusion equipment inserted. A second incision was made into the hepatic portal vein, creating an opening for the collection of worms.

The perfusion fluid consisted of 0.85% sodium chloride and sodium citrate. It was pumped through the abdominal aorta and collected at the hepatic portal vein having circulated through the mesenteric veins and the venous plexuses of the urinary bladder, carrying with it any worms present in these areas.

The fluid was then sucked into a bell jar using a standard vacuum pump and kept at 4^0C for one hour. It was then gently strained through a 105µm mesh sieve. The container bearing the worms was rinsed with phosphate buffered saline to ensure no worms were left attached to the container. The worms were then concentrated in one area of the sieve and transferred into a glass tube kept on ice. They were washed three times in cold phosphate buffered saline before being counted.

Meanwhile, tissue samples of liver and intestines were collected. For the liver, lung, spleen and brain tissues samples of $1cm^3$ were taken from each lobe while sections from the intestines were taken from the proximal, middle and distal segments of the colon. The samples were fixed in 10% neutral buffered formalin for at least 48 hours. At least three blocks of the tissues were prepared per organ.

3.7.2. Tissue processing

The samples were trimmed and processed through routine histological procedures as described in section 3.7.2.1. At least two paraffin sections were prepared per block of tissue at 4-5 micrometer thickness and stained with routine Haematoxylin and Eosin method as described in section 3.7.2.4.1.

3.7.2.1. Trimming, dehydration and infiltration

Approximately 2mm size of the fixed tissues were cut with a scalpel blade and transferred into a labeled tissue processing capsule. Using a pair of forceps, the capsule was immersed into an increasing concentration of ethanol; 80% for one hour, 95% for four hours, 95% for two hours, 100% for two hours, 100% for one hour and 100% for one hour. This process was aimed at dehydrating the tissue to enhance paraffin penetration.

It was followed by washing, three times using xylene, at one hour interval to clear the alcohol. The tissues were then placed in a paraffin wax bath placed in a vacuum oven at a temperature of 58-60°c to ensure complete paraffin infiltration. They were left in this oven for up to 3 hours at a pressure of 16-22psi pascals (per square inch). The paraffin bath was changed twice during the vacuum infiltration.

3.7.2.2. Casting /blocking

The capsule containg tissues was removed from the oven and centrally placed on a pre-warmed mold. Molten paraffin wax was poured onto the mold to completely cover the capsule and fill the mold. A labeled embedding ring was carefully put on the top and the mold transferred to a cold surface for wax block to solidify. After solidifying the blocks were separated from the mold and stored at 4°C awaiting sectioning.

3.7.2.3. Sectioning

A water bath was filled with water and 40mg of gelatin (section adhesive) added and set at 43-

46°C. Meanwhile, the tissue blocks to be sectioned were kept in an ice container at 4°C. The sectioning knife was carefully fixed onto the microtome and adjusted to cut 4-6 µm sections of the tissues. The tissue blocks were fastened onto the microtome stage using the tissue block holder and steadily cut to get ribbons which were transferred and floated on the warm water bath using dump carmel hair brushes.

Clean microscope slides, labeled with the animal number and name of the tissue were used to pick the tissue sections from the water bath by a gentle touch of the floating sections. This slides were then placed in a vertical staining rack and transferred into a dust free oven preset at 60°C for 30-60 minutes or at 45-50°C overnight to dry and allow for firm adhesion of the sections on glass slides.

3.7.2.4. Staining

The slides were removed from the drying oven and allowed to cool to room temperature. They were then stained with hematoxylin and eosin as described in section 3.7.2.4.1.

3.7.2.4.1. Haematoxylin and eosin staining

Sections were deparaffinised in two changes of xylene five minutes each and rehydrated by passing from absolute alcohol through decreasing concentrations of alcohol (100%, 95% and 80%) and back to water, a process that involved reversing steps used during dehydration.

The slides were stained with Harris haematoxylin for ten to fifteen minutes followed by washing with running tap water for one minute. They were washed with 0.5% acid alcohol using two dips, a process called differentiation, which turns the blue color of haematoxylin to red, followed by another washing with running tap water for one minute.

The slides were put in 2-3% lithium carbonate (ammonia water) for forty five seconds, a process called blueing to turn the red color to blue before counterstaining. They were washed in running tap water for one minute.

Counterstaining with Eosin was done for five minutes. Samples were then put through a dehydrating procedure using increasing levels of alcohol at double 95% and double 100% ethanol for fourteen deeps each. They were doubly cleared with xylene for five minutes each.

3.7.2.5. Cover slip

Using a Pasteur pipette, a thin vertical or horizontal line of mounting media, which is a mixture of distyrene, a plasticizer, and xylene (DPX) was placed on a dust-free cover slip. Then using forceps the cover slip was gently positioned on the slide and the mounting media allowed to solidify and the excess cleaned with xylene.

3.8. Examination of histological slides

In order to statistically score the severity of lesions in this study, histological indices associated with schistosomiasis-egg induced granulomatous reactions in the liver and intestines were

quantified. These included counting the number of hepatic and intestinal granuloma, measuring the size of hepatic and intestinal granuloma and counting the number of eosinophils attracted to hepatic granuloma.

3.8.1. Quantification of hepatic and intestinal granuloma

Liver sections from schistosomiasis infected groups, stained with heamatoxylin and eosin were observed under light microscope. All granulomas with a visible egg at the centre, occurring in twenty five randomly established fields on the slides were counted. Visible granulomas in the sections of the colon were counted. Five slides per animal were examined and the mean number in each animal established. The mean number per group was derived from the mean number per individual animals in each group.

3.8.2. Measurement of hepatic and intestinal granuloma

The size of hepatic and intestinal granuloma was measured using an ocular micrometer attached to a light microscope. Sizes were measured by averaging the vertical and horizontal diameters of a granuloma with a visible centrally placed schistosome egg (Farah *et al*, 2000). For standardization, all measurements were done at a magnification of x100.

3.8.3. Quantification of eosinophils attracted to hepatic granuloma

Five hepatic granulomas were randomly picked from each slide examined under light microscope. The number of eosinophils in each granuloma was then counted and the mean count for each animal and the subsequent mean for each group quantified.

3.9. Assessment of worm burden

Adult worms of both sexes recovered from the infected animals after perfusion were counted.

3.10. Data analysis

The significance of differences in mean of granuloma counts and sizes, eosinophil counts, eggs lodged in intestinal wall and worm burden between schistosomiasis-only infected (Group A) schistosomiasis-malaria co-infected (Group B) and schistosomiasis (treated) and malaria infected (Group C) was calculated using ANOVA. Post Hoc dunn's multiple comparison test was used to test the p values between selected groups. Differences were considered significant at a *P* value of < 0.05.

CHAPTER FOUR

RESULTS

4.1 MALARIA

4.1.1. Onset of clinical signs of malaria in experimental baboons with and without an existing schistosomiasis

Existing chronic schistosomiasis delayed onset of clinical signs of malaria in experimental baboons. The mean emergence of clinical signs associated with malaria disease varied among the three groups infected with malaria (Figure 1). Animals in Group D, which was infected with malaria only (MO) showed an early onset of clinical signs of the disease compared to animals in Group B and C who had a delayed onset. These signs included lethargy, reduced physical activity, reduced feed intake and prolonged sitting position. This signs were observed upto day 10, after which the asymptomatic cases survived up to day 30 post infection without clinical signs.

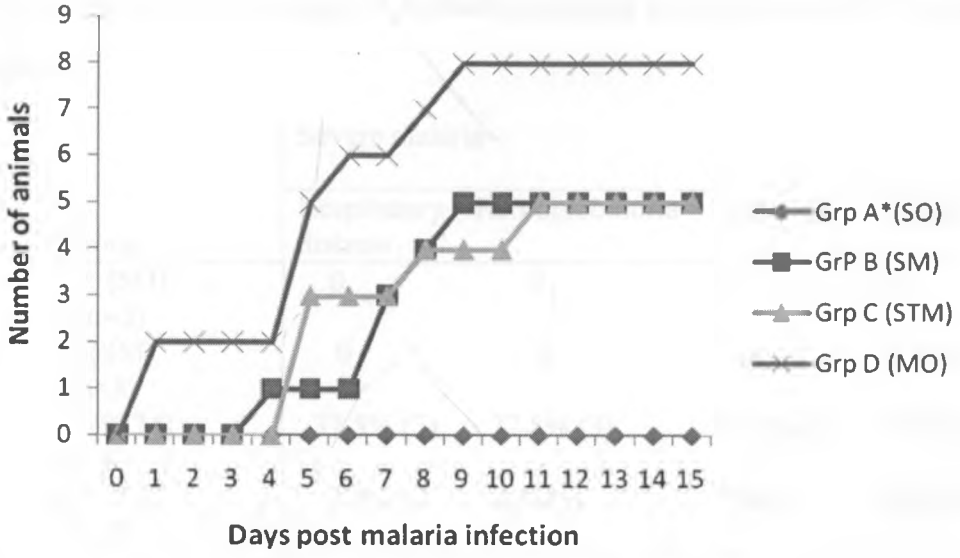

Figure 1. Onset of malaria clinical signs in baboons with and without existing schistosomiasis.
*Group A was not infected with Malaria. **SO**-Schistosomiasis only, **SM**-Schistosomiasis+Malaria, **STM**-Schistosomiasi (treated) +Malaria, **MO**-Malaria only

4.1.2. Severity of malaria infection in baboons with and without schistosomiasis co-infection

Following infection with malaria, animals showed varied degree of signs of the disease, manifesting either as severe, mild or asymptomatic outcome. A high number of animals in the malaria-only infected Group D (75%) manifested signs associated with severe malaria which included respiratory distress, hemoglobinuria, vomiting and anemia presenting as pallor of mucous membranes. On the other hand, the schistosomiasis-malaria co-infected Group B had no cases of severe malaria. Animals treated for schistosomiasis, and then infected with malaria (Group C) had a moderate malaria clinical disease outcome (Table 2).

Table 2. The mean percentage of animals presenting with either severe or mild clinical signs of malaria..

	Severe malaria				Mild malaria
Group	Respiratory distress	Hemoglobinuria	Vomiting	Anemia	Lethargy
A* (SO) (n=3)	0	0	0	0	0
B (SM) (n=8)	0	0	0	12.5% (1)	37.5%(3)
C (STM) (n=8)	37.5% (3)	37.5% (3)	37.5% (3)	37.5%(3)	25% (2)
D (MO) (n=8)	75% (6)	75%(6)	75%(6)	62.5% (5)	0

*Group A was not infected with Malaria
SO –Schistosomiasis only, SM- Schistosomiasis+Malaria,
STM-Schistosomiasis (treated) +Malaria, **MO**-Malaria only

4.1.2.1. Humane end points in baboons infected with either or both malaria and schistosomiasis

Animals recorded for humane end-points were five out of eight (62.5%) in the group infected with malaria only (Group D). This group also had a comparatively high percentage of animals' succumbing to the disease early (Table 3).

Table 3. Humane end points; The number of animals recorded for humane end-points.

Group	Animals with a humane end-point	
	Acute[1]	Subacute[2]
A* (SO) (n=3)	0	0
B (SM) (n=8)	0	12.5%(1)
C (STM) (n=8)	12.5% (1)	12.5%(1)
D (MO) (n=8)	37.5% (3)	25% (2)

[1]Humane end-points occurring between days 0-10 post Malaria infection.
[2]Humane end-points occurring between days 11-20 post Malaria infection.
*Group A was not infected with Malaria.
SO-Schistosomiasis only, **SM**-Schistosomiasis+Malaria,
STM-Schistosomiasis (treated) +Malaria, **MO**- Malaria only

4.1.3. Organ pathology in baboons infected with schistosomiasis with and without malaria super-infection

4.1.3.1. Pulmonary pathology

At post mortem, experimental animals infected with malaria alone (Group D) presented with various gross lesions in the lungs. This included, in a descending order of severity; congestion and edema, diffuse petechiations and focal petechiations. Pulmonary congestion and edema being a life threatening outcome of malaria infection was used to assess the severity of pulmonary involvement in the baboons. Malaria-only infected Group D had all the animals (100%) presenting with lung edema and congestion at post mortem, compared to a 25% case fatality in the schistosomiasis-malaria co-infected Groups B and C (Figure 2).

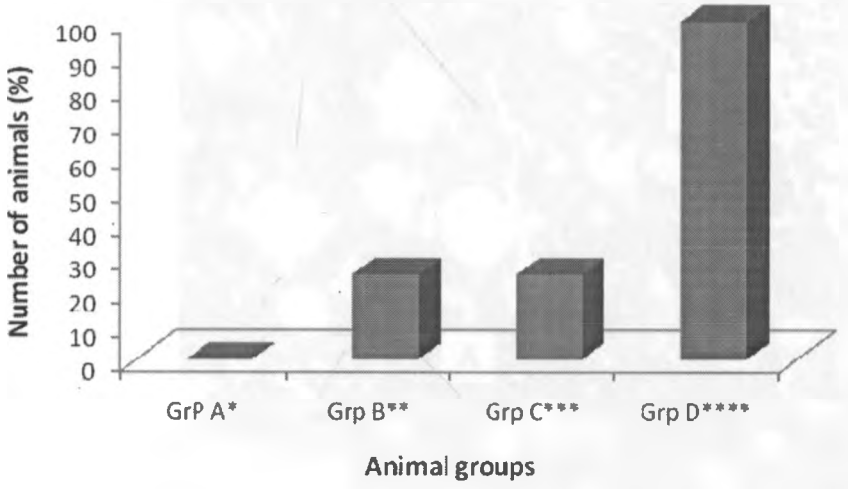

Figure 2. Frequency of pulmonary congestion and edema in baboons infected with malaria with and without schistosomiasis co-infection.

****Malaria only infected group
***Schistosomiasis (treated) + Malaria co-infected group
**Schistosomiasis +Malaria
* Schistosomiasis only

Microscopically, the schistosomiasis-malaria co-infected animals in Groups B and C had interstitial cellular infiltration, emphysema, collapsed alveoli, thickened alveoli septa and scattered hemorrhages with the normal architectural pattern of the lung lost (Figure 3). These changes were also observed in animals infected with malaria-only (Group D) who also had sequestration of parasitized and non-parasitized red blood cells in pulmonary vessels and hemorrhages accompanied by pigmentation, as were animals treated for schistosomiasis and infected with malaria (Figure 5). Animals co-infected with schistosomiasis and malaria did not show sequestration in pulmonary vessels (Figure 4). Schistosomiasis-only infected animals did not show any of the mentioned lesions.

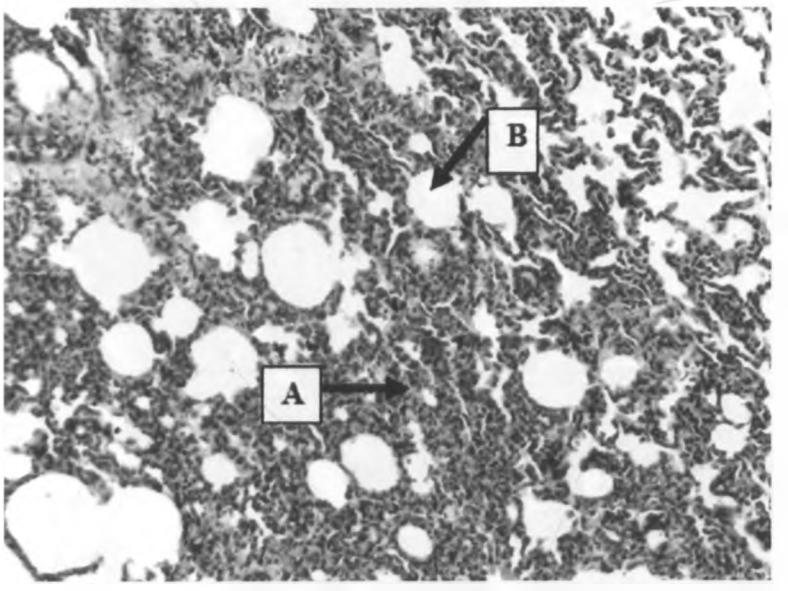

Figure 3. A photomicrograph of a section of the lung from a baboon co-infected with schistosomiasis and malaria showing alveolar collapse and thickening of alveolar walls due to cellular infiltration **(A)** and emphysema **(B)**. (H&E. ×200)

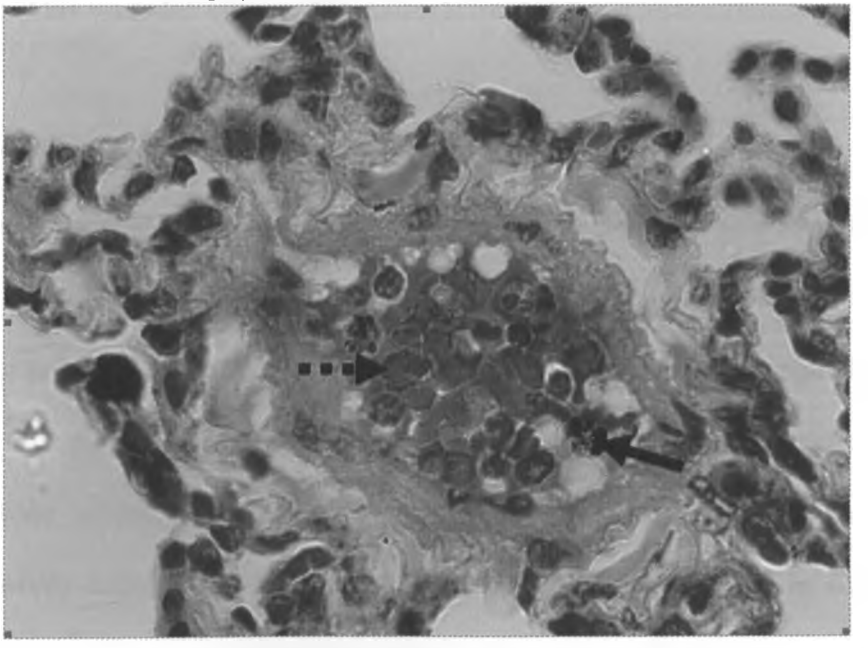

Figure 4. A photomicrograph of a pulmonary blood vessel from a schistosomiasis-malaria co-infected animal. Note the absence of parasitized red blood cells (broken arrow) and low pigment deposition (solid arrow) (H&E. ×1000).

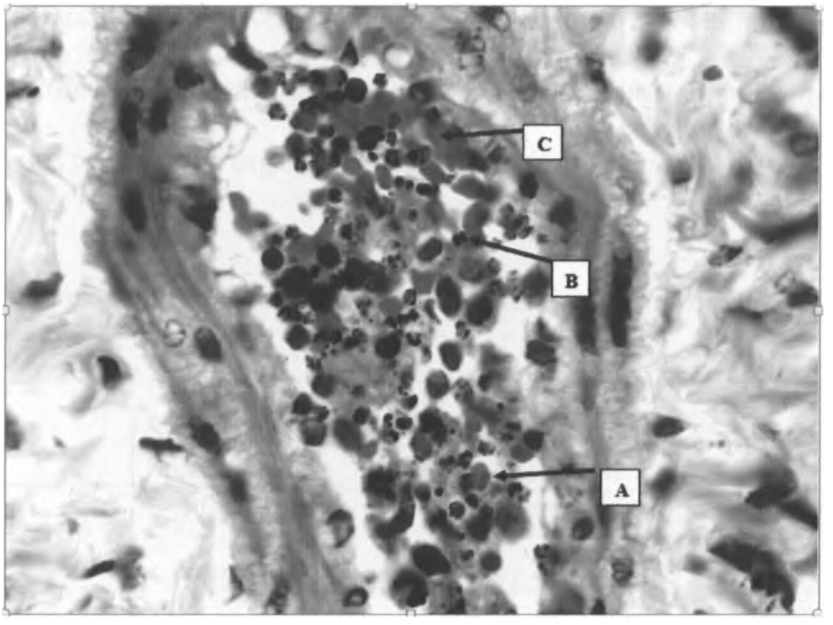

Figure 5. A photomicrograph of a section of a pulmonary vessel from a baboon infected with malaria alone showing parasitized red blood cells **(A)**, malaria pigment **(B)** and rosetting of red blood cells **(C)**. This was also observed in animals treated for schistosomiasis and infected with malaria (H&E. ×1000).

4.1.3.2. Splenic pathology

The three major gross splenic lesions observed in the experimental animals at post mortem were change in size, color and parechymal consistency. The sizes of the spleen in these baboons were either normal or enlarged while bulging of the pulp was a parenchymal change observed on the cut surface of the spleen. Splenomegally was observed in experimental animals infected with malaria, or those co-infected with schistosomiasis and malaria, but it was absent in schistosomiasis-only infected animals (Table 4). The spleen was dark red in animals infected with malaria alone, and this was due to congestion, hyperemia and deposition of hemozoin. This color change was also observed in animals treated for schistosomiasis and infected with malaria but was moderate and was mild in animals co-infected with schistosomiasis and malaria. It was

absent in schistosomiasis-only infected animals. The spleen showed bulging pulp on the cut surface in animals infected with malaria alone. The cut surface bulged and oozed dark viscous pulp and this was due to increased organ pathology attributed to splenitis.

Table 4. Splenic pathology in animals infected with malaria with or without schistosomiasis co-infection

	Splenic Pathology	
Group	Splenomegally	Pulpy spleen
A (SO) (n=3)	0%	0%
B (SM) (n=8)	100% (8)	12.5% (1)
C (STM) (n=8)	100% (8)	0%
D (MO) (n=8)	100% (8)	62.5% (5)

SO: Schistosomiasis only, SM: Schistosomiasis +Malaria
STM: Schistosomiasis (treated) +Malaria, MO: Malaria only

Microscopically, Spleen from schistosomiasis infected animals had no lesions (Figure 6). Spleen from animals infected with malaria (MO, SM and STM) had active phagocytosis in the red pulp with dark pigmentation and presence of parasitized and non-parasitized red blood cells in the red pulp. In addition, animals infected with malaria alone and those treated for schistosomiasis and then infected with malaria had sequestration of parasitized red blood cell in splenic arteries (Figure 9), hyperplasia of the lymphoid follicles and exhaustion of cells (B lymphocytes) in the white follicles seen as loss of the typical blue coloration of the white pulp. This was replaced with a paler blue color (Figures 7 and 8).

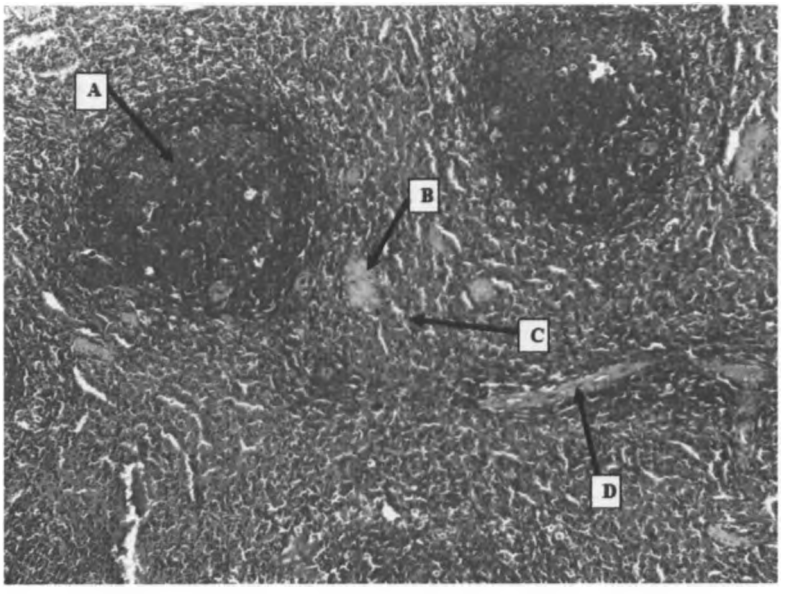

Figure 6. A photomicrograph of a section of the spleen from a baboon with chronic schistosomiasis showing the normal architectural organization; the white pulp **(A)**, splenic trabecula **(B)**, the red pulp **(C)** and splenic smooth muscle **(D)**. (H&E. ×100).

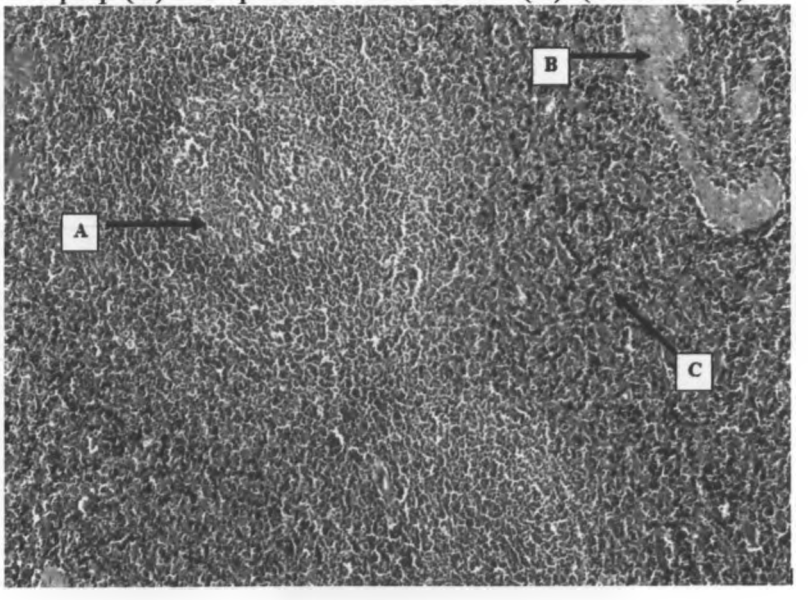

Figure 7. A photomicrograph of a section of the spleen from an animal co-infected with malaria and schistosomiasis showing lymphoid hyperplasia and slight paleness of the germinal centre **(A)**. The red pulp contains pigmentation due to malaria infection **(C)**.Note the splenic smooth muscles **(B)**. (H&E. ×100).

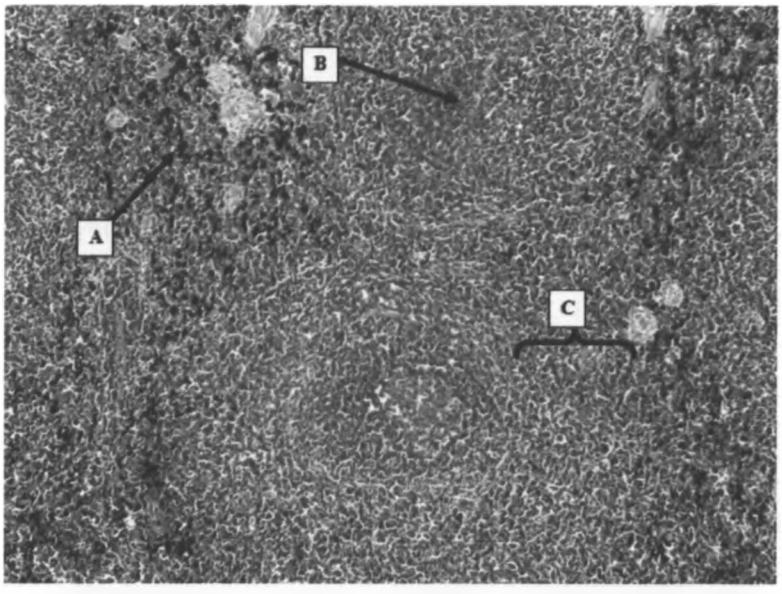

Figure 8. A photomicrograph of a section of the spleen from a baboon infected with malaria only showing the red pulp containing dark brown malaria pigment **(A)**. Note the decreasing size of the lymphoid follicle due to lymphocyte depletion **(B, C)**. (H&E. ×100).

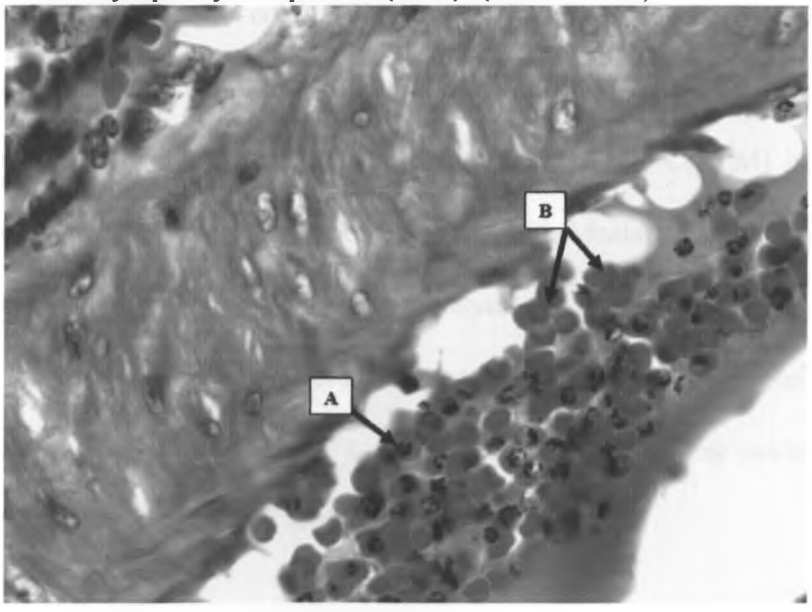

Figure 9. A photomicrograph from a splenic artery of a baboon treated for schistosomiasis then infected with malaria showing sequestration of parasitized red blood cells **(A)** and rossetting **(B).** (H&E. ×1000).

4.1.3.3. Hepatic pathology

Grossly, there was enlargement of the liver in all animals experimentally infected with either schistosomiasis or malaria or co-infected with both diseases. In addition to hepatomegally, malaria-only infected Group D (MO) animals had dark grey and hyperemic liver. The same color change was moderate in animals treated for schistosomiasis and malaria super-infected. It was mild in schistosomiasis-malaria co-infected Group B (SM) animals and absent in schistosomiasis-only infected Group A (SO) animals.

Microscopically, the schistosomiasis infected Groups A(SO), B(SM) and C(STM) showed granulomatous reactions (Andrade, 1987), periportal cellular infiltration (Figure 10), dilated sinusoids due to thinning hepatic cords (Figure 11). Double nucleated hepatocytes were a common occurrence in all the experimental animals. In addition to these histologic findings, the schistosomiasis-malaria co-infected animals in Group B(SM) and C(STM) had pigmentation with Kupffer cells hypertrophy. Animals infected with malaria only had degenerating hepatocytes, cytoplasmic vacuolation of hepatocytes, hypertrophied kupffer cells and sequestration of parasitized and non-parasitized red blood cells in hepatic vessels with accompanying Malaria pigment (Figures 12 and 13). Similar findings were moderated in animals treated for schistosomiasis and infected with malaria.

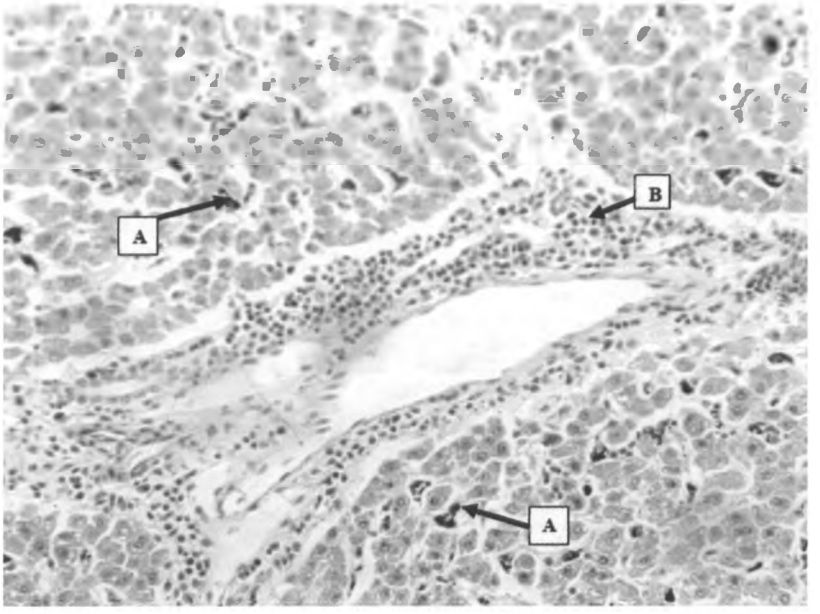

Figure 10. A photomicrograph of the liver from a schistosomiasis-malaria co-infected baboon showing activated kupffer cells **(A)** and periportal cellular infiltration **(B)**. (H&E. ×200).

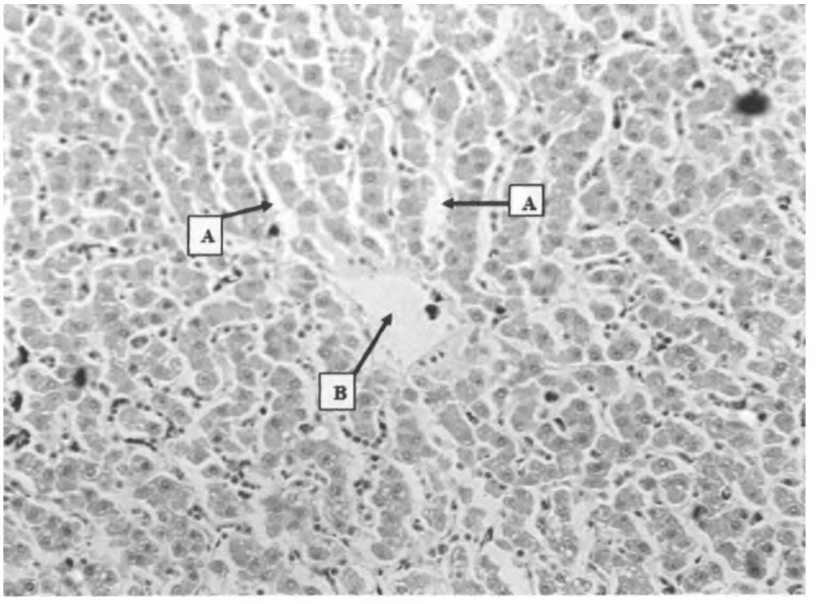

Figure 11. A photomicrograph of the liver from a schistosomiasi-malaria co-infected baboon showing dilated sinusoids **(A)** and the central vein **(B)** (H&E ×200).

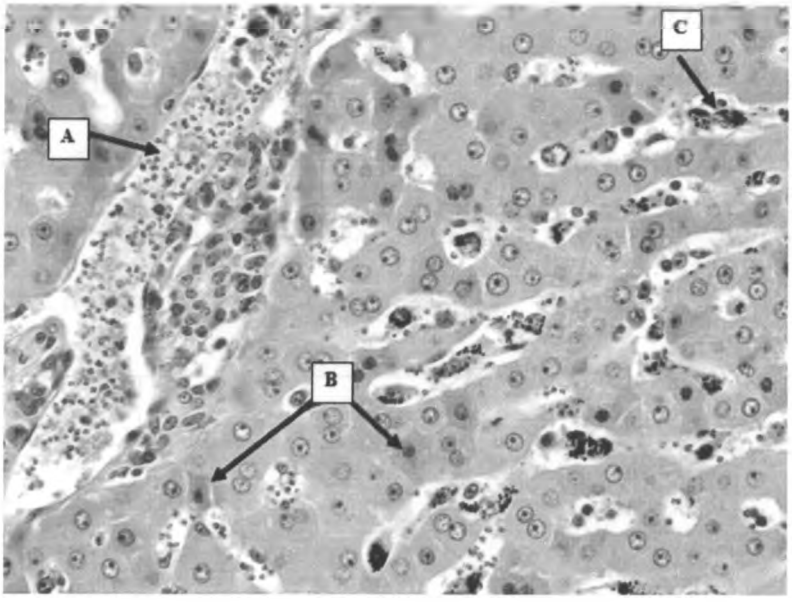

Figure 12. A photomicrograph of a section of the liver from a baboon infected with malaria alone showing sequestration of parasitized red blood cells and malaria pigment in the portal vein **(A)**, degenerating hepatocytes with pyknotic nucleus and an acidophilic cytoplasm **(B)** and activated kuppffer cells **(C)** (H&E ×400).

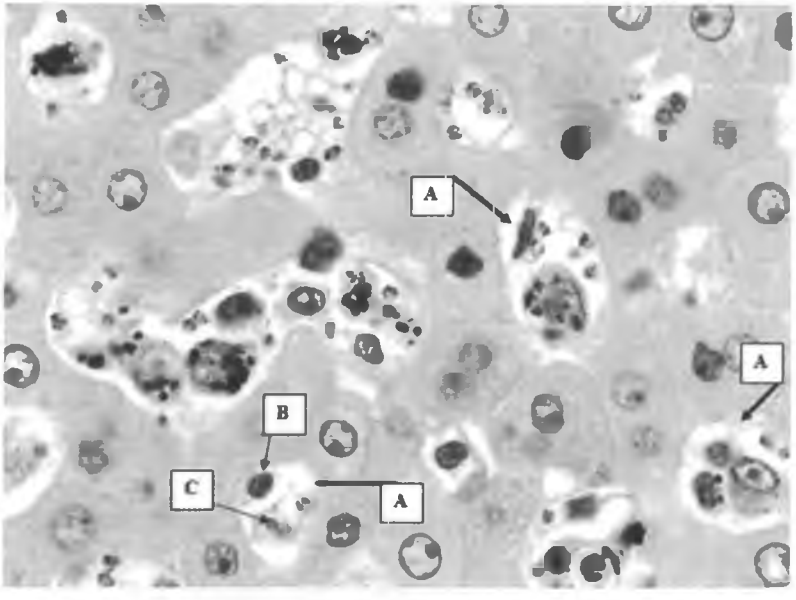

Figure 13. A photomicrograph of a section of the liver from a baboon infected with malaria alone showing cytoplasmic vacuolation **(A)** with pyknotic nucleus **(B)** and malaria pigment in hepatocyte cytoplasm **(C)** (H&E ×1000).

4.1.3.4. Colonic Pathology

At post mortem, several intestinal gross pathologic changes were observed. This included mucosal hemorrhages, mucosal congestion and nodular formations associated with schistosoma granulomas. Animals co-infected with schistosomiasis and malaria showed severe colonic gross pathology represented by mucosal hemorrhages (Table 5).

Table 5. Colonic pathology in baboons infected with schistosomiasis with and without malaria co-infection.

Group	Mucosal hemorrhages	Mucosal congestion	Nodular formations
A (SO) (n=3)	0	100% (8)	100% (8)
B (SM) (n=8)	62.5% (5)	100% (8)	100% (8)
C (STM) (n=8)	25% (2)	87.5% (7)	50% (4)
D (MO) (n=8)	0	37.5% (3)	0

SO: Schistosomiasis only, **SM:**Schistosomiasis+Malaria
STM: Schistosomiasis (treated)+Malaria, **MO:**Malaria only.

Microscopically, animals infected with schistosomiasis had granulomatous reactions in the colonic wall characterized by deposition of proteinaceous material (collagenous matrix) on the mucosae and cellular infiltration around the granuloma consisting mainly of lymphocytes, macrophages, eosinophils and plasma cells (Andrade, 1987). Most of these granulomatous reactions were located in the *Muscularis mucosae* (Figure 14). A large number of schistosomal eggs were found lodged in the lamina propria, some eliciting no inflammatory reactions (Figure

15). Animals infected with malaria only, and those treated for schistosomiasis and then infected with malaria had their intestinal vessels sequestred with parasitized and non-parasitized red blood cells with cytoadherence (Figures 16 and 17). This was absent in the other groups (Figure 18).

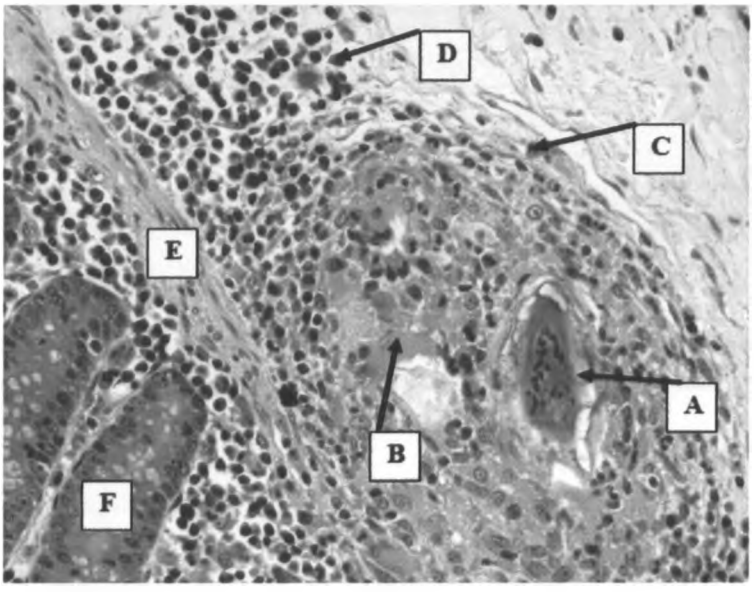

Figure 14. A photomicrograph of a colonic granuloma from a baboon infected with schistosomiasis showing schistosomal egg **(A)**, proteinaceous material **(B)**, eosinophils **(C)** and mononuclear cells **(D)**. Note the *Muscularis mucosae* **(E)** and a crypt **(F)** (H&E. ×200).

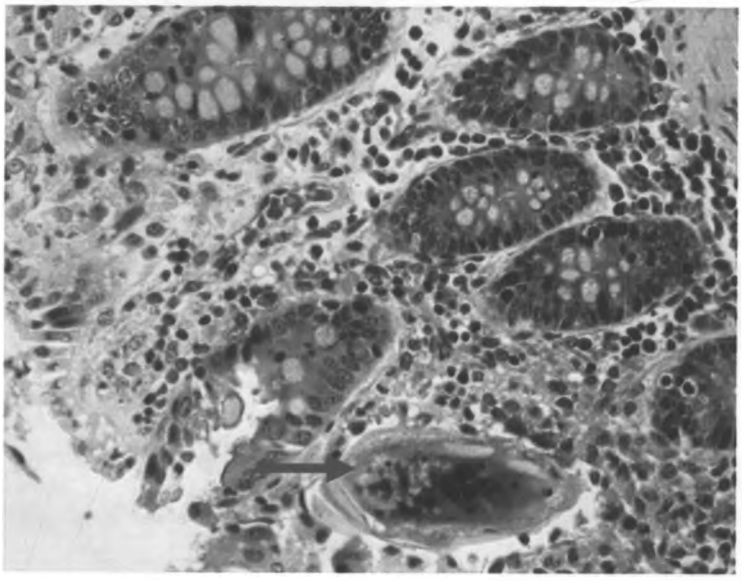

Figure 15. A photomicrograph of a colonic section from an animal infected with schistosomiasis showing an egg lodged in the lamina propria (arrow).Note the egg has not elicited cellular response. (H&E Stain. ×200).

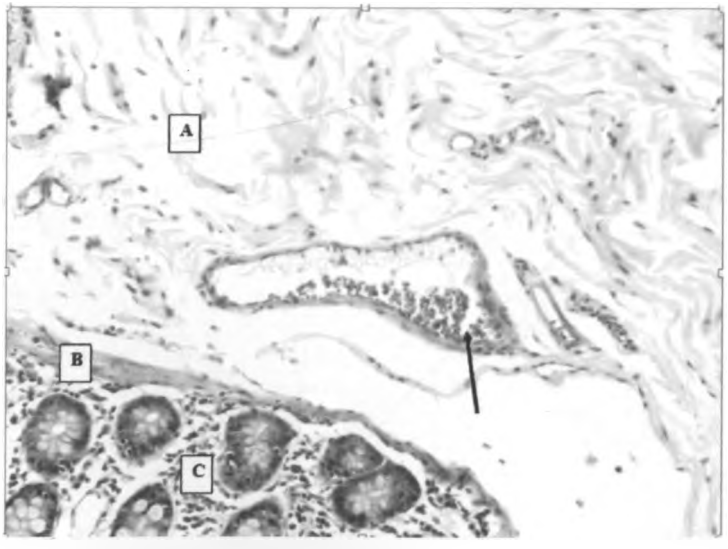

Figure 16. A photomicrograph of the colon from a baboon infected with malaria alone showing sequestration and cytoadherence in a blood vessel (arrow). Note the connective tissue layer **(A)**, *Muscularis mucosae* **(B)** and lamina propria **(C)**. (H&E. ×200).

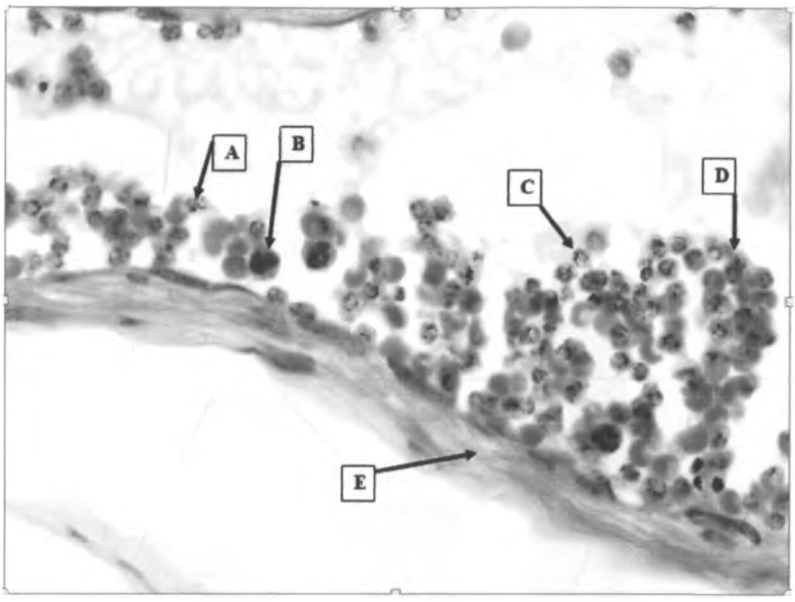

Figure 17. A photomicrograph from an intestinal blood vessel of a malaria-only infected baboon showing sequestration and cytoadherence. Note the malaria pigment **(A)**, mononuclear cells **(B)**, parasitized red blood cells **(C)** rossetting red blood cells **(D)** attached to the vascular endothelium **(E)** (H&E. ×1000).

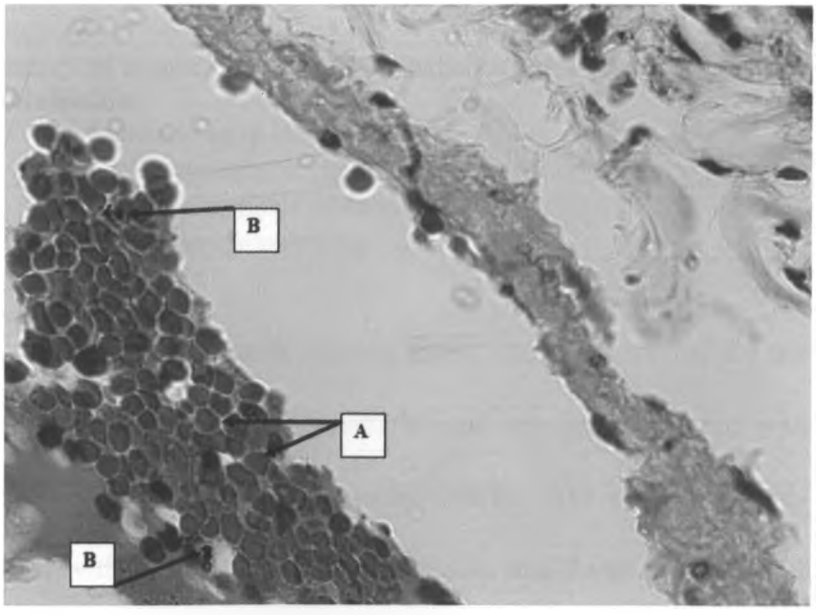

Figure 18. A photomicrograph of an intestinal vessel from a schistosomiasis-malaria co-infected baboon showing absence of parasitized red blood cells **(A)** with minimal pigment deposition **(B)**. (H&E. ×1000).

4.1.3.5. Cerebral Pathology.

Cerebral congestion was observed more in animals infected with malaria only (37.5%), compared to the other groups (Figure 19).

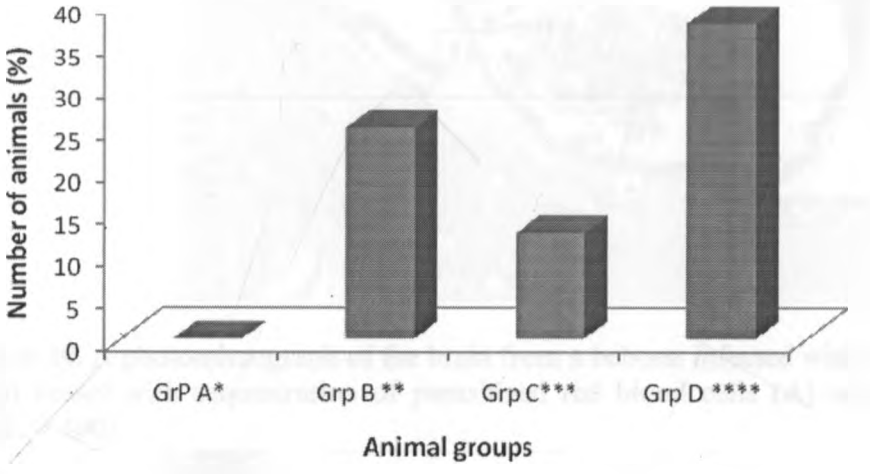

Figure 19. Frequency of cerebral pathology in baboons infected with malaria with and without schistosomiasis co-infection.

****Malaria only infected group
***Schistosomiasis (treated) + Malaria co-infected group
**Schistosomiasis +Malaria
* Schistosomiasis only

Microscopically, animals infected with malaria alone, and those treated for schistosomiasis and then infected with malaria had cerebral capillaries and venules sequestred with both parasitized and non-parasitized red blood cells and especially in the grey matter (Figures 20, 21 and 22). Animals co-infected with malaria and schistosomiasis, and those infected with schistosomiasis did not show sequestration of cerebral vessels with red blood cells (Figures 23 and 24).

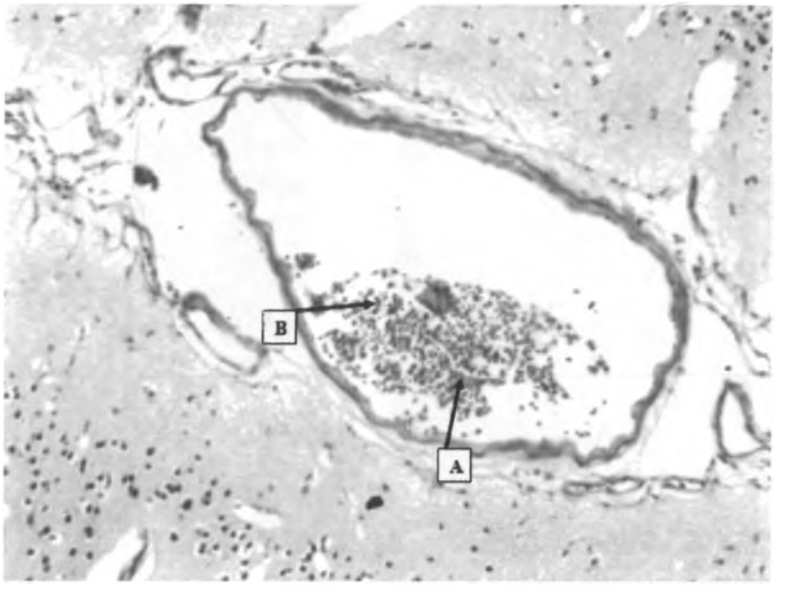

Figure 20. A photomicrograph of the brain from a baboon infected with malaria alone showing a blood vessel with sequestration of parasitized red blood cells **(A)** with malaria pigment **(B).** (H&E. ×400).

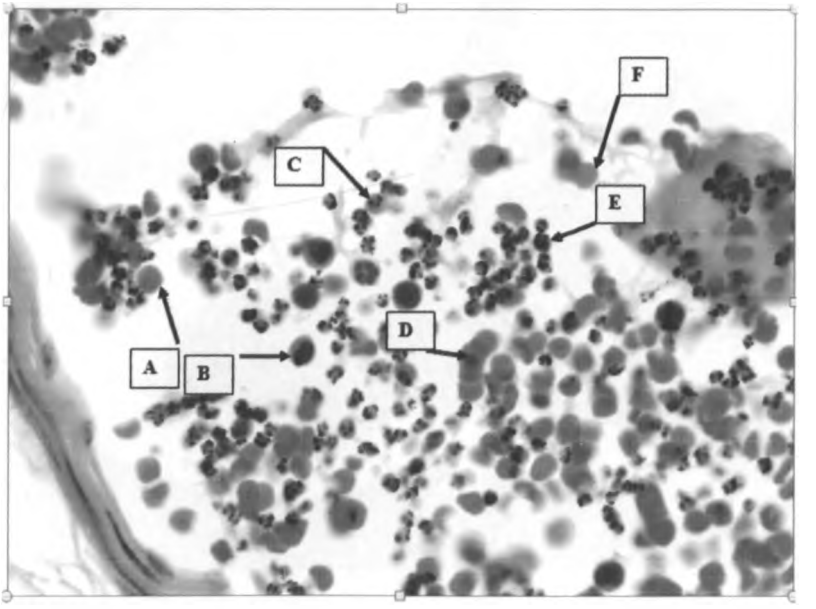

Figure 21. A photomicrograph of a brain vessel showing an immature erythrocyte appearing larger in size and bluish staining **(A),** mononuclear cell **(B),** parasitized red blood cell **(C),** rosseting of red blood cells **(D),** malaria pigment **(E)** and a mature red blood cell **(F)** (H&E. ×1000).

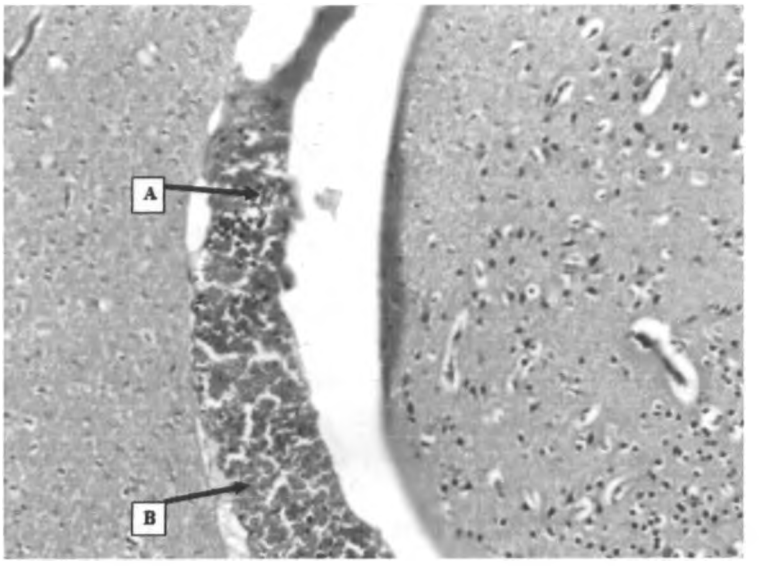

Figure 22. A photomicrograph of a brain section from a schistosomiasis (treated) then malaria infected baboon showing malaria pigment **(A)** and sequestration of red blood cells **(B)** (H&E. ×400).

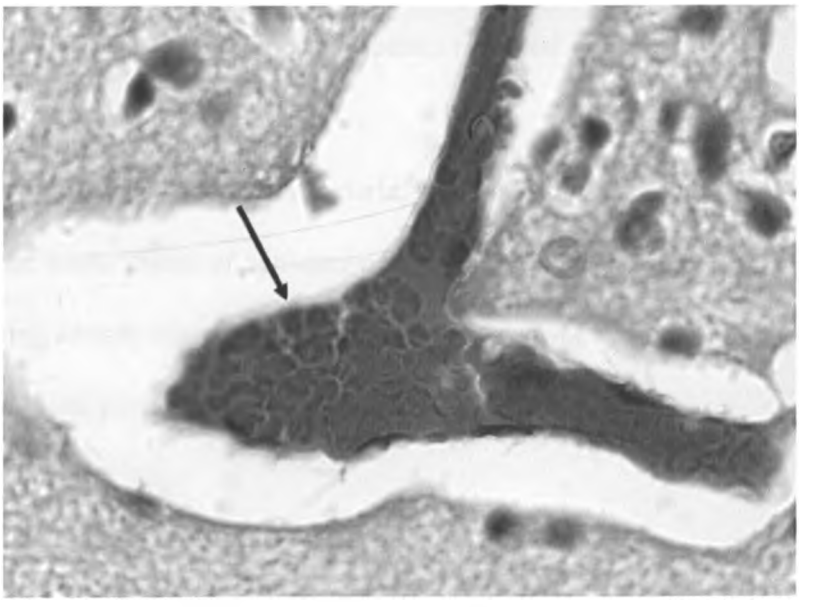

Figure 23. A photomicrograph of a brain vessel from a schistosomiasis-malaria co-infected baboon showing absence of parasitized red blood cells (arrow) (H&E. ×1000).

4.2. SCHISTOSOMIASIS

4.2.1. Effects of malaria infection on animals with chronic schistosomiasis

Introduction of malaria to animals with chronic schistosomiasis reverted some animals to the acute phase of the disease. After challenging animals with schistosomiasis at the beginning of the experiment, several of them manifested acute schistosomiasis characterized by bloody mucoid diarrhea. The number of animals manifesting this acute phase of schistosomiasis varied among the experimental groups. Group A (SO) had two animals (66.6%), Group B (SM) had seven animals (87.5%), while Group C (STM) had six animals (75%). The rest of the animals in each of the three groups did not show signs of acute Schistosomiasis. This acute phase was observed between week six and week ten (Farah *et al,* 1996). Thereafter the animals went to chronic schistosomiasis and the above mentioned symptoms resolved.

When the animals were challenged with malaria, some animals in the co-infected groups showed a recurrence of the acute phase of schistosomiasis (Figure 25). Group B (SM) had one animal (12.5%) manifesting bloody diarrhea for two days while Group C (STM) had two animals (25%) showing similar clinical picture for two days.

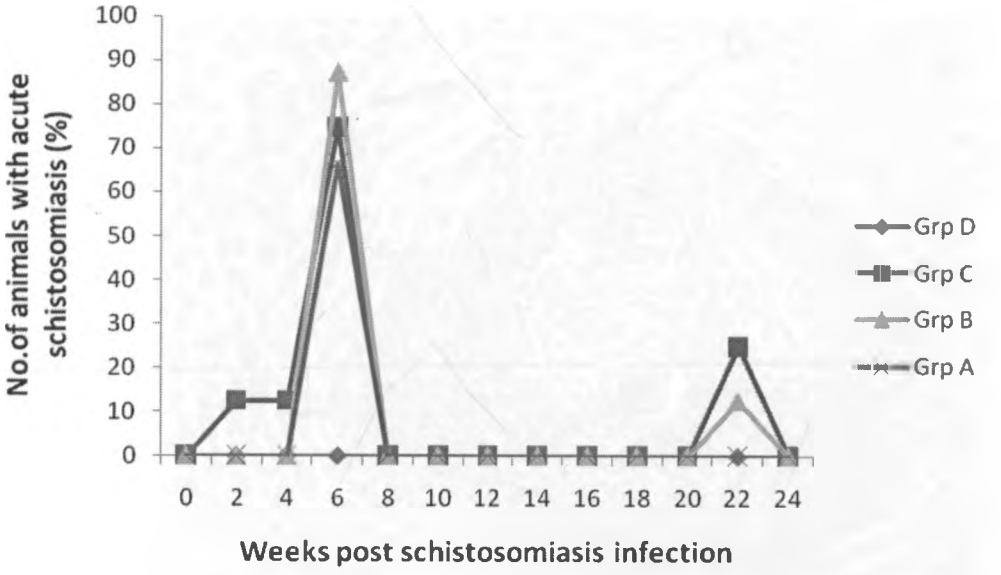

Figure 24. Severity of schistosomiasis in baboons before and after infection with malaria
Day zero-Schistosomiasis infection point, Week 19- Malaria infection point, **SO**-Schistosomiasis only, **SM**-Schistosomiasis+Malaria, **STM**-Schistosomiasis (treated) +Malaria, **MO**-Malaria only.
*Group D was Schistosomiasis-free.

4.2.2. Schistosomiasis egg-induced hepatic granulomatous response in baboons

Schistosomiasis infected animals developed egg-induced granuloma in the liver and intestines which consisted of cellular infiltrates mainly lymphocytes, macrophages, eosinophils, giant cells and plasma cells arranged around the schistosome egg (Andrade, 1987). The hepatic cords around the eggs had disrupted architectural organization, assuming a concentric appearance around the granuloma. The sinusoids were also compressed and appeared eccentric (Figure 26).

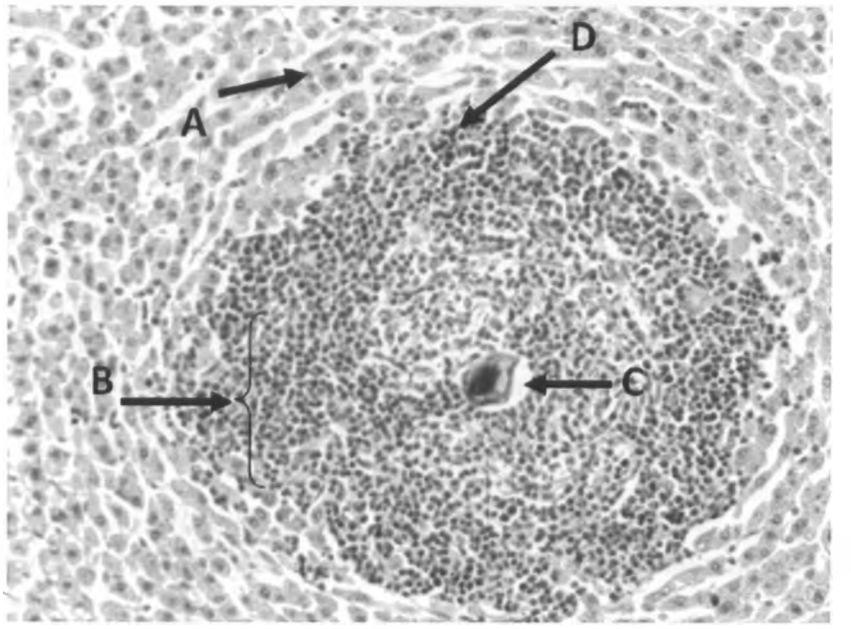

Figure 25. A photomicrograph of the liver from a schistosomiasis infected baboon showing a typical egg granuloma; **A;** hepatocyte, **B;** eosinophils, **C;** schistosome egg, **D;** mononuclear cells. Note the normal architecture of the hepatic cords and sinusoids around the granuloma is disrupted. (H&E. ×200).

4.2.3. Histological quantification of hepatic granuloma pathology

4.2.3.1. The mean number of schistosomiasis egg-induced hepatic granuloma in baboons with and without malaria co-infection

Animals co-infected with schistosomiasis and malaria had a significant ($p=0.0167$) low number of hepatic granulomas (8.88 ±1.27) compared with animals infected with schistosomiasis alone that had a mean number of 25.67±3.92 hepatic granulomas. The schistosomiasis treated and malaria infected group had a significant low number of hepatic granulomas (2.25±0.94) compared to schistosomiasis only infected group and schistosomiasis-malaria co-infected animals. (Table 6 and 7).

Table 6. Comparative mean number of schistosomiasis egg-induced hepatic granuloma in baboons with and without malaria co-infection.

Group	Mean±SE
A (SO) (n=3)	25.67±3.92
B (SM) (n=8)	8.88 ±1.27
C (STM) (n=8)	2.25±0.94

SO: Schistosomiasis only, SM: Schistosomiasis +Malaria
STM: Schistosomiasis (treated) +Malaria.
SE: Standard error

Table 7. Comparative variation, given as p-values, in the number of hepatic granuloma between different groups of baboons infected with schistosomiasis with or without malaria co-infection.

Groups	P- values
A/B	0.0167
A/C	0.0143
B/C	0.0157

4.2.3.2. The mean size of schistosomiasis egg-induced hepatic granuloma in baboons with and without malaria co-infection

There was no significant difference (p=0.4250) in the size of hepatic granuloma between schistosomiasis infected animals and those co-infected with schistosomiasis and malaria, or the schistosomiasis treated and malaria super-infected animals (p=0.4142). In deed, granuloma sizes between different pairs of experimental groups did not reflect any significant variation. Animals in Group A, that was infected with schistosomiasis alone had a granuloma mean size of 125±17.31 micrometers, Group B animals (SM) had a granuloma mean size of 126±3.40 micrometers while Group C had 86.98±38.73 micrometers in size (Table 8 and 9).

Table 8. Comparative mean size of schistosomiasis egg-induced hepatic granuloma in baboons with and without malaria co-infection.

Group	Mean±SE
A (SO) (n=3)	125±17.31
B (SM) (n=8)	126±3.40
C(STM) (n=8)	86.98±38.73

SO: Schistosomiasis only, SM: Schistosomiasis +Malaria
STM: Schistosomiasis (treated) +Malaria.
SE: Standard error.

Table 9. Comparative variation, given as p-values, in the size of hepatic granuloma between different groups of baboons infected with schistosomiasis with or without malaria co-infection.

Groups	P- values
A/B	0.4250
A/C	0.4142
B/C	0.3446

4.2.3.3. The mean number of eosinophils attracted to schistosomiasis egg-induced hepatic granuloma in baboons with and without malaria co-infection

There was a significant difference (p=0.0167) in the number of eosinophils attracted to hepatic granuloma between animals infected with schistosomiasis alone and those co-infected with schistosomiasis and malaria with the latter showing a decreased population of eosinphils (6.3±1.76). Animals treated for schistosomiasis then infected with malaria also had a significant (p=0.0412) low eosinophil counts (5.4±2.84) compared to schistosomiasis-only infected animals (26.3±12) (table 10 and 11). However there was no significant difference in eosinophil counts between Group B (SM) and Group C (STM) (p=0.6365).

An illustration on the difference in the eosinophilic cell population in a granuloma from a schistosomiasis-only infected animal and one from a schistosomiasis-malaria co-infected one is shown in figures 26 and 28.

Table 10. Comparative mean number of eosinophils attracted to hepatic granuloma in baboons with and without malaria co-infection.

Group	Mean±SE
A (SO) (n=3)	26.3±12
B (SM) (n=8)	6.3±1.76
C (STM (n=8)	5.4±2.84

SO: Schistosomiasis only, SM: Schistosomiasis +Malaria
STM: Schistosomiasis (treated) +Malaria.
SE: Standard error

Table 11. Comparative variation, given as p-values, in the number of eosinophils attracted to hepatic granuloma between different groups of baboons infected with schistosomiasis with or without malaria co-infection.

Groups	P-values
A/B	0.0167
A/C	0.0412
B/C	0.6365

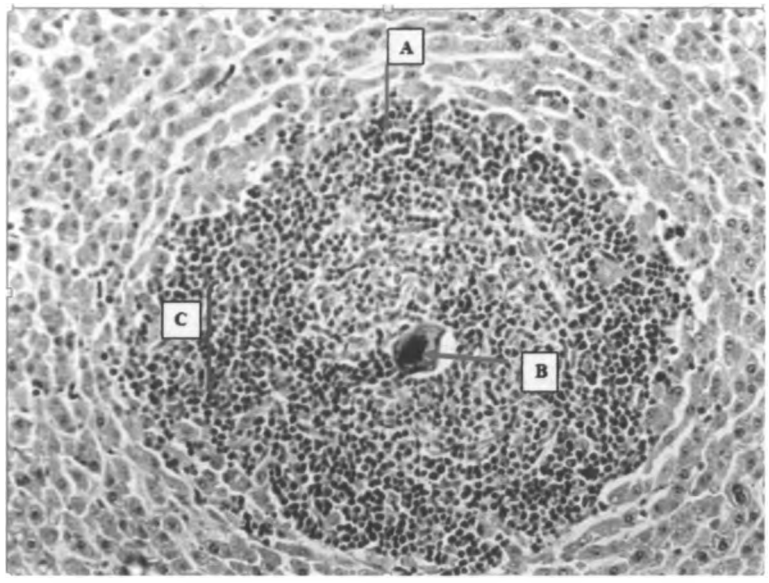

Figure 26. A photomicrograph of hepatic granuloma from a baboon infected with schistosomiasis alone showing marked eosinophil response represented by red colored spots **(C)**, schistosomal egg **(B)** and mononuclear cells **(A)** (H&E. ×200).

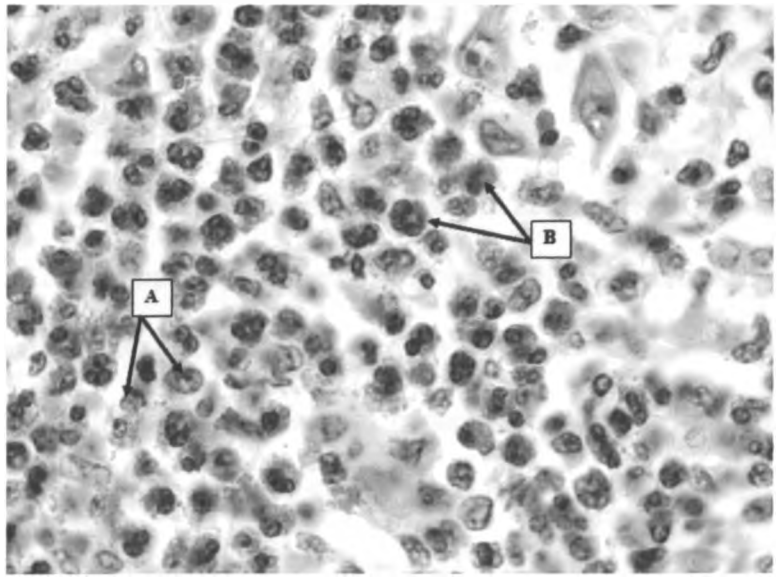

Figure 27. A photomicrograph of a section of hepatic granuloma from a schistosomiasis-only infected baboon showing macrophages **(A)** and eosinophils **(B)**. (H&E. ×1000).

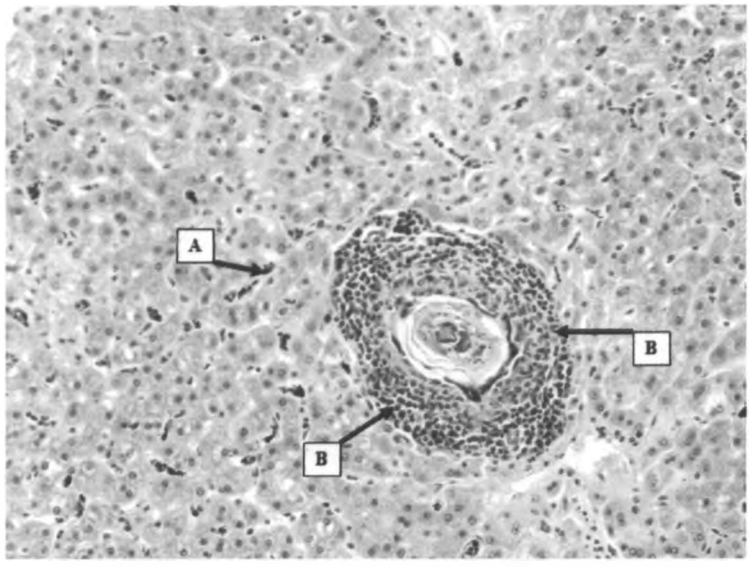

Figure 28. A photomicrograph of hepatic granuloma from a baboon co-infected with schistosomiasis and malaria showing minimal eosinophil numbers **(B)**. Note the activated kupffer cells **(A)** (H&E. ×200).

4.2.4. Histological quantification of colonic granuloma pathology

4.2.4.1. The mean number of schistosomiasis egg-induced colonic granuloma in baboons with and without malaria co-infection

Animals co-infected with schistosomiasis and malaria had a low mean number of colonic granuloma (8.75± 3.38) compared to animals infected with schistosomiasis alone (19± 6.11). However, the variation between these two groups was not significant (p=0.0827). Animals treated for schistosomiasis then infected with malaria showed a significant (p =0.0350) low number of colonic granuloma (1.75±0.67) when compared to schistosomiasis-malaria co-infected animals or schistosomiasis infected animals (Table 12 and 13).

Table 12. Comparative mean number of schistosomiasis egg-induced colonic granuloma in baboons with and without malaria co-infection

Group	Mean±SE
A (SO) (n=3)	19± 6.11
B (SM) (n=8)	8.75± 3.38
C (STM) (n=8)	1.75±0.67

SO: Schistosomiasis only, SM: Schistosomiasis +Malaria
STM: Schistosomiasis (treated) +Malaria.
SE: Standard error.

Table 13. Comparative variation, given as p-values, in the number of colonic granuloma between different groups of baboons infected with schistosomiasis with or without malaria co-infection.

Groups	P-values
A/B	0.0827
A/C	0.0143
B/C	0.0350

4.2.4.2. The mean size of schistosomiasis egg-induced intestinal granuloma in baboons with and without malaria co-infection

Animals co-infected with schistosomiasis and malaria had their colonic granuloma smaller in size (126.74±19.95 micrometers) compared with schistosomiasis only infected animals (166.97 ±9.81 micrometers). The variation was however not significant (p=0.1025). Schistosomiasis treated then malaria infected group had a mean granuloma size of 89.53±27.75 micrometers (table 14 and15). Overally, there was no significant difference in the size of intestinal granulomas between different pairs of the experimental groups.

Table 14. Comparative mean size of schistosomiasis egg-induced colonic granuloma in baboons with and without malaria co-infection.

Group	Mean±SE
A (SO) (n=3)	166.97 ±9.81
B (SM) (n=8)	126.74±19.95
C (STM) (n=8)	89.53±27.75

SO: Schistosomiasis only, SM: Schistosomiasis +Malaria
STM: Schistosomiasis (treated) +Malaria.
SE: Standard error.

Table 15. Comparative variation, given as p-values, in the size of colonic granuloma between different groups of baboons infected with schistosomiasis with or without malaria co-infection.

Groups	P-values
A/B	0.1025
A/C	0.1025
B/C	0.2936

4.2.4.3. The mean number of schistosome eggs occurring in colonic tissue of baboons infected with schistosomiasis with and without malaria co-infection

The number of schistosome eggs lodged in colonic tissue of animals co-infected with schistosomiasis and malaria was significantly (p=0.0143) lower (18.5±5.80) compared with the number occurring in schistosomiasis-only infected animals (37±11.50). Schistosomiasis treated, then malaria infected animals had very few eggs lodged in colonic tissue (0.75±0.37) (Table 16 and 17).

Table 16. Comparative mean number of schistosome eggs occurring in colonic tissue of baboons infected with schistosomiasis with and without malaria co-infection.

Group	Mean±SE
A (SO) (n=3)	37±11.50
B (SM) (n=8)	18.5±5.80
C (STM) (n=8)	0.75±0.37

SO: Schistosomiasis only, SM: Schistosomiasis +Malaria
STM: Schistosomiasis (treated) +Malaria.
SE: Standard error.

Table 17. Comparative variation, given as p-values, in the number of schistosome eggs occurring in colonic wall between different groups of baboons infected with schistosomiasis with or without malaria co-infection.

Groups	P values
A/B	0.0143
A/C	0.0143
B/C	0.0274

4.2.5. Schistosome worm numbers in baboons with and without malaria co-infection

Animals co-infected with schistosomiasis and malaria had a low number of worms recovered after perfusion (257±10.83) compared with the schistosomiasis-only infected animals (296±17.37). This variation was however not significant (p=0.0874). Animals treated for schistosomiasis then infected with malaria had significantly low worm numbers (115.5±32.71) compared with animals in Group A and B. (Table 18 and 19).

Table 18. Comparative mean number of adult schistosome worms recovered in baboons infected with schistosomiasis with and without malaria co-infection.

Group	Mean±SE
A (SO) (n=3)	296±17.37
B (SM) (n=8)	257±10.83
C (STM) (n=8)	115.5±32.71

SO: Schistosomiasis only, SM: Schistosomiasis +Malaria
STM: Schistosomiasis (treated) +Malaria.
SE: Standard error.

Table 19. Comparative variation, given as p-values, in the number of adult schistosome worms recovered in baboons infected with schistosomiasis with or without malaria co-infection.

Groups	P-values
A/B	0.0874
A/C	0.0247
B/C	0.0460

CHAPTER FIVE

DISCUSSION, CONCLUSION, RECOMMENDATIONS

5.1. Discussion

Plasmodium falciparum and *Schistosoma species* are co-endemic parasitic diseases most predominant in Sub-Saharan Africa, but whose host impact of dual infection is unknown. This study was therefore aimed at investigating the clinical and pathological outcome of either disease in a co-infection, using the baboon as an animal model.

5.1.1. Clinical outcome of malaria

This study has shown that baboons with an existing *S. mansoni* infection had a moderated malaria outcome. Introduction of *P. knowlesi* to these baboons was characterized by a delayed onset of clinical malaria and reduced disease severity. This finding agrees with co-infection studies done on other experimental animals using different strains of protozoa parasites that have supported the hypothesis that such co-infections are protective to the host. Findings from Yoshida *et al*, 2000 on A/J mice co-infected with *S. mansoni* and *P. chabaudi* showed that the co-infected mice had an increased survival rate compared to mice infected with *P. chabaudi* only.

Similarly, rats co-infected with *Strongyloides ratti* and *Trypanosoma brucei* were shown to survive longer than those infected with *T. brucei* infection alone (Onah *et al*, 2004). Co-infection studies done on humans have too generated similar results; one study reported a protective effect

of *S. haematobium* on *P. falciparum* infection (Briand *et al*, 2005, Lyke *et al*, 2005). Several other studies yielded similar results (Nacher *et al*, 2001, Nacher *et al*, 2000).

However, other studies have produced contrasting results. Kanyungo *et al*, 2009 showed that co-infection aggravated malaria severity in Balb/C mice co-infected with *S.mansoni* and *P.berghei.* In humans, the risk of clinical malaria was shown to be reduced in helminth-free children (Spiegel *et al*, 2003).

It is hypothesized that the co-infection reduced the virulence of the malaria parasite hence the delayed onset of clinical disease and lowered disease severity. It is possible that there was mild erythrocyte destruction due to lower parasitization and mild bone marrow depression. Indeed co-infected animals showed reduced cases of anemia. This could have been brought about by interference in the schizogenesis of the malaria parasite in the red blood cells. Such hypothesis agrees with Nacher *et al*, 2000 suggestion that helminth infections reduce the number of mature malaria schizonts thereby preventing sequestration and cytoadherence which are the core determinants of malaria severity.

5.1.2. Clinical outcome of schistosomiasis

The acute clinical manifestations of schistosomiasis in experimental baboons have been shown to be characterized by bloody diarrhea and weight loss (Farah *et al*, 1997). With chronicity, these symptoms resolve (Boros *et al*, 1975).

In this study, introduction of malaria to baboons with experimental chronic schistosomiasis reverted a portion of the animals (one animal in the schistosomiasis-malaria co-infected group and two animals in the schistosomiasis treated and malaria infected group) to the acute phase of the disease. Statistically, this was not significant and may not be strongly attributed to malaria super-infection.

5.1.3. Organ pathology

5.1.3.1. Liver

Animals infected with malaria alone showed hyperemic and dark grey liver as described by Ozwara *et al,* 2003, while those co-infected with malaria and schistosomiasis had mild color change. Schistosomiasis treated, then malaria infected animals had a moderate color change. This color change is due to congestion and malaria pigment hemozoin (Chen *et al*, 2001). It therefore shows that the amount of hemozoin produced by malaria parasites in the livers of schistosomiasis-malaria co-infected animals was lower compared with malaria-only infected animals.

Hemozoin has been shown to cause the production of pro-inflammatory cytokines witnessed in malaria infections (Prato *et al*, 2005). Its presence also activates macrophages which in turn produce pro-inflammatory cytokines (MacPherson *et al,* 1985). This means that severity due to pro-inflammatory cytokine-mediated pathology was mild in the co-infected animals. Hyperemia in these animals was an indication of increased circuculatory disturbances, which could have been due to loss of vascular endothelial intergrity and thrombocytopaenia (Pain *et al*, 2001).

Microscopically, animals infected with malaria alone presented a severe microscopic lesions of the liver marked by degeneration of hepatocytes, cytoplasmic vacuolation of hepatocytes, hypertrophied kupffer cells and sequestration of parasitized and non-parasitized red blood cells in hepatic vessels (Ozwara *et al,* 2003). Sequestration of red blood cells in these vessels interferes with the flow of oxygen and nutritional elements to parts of the organ distal to the point of sequestration, thereby compromising organ perfusion, creating an anoxic environment which allows for optimal growth of the malaria parasite and protects the parasite from splenic clearance (Marsh *et al*, 1988). There is accumulation of waste metabolites. This leads to cellular degeneration. Activated macrophages produce pro-inflammatory cytokines (MacPherson *et al,* 1985) that increase the severity of organ involvement.

This study has shown that hepatic lesions were severe in animals infected with malaria alone. It is likely that the virulence of the malaria parasite was low in the co-infected animals. This led to mild hepatic lesions. This is in contrast to other studies that have suggested that hepatic pathology is severe in a schistosomiasis-malaria co-infection (Whittle *et al,* 1969, Booth *et al,* 2004).

5.1.3.2. Spleen

Animals infected with malaria alone had their spleens appearing more dark red, while those of animals co-infected with schistosomiasis and malaria had a mild color change. Animals treated for schistosomiasis and then infected with malaria had a moderate color change. This color change is due to congestion, hyperemia and deposition of hemozoin. Hence the amount of

malaria pigment (hemozoin) produced was more in the malaria-only infected animals and so an increase in the severity of the disease due to hemozoin-induced damage. Vascular endothelial damage and thrombocytopaenia was severe in this animals hence the hyperemic state of the organ. Severe parechymal changes of the spleen, characterized by a bulging pulp upon cutting the organ (Ozwara *et al,* 2003), a phenomenon attributed to splenitis occurred in the malaria-only infected group.

Microscopically, animals infected with malaria alone showed severe lymphoid hyperplasia and cellular exhaustion. The area covered by the lymphoid follicles was increased due to hyperplasia. The staining of the follicles become pale blue from the typical dark blue coloration, and this was due to decrease in the total population of lymphocytes following continued activation, as has been suggested by Britta *et al*, 2005. This finding was moderate in the schistosomiasis treated then malaria infected animals and mild in the schistosomiasis-malaria co-infected animals.

Brinkmann *et al,* 1984 work agrees with this finding as he associated splenomegally in malaria infection with lymphoid and reticulo-endothelial hyperplasia. Sequestration of infected red blood cells was observed in splenic arteries of animals infected with malaria alone and those treated for schistosomiasis and infected with malaria. Such interferes with the flow of oxygen and nutritional elements to parts of the organ distal to the point of sequestration, compromising organ perfusion, creating an anoxic environment which favors the parasites growth and protects it from splenic clearance (Marsh *et al*, 1998). There is accumulation of metabolites since cellular metabolism is compromised. This leads to cellular degeneration and loss of function. Increased

activated macrophages seen in animals infected with malaria alone meant increased production of pro-inflammatory cytokines with subsequent increased disease severity.

Animals infected with malaria alone therefore presented with severe splenic lesions. This could have been due to the possibility of mild parasite virulence in the co-infected animals.
In contrast, other studies have shown that a co-infection between the two diseases enhances splenic lesions (Booth *et al,* 2004, Britta *et al,* 2005, Fulford *et al,* 1991, Whittle *et al,* 1969).

5.1.3.3. The colon

There was increased gross colonic pathology in the co-infected animals. This was manifested by increased mucosal hemorrhages. This shows that infections of the gastral tract, in this case, the schistosoma egg-induced granulomatous reactions may complicate malaria.

Microscopically, animals infected with malaria alone and those treated for schistosomiasis and then infected with malaria had sequestration and cytoadherence of parasitized red blood cells in intestinal vessels. Such sequestration compromises local microcirculation creating an anoxic environment with degeneration of tissue and loss of function, as suggested by Prasad and Virk, 1993. Animals infected with malaria alone showed increased microcirculatory disturbances indicating a high virulence of the parasite in this group of animals.

5.1.3.4. Lungs

Lung lesions were severe in malaria-only infected animals and mild in the co-infected animals. Group D, which was a malaria-only group had all the animals (100%) presenting with pulmonary edema at post mortem. Microscopically, these animals (Group D) together with animals that were treated for schistosomiasis and then infected with malaria (Group C) had their pulmonary capillaries and venules packed with parasitized red cells (Ozwara *et al,* 2003). This observation was absent in schistosomiasis-malaria co-infected animals. Such sequestration of infected erythrocytes in pulmonary vessels interfered with the local microcirculation, as suggested by Boulos *et al,* 1993, inducing an anoxic environment, interfering with cellular metabolism due to diminishing nutritional supply and eventually leading to cellular degeneration.

This study agrees with Nacher *et al,* 2000 that minimal changes in the red blood cells and local microcirculation witnessed in the co-infected animals protected them from severe lung lesions. Results from this study have also shown that acute pulmonary edema, which is an infrequent but nearly fatal complication of *P. falciparum* malaria, was less in the co-infected animals. Similarly, Claudia *et al,* 2007 showed that co-infection with *T. cruzi* protected mice from extensive pulmonary edema induced by malaria parasites.

5.1.3.5. Brain

Cerebral involvement manifesting as congestion was mild in the co-infected animals and severe in animals infected with malaria alone. Microscopically, animals infected with malaria alone (Group D) and those treated for schistosomiasis and then infected with malaria (Group C) had

sequestration of parasitized red blood cells in cerebral vessels (Ozwara *et al,* 2003). Increased cytoadherence, rosetting and thrombosis with subsequent occlusion of the microcirculation, as witnessed in animals infected with malaria alone results in cerebral anoxia and a severe clinical disease outcome (MacPherson *et al*, 1985). This lesion was lacking in animals co-infected with schistosomiasis and malaria. These findings agree with work done on C57BL/6 mice co-infected with *S. mansoni* and *P. berghei* (Kirsten *et al,* 2010) where co-infection was shown to protect the mice against brain lesions.

5.1.4. Hepatic granuloma number and size

In this study, animals co-infected with schistosomiasis and malaria had a significantly ($p<0.05$) low number of mean hepatic granulomas counted (8.88±1.27) compared to the schistosomiasis-only infected animals (25.67±3.92). This could be attributed to the observed low number of adult worms recovered in the schistosomiasis-malaria co-infected group, meaning there were comparatively fewer eggs laid. In deed, histopathological observations from the colon showed that the number of schistosomal eggs observed in colonic wall was in the co-infected animals. It is therefore hypothesized that this reduction in number of eggs laid could be attributed to a lowered fecundity of the female worms. Williams *et al*, 1994 and Couissinier-Paris and Dessein, 1995 have indicated that Th1 responses occasioned by malaria infection (Stevenson and Tam, 1993) indeed target adult worms.

The size of hepatic granuloma was smaller in the schistosomiasis-malaria co-infected groups compared to the schistosomiasis-only infected animals. This variation was however not

significant ($p>0.05$). Other studies have recorded significant difference in granuloma sizes (Kanyungo *et al*, 2009, Abdel-Wahab *et al*, 1974). Jankovic *et al*, 1997 and Mentink-Kane *et al*, 2004 associated increased granuloma size with Th2 responses. Suppression of this Th2 response has been shown to occur during schistosomiasis-malaria co-infection (Helmby *et al*, 1998)

5.1.5. Eosinophilic counts in hepatic granuloma

Eosinophils are cells of the inate immune system responsible for especially combating multicellular parasites. Animals in schistosomiasis-only infected group had a significant ($p<0.05$) increase in eosinophilic response to granulomas compared with the schistosomiasis-malaria co-infected groups. Similar finding has been observed in other studies (Abdel-Wahab *et al*, 1974). It is hypothesized that malaria super-infection interfered with the cellular dynamics induced by schistosomiasis egg antigens and that recruit eosinophils. Sher *et al*, 1990 and Gleich and Adolphson, 1986 have shown that eosinophil responses are Th2 dependent. On the other hand, Helmby *et al*, 1998 associated malaria super-infection with suppression of these Th2 responses prevalent in chronic schistosomiasis.

Such a regulated eosinophilic response is however protective to the host. Activated eosinophils secrete a range of highly toxic proteins and free radicals that are very effective in killing pathogens, but if uncontrolled may cause tissue damage (Stvrtinová *et al*, 1995).

5.1.6. Colonic granuloma number and size

Animals co-infected with schistosomiasis and malaria had a smaller number of granulomas compared to the schistosomiasis-only infected subjects. The variation was considered not significant ($p>0.05$). On the other hand, the size of granuloma did not carry a significant variation though the co-infected animals had smaller sized granulomas compared to schistosomiasis-only infected animals. This gives a picture similar to the hepatic findings.

5.1.7. Schistosoma egg counts lodged in colonic wall

The number of schistosomal eggs lodged in colonic wall was significantly low ($p<0.05$) in animals co-infected with schistosomiasis and malaria compared with animals infected with schistosomiasis alone. This shows that there was low female fecundity in the schistosomiasis-malaria co-infected animals.

5.1.8. Worm Burden

Animals infected with both schistosomiasis and malaria had an insignificantly low ($p>0.05$) number of adult worms recovered after perfusion compared to schistosomiasis-only infected animals.This is in tandem with previous co-infection work done on mice by Kanyungo *et al*, 2009, that showed a significant reduction in worm burden in co-infected mice. Yole *et al*, 2007 too showed a reduction in worm burden in mice co-infected with *S. mansoni* and *Leishmania major*. Williams *et al*, 1994 and Couissinier-Paris and Dessein, 1995 have shown that Th1 responses occasioned by Malaria infection (Stevenson and Tam, 1993) target adult worms. It is possible that this led to a reduction in the survival rate of mature worms, or induced a self-cure

phenomenon where the hostile immune environment in the host leads to evacuation of adult worms.

5.2. Conclusion

1). ***An underlying experimental schistosomiasis infection in baboons reduced the severity of a subsequent malaria infection***

2). ***Introduction of malaria to baboons harboring chronic schistosomiasis exercabated lesions occurring in the intestines while reducing severity of hepatic lesions.***

3) Treatment of schistosomiasis in baboons co-infected with malaria worsened the malaria clinical outcome.

The null hypothesis which stated that:

Schistosomiasis and malaria co-infection doesn't affect disease outcome has been rejected and therefore the alternate hypothesis which stated that schistosomiasis and malaria co-infection does affect disease outcome accepted.

5.3. Recommendations

While further studies are needed to clearly understand the immunological interaction between schistosomiasis and malaria, it is recommended that during treatment of patients, especially those from areas where the two diseases are endemic, screening for both diseases should be done during therapy. For instance, this study has shown that both diseases cause hepatomegally and therefore treating one disease in a co-infection will not alleviate the problem. Also the immunological modulation offered by either disease to each other may be affected with single treatment, thereby worsening the intact infection.

REFERENCES

Abdella S. and Wickramasinghe S. (1998). A study of erythroid progenitor cells in the bone marrow of Gambian children with falciparum Malaria. Clinical and Laboratory Haematolology, **10**:33-40.

Abdel-Wahab M.F., Mahmoud S.S. and Good W. (1974). Suppression of schistosome granuloma formation by Malaria in mice. American Journal of Tropical Medicine and Hygiene, **23**:915-918.

Aikawa M., Iseki M., Barnwell J.W., Taylor D., Oo M.M. and Howard R.J. (1990). The pathology of human cerebral Malaria. American Journal of Tropical Medicine and Hygiene, **43**:30-37.

Anand A.C., Ramji C., Narula A.S. and Singh W. (1992). Malarial hepatitis: a heterogeneous syndrome? National Medical Journal of India, **5**:59-62.

Andrade Z.A. (1987). Pathology of human Schistosomiasis. Memorias Instituto oswaldo Cruz, **82**:17-23.

Auriault C., Gras-Masse H., Pierce R.J., Butter-worth A.E., Wolowezuk I., Capron M., Ouma J.H., Balloul J.M., Khalife J. and Neyrinck J.L. (1990): Antibody response of *Schistosoma mansoni* infected human subjects to the incombinant P 28 glutathione-S-transferase and its synthetic peptides. Journal of Clinical Microbiology, **9**:1918.

Aursudkij B., Wilairatana P., Vannaphan S., Walsh D.S., Gordeux V.R. and Looareesuwan S. (1998). Pulmonary edema in cerebral Malaria patients in Thailand. Southeast Asian Journal of Tropical Medicine and Public Health, **29**:541-5.

Barnwell, J. W., Howard, R. J., Coon, H.G. and Miller, L. H. (1983). Splenic requirement for antigenic variation and expression of the variant antigen on the erythrocyte membrane in cloned *Plasmodium knowlesi* Malaria. Infection and Immunity, **40**:985-994.

Barsoum R.S. (2000). Malaria acute renal failure. Journal of American Society for Nephrology, **11**:2147-54.

Barsoum R. and Sitprija V. (1996). Tropical nephrology. In: Schrier RW, Gottaschalk CW, editors. Diseases of the kidney, VI edition. Boston: Little Brown & Co.; 2221-68.

Beltran S. and Boissier J. (2008). "Schistosome monogamy: who, how and why?" Trends in Parasitology, **24**:386-91.

Bindseil E., Iburg T., *et al.* (2004). Distinguishing periportal fibrosis from portal fibrosis in hepatic Schistosomiasis. Trends in Parasitology, **20**:361-362.

Bledsoe G. H. (2005). "Malaria primer for clinicians in the United States" Southern Medical Journal, **98**:1197-204.

Bondi F.S. (1992). The incidence and outcome of neurological abnormalities in childhood cerebral malaria: a long term follow up of 62 surviviors. Transactions of the Royal Society of Tropical Medicine and Hygiene, **86**:17-19.

Boonpucknavig V., Srichaikul T. and Punyagupta S. (1984). Clinical pathology in Malaria. In: Handbook of Experimental Pharmacology. Volume 68/1 Berlin, Heidelberg, New York, Tokyo: Springer-Verlag.127-76.

Boonpucknavig V. and Soontornniyomkij V. (2003). Pathology of renal diseases in the tropics.Seminars in Nephrology, **23**: 88-106.

Booth M., Vennervald B.J., Kenty L. *et al.* (2004): Micro-geographical variation in exposure to *Schistosoma mansoni* and Malaria, and exacerbation of splenomegaly in Kenyan school-aged children. Biomed Central Infectious Diseases, **4**:13.

Boulos M., Costa J.M. and Tosta C.E. (1993). Pulmonary involvement in Malaria. Revista do instituto de medicina Tropical de Sao Paulo, **35**:93-102.

Boros D. L. (1989). Immunopathology of *Schistosoma mansoni* infection. Clinical Microbiology Reviews, **2**:250.

Boros D. L., Pelley R.P. and Warren K.S. (1975). Spontaneous modulation of granulomatous hypersensitivity in *Schistosomiasis mansoni.* Journal of Immunology, **114**: 1437-1441.

Braschi S., Borges W.C. and Wilson R.A. (2006). "Proteomic analysis of the schistosome tegument and its surface membranes". Memórias Do Instituto Oswaldo Cruz 101.

Brewster D.R., Kwiatkowski D. and White N.J. (1990). Neurological sequelae of cerebral Malaria in children. Lancet. **336**:1039-1043.

Briand V., Watier L., Hesran J., Garcia A., and Cot M. (2005). Coinfection with *Plasmodium falciparum* and *Schistosoma haematobium*: Protective effective of Schistosomiasis on Malaria in Senegalese children. American Journal of Tropical Medicine and Hygiene, **72**:702-707.

Brinkmann V., S.H. Kaufmann, M.M. Simon and H. Fischer. (1984). Role of macrophages in Malaria: O2 metabolite production and phagocytosis by splenic macrophages. Infection and Immunity, **44**: 743-6.

Britta C.U., Tran T.H., Nicholas P.D., Nguyen H.P., Rachel R., Emsri P., Margret J., Nguyen T.H.M., Delia B., Gareth D.H.T., David F., Nicholas J.W. and David J.R. (2005). Fatal *Plasmodium falciparum* Malaria causes specific patterns of splenic architectural disorganization. Infection and Immunity, **73**:1986-94.

Brown H., Hien T.T., Day N., Mai N.T., Chuong L.V., Chau T.T., Loc P.P., Phu N.H., Bethell D., Farrar J., Gatter K., White N. and Turner G. (1999). Evidence of blood-brain barrier dysfunction in human cerebral malaria. Neuropathology and Applied Neurobiology, **25**:331-340.

Burgmann H., Looareesuwan S., Kapiotis S., Viravan C., Vanijanonta S., Hollenstein U., Wiesinger E., Presterl E., Winkler S. and Graninger W. (1996). Serum levels of erythropoietin in acute *P falciparum* Malaria. American Journal of Tropical Medicine and Hygiene, **54**:280-283.

Capron C. and Capron A. (1994). Immunoglobulin E and Effector Cells in Schistosomiasis. Science, **264**:1876-1877.

Centre for Disease control (CDC). (2007).

Chen M.M., Shi L. and Sullivan D.J. Jr. (2001). Hemoproteus and Schistosoma synthesize heme polymers similar to Plasmodium hemozoin and β-hematin. Molecular and Biochemical Parasitology, **113**:1-8.

Chen Q., Schlichtherle M. and Wahlgren M. (2000). "Molecular aspects of severe Malaria". Clinical Microbiology Reviews, **13**:439-50.

Cheever A.W. (1969). Quantitative comparison of the intensity of *Schistosoma mansoni* infections in man and experimental animals. Transactions of the Royal Society of Tropical Medicine and Hygiene, **63**:781-795.

Cheever A.W. (1972). Pipe stem fibrosis of liver. Transactions of the Royal Society of Tropical Medicine and Hygiene, **66**:946-7.

Cheever A.W. (1978). Schistosomiasis and neoplasia. Journal of the National Cancer Institute, **61**:13-8.

Cheever A.W. and Andrade Z.A. (1967). Pathological lesions associated with *Schistosoma mansoni* infection in man. Transactions of the loyal society of Tropical Medicine and Hygiene, **61**: 626-639.

Chin, W., Contacos, P.G., Coatney, R.G. and Kimbal, H.R. (1965)."Naturally Acquired Quotidian-type Malaria in Man Transferable to Monkeys". Science, **149:**865.

Claire L., Mackintosh J. G., Beeson and Kevin M. (2004). Clinical features and pathogenesis of severe Malaria. TRENDS in Parasitology, **20**:597-603.

Clark I. and Chaudhri G. (1988). Tumor necrosis factor may contribute to the anemia of malaria by causing dyserythropoiesis. British Journal of Haematology, **70**:97-103.

Clark I. A., Rockett K. A. and Cowden, W. B. (1992). Possible central role of nitric oxide in conditions clinically similar to cerebral Malaria. Lancet, **340**:894-896.

Claudia M. Egima , Silene F. Macedo , Gisela R.S. Sasso , Charles Covarrubias , Mauro Cortez , Fernando Y. Maeda , Fabio T. Costa and Nobuko Yoshida. (2007): Co-infection with *Trypanosoma cruzi* protects mice against early death by neurological or pulmonary disorders induced by *Plasmodium berghei* ANKA; Malaria Journal, **6**:90.

Cogswell F.B. (1992). "The hypnozoite and relapse in primate Malaria". Clinical Microbiology Reviews, **5**: 26-35.

Cooke B., Coppel R. and Wahlgren M. (2000). Falciparum malaria: sticking up, standing out and out-standing. Parasitology Today, **10**:416-20.

Couissinier-Paris P. and Dessein A. J. (1995). Schistosoma-specific helper T cell clones from subjects resistant to infection by *Schistosoma mansoni* are Th1/2. European Journal of Immunology, **25**:2295-2302.

Cox F.E. (2001). Concomitant infections, parasites and immune responses. Parasitology, **122**:S23-S38.

Crompton D.W. (1999). "How much human helminthiasis is there in the world?" The Journal of Parasitology, **85**:397-403.

Damian R.T., De la Rose M.A., Murfin D.J., Rawlings C.A., Weina P.J. and Hue Y.P. (1992). Further development of the baboon as a model for acute *Schistosoma mansoni*. Memoirs Instituto Oswaldo Cruz, **87**:261-269.

Davis T.M., Suputtamongkol Y., Spencer J.L., Ford S., Chienkol N., Schulenburg W.E. and White N.J. (1992). Measures of capillary permeability in acute falciparum malaria: relation to severity of infection and treatment.Clinical Infectious Diseases, **15**:256-66.

Day N.P., Hien T.T., Schollaardt T., Loc P.P., Chuong L.V., Chau T.T., Mai N.T., PhuN.H., Sinh D.X., White N.J. and Ho M. (1999). The prognostic and pathophysiologic role of pro- and antiinflammatory cytokines in severe malaria. Journal of Infectious Diseases, **180**:1288-1297.

Day N., Phu N.P., Mai N.T.H., Chau T. T., Loc P. P., Chuong L. V., Sinh D. X., Holloway P., Hien T. T. and White N. J. (2000). Prognostic significance of acidosis in severe Malaria. Critical Care Medicine, **28**:1833-40.

De Aguirre Z., De Droogh E., Van den Ende J., Lynen L., De Praetere K., Demey H.E., De Schepper A., Hubens G. and Colebunders R. (1998). Splenic rupture as a complication of *P. falciparum* Malaria after residence in the tropics. Report of two cases. Acta Clinica Belgica, **53**:374-7.

Diallo T.O., Remoue F., Schacht A.M., Charrier N., Dompnier J.-P., Pillet S., Garraud O., N'Diaye A. A., Capron A., Capron M. and Riveau G. (2004): Schistosomiasis co-infection in humans influences inflammatory markers in uncomplicated *Plasmodium falciparum* malaria. *Parasite Immunol*; **26**: 365-369.

Dondorp A.M., Kager P.A., Vreeken J. and White N.J. (2000). Abnormal blood flow and red cell deformability in severe falciparum Malaria. Parasitology Today, **16**:228-32.

El-Garem A. A. (1998): Schistosomiasis digestion, **59**:589.

Essien E. (1989). The circulating platelets in acute Malaria infection. British Journal of Haematology, **72**:589-90.

Farah I.O. and Nyindo M. (1996). *Schistosoma mansoni* induces in the Kenyan baboon a novel intestinal pathology that is manifestly modulated by an irradiated cercarial vaccine. Journal of Parasitology, **82**:601-607.

Farah I.O., Nyindo M., King C.I., and Hau J. (2000). Hepatic granulomatous response to *Schistosoma mansoni* eggs in BALB/c mice and Olive baboons (*Papio cynocephalus anubis*). Journal of Comparative Pathology, **123**:7-14.

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

Fong Y.L., Cadigan F.C. and Coatney G.R. (1971). "A presumptive case of naturally occurring *Plasmodium knowlesi* Malaria in man in Malaysia". Transactions of the Royal Society of Tropical Medicine and Hygiene, **65**: 839-40.

Fulford A.J., Mbugua G.G., Ouma J.H., Kariuki H.C., Sturrock R.F. and Butterworth A.E. (1991). Differences in the rate of hepatosplenomegaly due to *Schistosoma mansoni* infection between two areas in Machakos District, Kenya. Transactions of the Royal Society of Tropical Medicine and Hygiene, **85**:481-488.

Garcia L.S., Shimizu R.Y. and Palmer J.C. (1999). Algorithms for detection and identification of parasites. Manual of clinical microbiology. 7th edition. Washington, D.C.: American Society for Microbiology Press: 1336-54.

Gatlin M.R., Black C.L., Mwinzi P.N., Secor W.E., Karanja D.M. and Colley D.G. (2009). "Association of the gene polymorphisms IFN-gamma +874, IL-13 -1055 and IL-4 -590 with patterns of reinfection with *Schistosoma mansoni*". Public Library of Science Neglected Tropical Diseases, **3** (2): e375.

Gleich G.J. and Adolphson C.R. (1986). The eosinophilic leukocyte: structure and function. Advanved Immunology, **39**:177-253.

Gordeuk, V. R., Thuma P., Brittenham G., McLaren C., Parry D., Backenstose A., Biemba G., Msiska R., Holmes L., McKinley E., Vargas L., Gilkeson R. and Poltera A.A. (1992). Effect of iron chelation therapy on recovery from deep coma in children with cerebral Malaria. New England Journal of Medicine, **327**:1473-1477.

Grau G.E., Taylor T.E., Molyneux M.E., Wirima J. J., Vassalli P., Hommel M., and Lambert P. H. (1989). Tumor necrosis factor and disease severity in children with falciparum malaria. New England Journal of Medicine, **320**:1586-1591.

Grau G.E., Mackenzie C.D., Carr R.A., Redard M., Pizzolato G., Allasia C., Cataldo C., Taylor T.E. and Molyneux M.E. (2003). Platelet accumulation in brain microvessels in fatal pediatric cerebral Malaria. Journal of Infectious Diseases, **187**:461-6.

Haas W. and Schmitt R. (1982). Characterization of chemical stimuli for the penetration of *Schistosoma mansoni* cercariae. Effective substances, host specificity. Z Parasitenkd, **66**:293-307.

Hancock K., Mohamed Y.B., Haichou X., Noh J., Dotson E.M. and Tsang V.C.W. (1997): A recombinant protein from *Schistosoma mansoni* useful for the detection of *S. mansoni* and *S. haematobium* antibodies. Journal of Parasitology, **83**:612.

Helmby H., Kullberg M. and Troye-Blomberg M. (1998). Altered immune responses in mice with concomitant *Schistosoma mansoni* and *Plasmodium chabaudi* infections. Infection and Immunity, **66**:5167-5174.

Hesse M., Piccirillo C. A., Belkaid Y., Prufer J., Mentink-Kane M., Leusink M., Cheever A.W., Shevach E.M. and Wynn T.A. (2004). The pathogenesis of Schistosomiasis is controlled by cooperating IL-10-producing innate effector and regulatory T cells. Journal of Immunology, **172**:3157-3166.

Hutt M.S.R. (1971). Some aspects of liver disease in Ugandan Africans. Transactions of the Royal Society of Tropical Medicine and Hygiene, 65:273-81.

Huynh V. T., Piet A. K. and Hans P. S. (2006). Hypoglycemia in *falciparum* malaria: is fasting an unrecognized and insufficiently emphasized risk factor? Trends in Parasitology, **22**: 410-415.

Ikekpeazu E.J., Neboh E.C., Aguchime N.C., Maduka I.C. and Aronu E.A. (2010). Malaria Parasitaemia: Effect on Serum Sodium and Potassium Levels. International Journal of Tropical Medicine, **5**:46-49.

Jacobs W., Bogers J., Timmermans J.P., Deelder A. and Van Marck E. (1998). Adhesion molecules in intestinal *Schistosoma mansoni* infection. Parasitology Research, **84**:276-280.

Jankovic D., Kullberg M. C., Dombrowicz D., Barbieri S., Caspar P., Wynn T. A., Paul W. E., Cheever A. W., Kinet J. P. and Sher A. (1997). Fc epsilonRI-deficient mice infected with *Schistosoma mansoni* mount normal Th2-type responses while displaying enhanced liver pathology. Journal of Immunology, **159**:1868-1875.

Jootar S., Chaisiripoomkeeree W., Pholvicha P. Leelasiri A., Prayoonwiwat W., Mongkonsvitragoon W. and Srichaikul T. (1993). Suppression of erythroid progenitor cells during malaria infection in Thai adults caused by serum inhibitor. Clinical Laboratory Haematology, **15**:87-92.

Joshi Y.K., Tandon B.N., Acharya S.K., Babu S. and Tandon M. (1986). Acute hepatic failure due to Plasmodium falciparum liver injury. Liver, **6**:357-60.

Kamel J.A., Elwi A.M., Cheever A.W., Mossimann J.E. and Danner R. (1978). *Schistosoma mansoni* and *Schistosoma haematobium* infectionin Egypt.IV. Hepatic lesions. American Journal of Tropical Medicine and Hygiene, **27**:939-43.

Kano S. and Aikawa M. (1999). Pathology and pathophysiology of Malaria. Japanese Journal of Tropical Medicine and Hygiene, **27**:471-476.

Kanyungo M. S., Ozwara H., Mutahi W. and Yole D. (2009). Parasitological and immunopathological responses in Balb/C mice with concomitant *Schistosoma mansoni* And *Plasmodium berghei* infections. The Internet Journal of Tropical Medicine. Volume 5 Number 2.

Karanikas G., Zedwitz-Liebenstein K., Eidherr H., Schuetz M., Sauerman R., Dudczak R., Winkler S., Pabinger I. and Kletter K. (2004). Platelet kinetics and scintigraphic imaging in thrombocytopenic Malaria patients. Thrombosis and Haemostasis, **91**:553-557.

Katz N., Chaves A. and pellegrino J. (1972). A simple device for quantitative thick smear technique in *Schistosomiasis mansoni*. Revista do instituto de medicina Tropical de Sao Paulo, **14**:397-400.

Kennedy P.G.E., Johnson R.I., White N.J. and Looareesuwan S. (1987). Cerebral malaria. Infections of the nervous system. Butterworths, London, pp 118–143.

King C.H., Magak P., Salam E.A., Ouma J.H., Kariuki H.C. and Blanton R.E. (2003). Measuring morbidity in *Schistosomiasis mansoni*: relationship between image pattern, portal vein diameter and portal branch thickness in large-scale surveys using new WHO coding guidelines for ultrasound in Schistosomiasis. Tropical Medicine and International Health, **8**:109-117.

Kirsten B., Klaus D., Peter L., Bastian P., Rolf F., Benjamin M., Anne B. and Wolfgang H. H. (2010). Schistosoma co-infection protects against brain pathology but does not prevent severe disease and death in a murine model of cerebral Malaria. International Journal for Parasitology, **41**:21-31.

Kitani A., Fuss I., Nakamura K., Kumaki F., Usui T., and Strober W. (2003). Transforming growth factor (TGF)-ß1-producing regulatory T cells induce Smad-mediated interleukin 10 secretion that facilitates coordinated immunoregulatory activity and amelioration of TGF-ß1-mediated fibrosis. Journal of Experimental Medicine, **198**:1179-1188.

Kreil A., Wenisch C., Brittenham G., Looareesuwan S. and Peck-Radosavljevic M. (2000). Thrombopoietin in *Plasmodium falciparum* Malaria. British Journal of Haematology, **109**:534-536.

Kwiatkowski D., Hill A.V., Sambou I., Twumasi P., Castracane J., Monogue K.R., Cerami A., Brewster D.R. and Greenwood B.M. (1990). TNF concentration in fatal cerebral, non-fatal cerebral, and uncomplicated Plasmodium falciparum malaria. Lancet, **336**:1201-1204.

Kyes S., Horrocks P. and Newbold C. (2001). Antigenic variation at the infected red cell surface in Malaria. Annual Review of Microbiology, **55**:673-707.

Kyes S.A., Rowe J.A., Kriek N., Newbold C. and Rifins I. (1999). A second family of clonally variant proteins expressed on the surface of red cells infected with *plasmodium falciparum.* Proceedings of the National Academy of Sciences of the United States America, **96**:9333-8.

Legesse M., Erko B. and Baicha F. (2004). Increased parasitaemia and delayed parasite clearance in *Schistosoma mansoni,* and *Plasmodium berghei* co-infected mice. Acta Tropical, **91**:161-166.

Leshem E., Maor Y., Meltzer E., Assous M. and Schwartz E. (2008). Acute Schistosomiasis outbreak: clinical features and economic impact. Clinical Infectious Diseases, **47**:1499-506.

Lewinsohn R. (1975). Anaemia in mice with concomitant *Schistosoma mansoni* and *Plasmodium berghei yoelii* infection. Transactions of the Royal Society of Tropical Medicine and Hygiene, **69**:51-56.

Looareesuwan S., Davis T., Pukittayakamee S., Supanaranond W, Desakorn V, Silamut k., Krishna S, Boonamrung S and White NJ (1991). Erythrocyte survival in severe falciparum Malaria. Acta Tropical, **48**:263-70.

Looareesuwan S., Wilairatana P., Krishna S., Kendall B., Vannaphan S., Viravan C. and White N.J. (1995.) Magnetic resonance imaging of the brain in patients with cerebral malaria. Clinical Infectious Diseases, **21**:300-309.

Loverde P.T. and Chen L. (1991). "Schistosome female reproductive development". Parasitology Today, **7**:303-8.

Luijckx G.J., De Krom M.C. and Takx-Kohlen B.C.(1992). Does chloroquine cause seizures? Presentation of three new cases and a review of the literature. Seizure: the journal of the British Epilepsy Association **1**:183-185.

Lwin M., Last C., Targett G.A. and Doenhoff M.J. (1982). Infection of mice concurrently with *Schistosoma mansoni* and rodent Malarias: contrasting effects of patent *S. mansoni* infections on *Plasmodium chabaudi, P. yoelii* and *P. berghei*. Annals of Tropical Medicine and Parasitology, **76**:265-273.

Lyke K.E., Dicko A., Dabo A., Sangare L., Kone A., Coulibaly D., Guindo A., Traore K., Daou M., Diarra I., Sztein M. B., Plowe C. V. and Doumbo O. K. (2005). Association of *Schistosoma haematobium* infection with protection against acute *Plasmodium falciparum* malaria in Malian children. American Journal of Tropical Medicine and Hygiene, **73:**1124-1130.

Machado-Silva J.R., Galvao C., Oliveira R.M.F., Presgrave A.F. and Gomes D.C. (1995). *"Schistosoma mansoni* sambon, 1907: Comparative morphological studies of some Brazilian Strains". Revista do instituto de medicina Tropical de Sao Paulo, **37**:441-447.

MacPherson G.G., Warrell M.J., White N.J., Looareesuwan S. and Warrell D.A. (1985). Human cerebral malaria. A quantitative ultrastructural analysis of parasitized erythrocyte sequestration. American Journal of Pathology, **119**:385-401.

Magowan C., Wollish W., Anderson L. and Leech J. (1988). Cytoadherence by *Plasmodium falciparum* infected erythrocytes is correlated with expression of a family of variable proteins on infected erythrocytes. Journal of Experimental Medicine, **168**:1307-20.

Mahakur A.C., Panda S.N., Nanda B.K., Bose T.K., Satpathy S.R. and Misra Y. (1983). Malarial Acute Renal Failure. Journal of the Association of Physicians of India, **31**:633-616.

Manoukian N. and Borges D.R. (1984). Prealbumin, prekallikrein and prothrombin in hepatosplenic Schistosomiasis. Increased turnover of the clotting? Revista do instituto de medicina Tropical de Sao Paulo, **26**:237-40.

Maquir P., Prudhomme J. and Sheman I. (1991). Alterations in erythrocyte membrane phospholipid organization due to the intracellular growth of the human Malaria parasite *P.falciparum.* Parasitology, **102**:179-86.

Marsh K. (1992). Malaria-a neglected disease? Parasitology, **104**: S53-5.

Marsh K., Forster D., Waruiru C., Mwangi I., Winstanley M., Marsh V., Newton C., Winstanley P., Warn P., Peshu N., Pasvol G. and Snow R. (1995). Indicators of life threatening Malaria in African children. New England Journal of Medicine, **332**:1399-404.

Marsh K., Marsh V.M., Brown J. Whittle H.C. and Greenwood B.M. (1988). *Plasmodium falciparum*: the behavior of clinical isolates in an in vitro model of infected red blood cell sequestration. Experimental Parasitology, **65**:202-208.

Mashaal H.A.H. (1986). Splenomegaly in Malaria. Indian Journal of Malariology, **23**:1-18.

Mathew R. C. and Boros D. L. (1986). Anti-L3T4 antibody treatment suppresses hepatic granuloma formation and abrogates antigen-induced interleukin-2 production in *Schistosoma mansoni* infection. Infection and Immunity, **54**:820.

McGarvey St., Aligui G. and Daniel B.L. (1992): Child growth and *Schistosomiasis japonica* in north eastern leyte, Philippines. I. Cross sectional results. American Journal of Tropical Medicine and Hygiene, **46**: 571.

Mendis K., Sina B., Marchesini P. and Carter R. (2001). "The neglected burden of *Plasmodiu vivax* Malaria" (PDF). American Journal of Tropical Medicine and Hygiene, **64**:97-106.

Mens P.F., Schoone G.J., Kager P.A. and Schallig H.D. (2006). "Detection and identification of human Plasmodium species with real-time quantitative nucleic acid sequence-based amplification". Malaria Journal, **5**:80.

Mentink-Kane M.M., Cheever A.W., Thompson R.W., Hari D.M., Kabatereine N.B., Vennervald B.J., Ouma J.H., Mwatha J.K., Jones F.M., Donaldson D.D., Grusby M.J., Dunne D.W. and Wynn T.A. (2004). "IL-13 receptor alpha 2 down-modulates granulomatous inflammation and prolongs host survival in Schistosomiasis". Proceedings of the National Academy of Sciences of the United States of America, **101**:586-90

Mohan K., Dubey M.L., Ganguly N.K. and Mahajan R.C. (1995). *Plasmodium falciparum* role of activated blood monocytes in erythrocyte membrane damage and red cell loss during Malaria. Experimental Parasitology, **80**:54-63.

Murthy G.L., Sahay R.K., Sreenivas D.V. Sundaram C. and Shantaram V. (1998). Hepatitis in falciparum malaria. Tropical Gastroenterology, **19**:152-4.

Mwatha J.K., Jones F.M., Mohamed G., Naus C.W., Riley E.M., Butterworth A.E., Kimani G., Kariuki C.H., Ouma J.H., Koech D. and Dunne D.W. (2003). Associations between anti-schistosoma mansoni and anti-plasmodium falciparum antibody responses and hepatomegally in Kenyan school children. Journal of Infectious Diseases, **187**:1337-1341.

Nacher M., Singhasivanon P., Yimsamran S., Manibunyong W., Thanyavanich N., Wuthisen R. and Looareesuwan S. (2002). Intestinal helminth infections are associated with increased incidence of *Plasmodium falciparum* Malaria in Thailand. Journal of Parasitology, **88**: 55-58.

Nacher M., Singhasivanon P., Silachamroon U., Treeprasertsuk S., Vannaphan S., Traore B., Gay F. and Looareesuwan S. (2001). Helminth infections are associated with protection from Malaria-related acute renal failure and jaundice in Thailand. American Journal of Tropical Medicine and Hygiene, **65**:834-836.

Nacher M., Gay F., Singhasivanon P., Krudsood S., Treeprasertsuk S., Mazier D., Vouldoukis I. and Looareesuwan S. (2000). *Ascaris lumbricoides* infection is associated with protection from cerebral Malaria. Parasite Immunology, **22**:107-113.

Newton C.R., Crawley J., Sowumni A., Waruiru C., Mwangi I., English M., Murphy S., Winstanley P.A., Marsh K. and Kirkham F.J. (1997). Intracranial hypertension in Africans with cerebral malaria. Archives of Disease in Childhood, **76**:219-226.

Newton and Krishna (1998). Severe falciparum Malaria in children: current understanding of pathophysiology and supportive treatment. Pharmacology and Therapeutics, **79**:1-53.

Nguansangiam S., Day N.P.J., Hien T.T., Mai N.T.H., Chaisri U. and Riganti M. (2007). A quantitative ultrastructural study of renal pathology in fatal *Plasmodium falciparum* Malaria. Tropical Medicine and International Health, **12**:1037-50.

Njenga M.N., Farah I.O., Muchemi G.K. and Nyindo M. (1998). Peri-portal fibrosis of liver due to natural or experimental infection with *Schistosoma mansoni* occurs in the Kenyan baboon. Annals of Tropical Medicine and parasitology, **92**:183-187.

Nyindo M., Kariuki T.M., Mola P.W., Farah I.O., Elson L., Blanton R.E. and King C.L. (1999). Role of adult worm antigen-specific immunoglobulin E in acquired immunity to *Schistosoma mansoni* infection in baboons. Infection and immunity, **67**:636-642.

Ockenhouse C.F., Klotz F.W., Tandon N.N. and Jamieson G.A. (1991). Sequestrin, a CD36 recognition protein on *Plasmodiun falciparum* Malaria-infected erythrocytes identified by anti-idiotype antibodies. Proceedings of the National Academy of Sciences of the United States of America, **88**:3175-9.

Ogbonna A. and Uneke C.J. (2008). Artemisinin-based combination therapy for uncomplicated Malaria in sub-Saharan Africa: the efficacy, safety, resistance and policy implementation since Abuja 2000. Transactions of the loyal society of Tropical Medicine and Hygiene, 102:621-7.

Oliveira M.F., d'Avila J.C., Torres C.R., Oliveira P. L., Tempone A. J., Rumjanek F. D., Braga M.S, Silva J. R., Dansa-Petretski M., Oliveira M. A., de Souza W. and Ferreira S. T. (2000). "Haemozoin in *Schistosoma mansoni*". Molecular and Biochemical Parasitology, **111**:217-21.

Onah D.N., Onyenwe I.W., Ihedioha J.I. and Onwumere O.S. (2004). Enhanced survival of rats concurrently infected with *Trypanosoma brucei* and *Strongyloides ratti.* Veterinary Parasitology, **119**:165-176.

Ongom V.L. and Bradley D.J. (1972). The epidemiology and consequences of *Schistosoma mansoni infection* in West Nile, Uganda. I. Field studies of a community at Panyagoro. Transactions of the Royal Society of Tropical Medicine and Hygiene, **66**:835-851.

Ozwara H., Jan A. M. L., Jenneby M., Farah I. O., Yole D. S., Mwenda J. M., Horst W., and Alan W. T. (2003). Experimental infection of the olive baboon (*papio anubis*) with *plasmodium knowlesi*: severe disease accompanied by cerebral involvement. American Journal of Tropical Medicine and Hygiene, **69**:188-94.

Pain, A., Böhme, U., Berry, A.E., Mungall, K., Finn, R.D., Jackson, A.P., Mourier, T., Mistry, J., Pasini, E.M., Aslett, M.A., Balasubrammaniam, S., Borgwardt, K., Brooks, K., Carret, C., Carver, T.J., Cherevach, I., Chillingworth, T., Clark, T.G., Galinski, M.R., Hall, N., Harper, D., Harris, D., Hauser, H., Ivens, A., Janssen, C.S., Keane, T., Larke, N., Lapp, S., Marti, M., Moule, S., Meyer, I., Ormond, D., Peter, N., Sanders, M., Sanders, S., Sargeant Smith, F., Squares, R., Thurston, S., Tivey, A.R., T.J, Simmonds, M, Walker, D., White, B., Zuiderwijk, E., Churcher, C., Quail, M.A., Cowman, A.F., Turner, C.M., Rajandream, M.A., Kocken, C.H., Thomas, A.W., Newbold, C.I., Barrell, B.G. and Berriman, M. (2008): The genome of the simian and human Malaria parasite *Plasmodium knowlesi.* Nature, **455**:799-803.

Pain A., Ferguson D.J., Kai O., Urban B.C., Lowe B., Marsh K. and Roberts D.J. (2001). Platelet mediated clumping of *Plasmodium falciprum*-infected erythrocytes is a common adhesive phenotype and is associated with severe Malaria. Proceedings of the National Academy of Sciences of the United States of America, **98**: 1805-10.

Pattanasin S., Proux S., Chompasuk D., Luwiradaj K., Jacquier P., Looareesuwan S. and Nosten F. (2003). "Evaluation of a new Plasmodium lactate dehydrogenase assay (OptiMAL-IT) for the detection of Malaria". Transactions of the Royal Society of Tropical Medicine and Hygiene, **97**:672-4.

Pernod G., Polack B., Peyron F., Luisy A., Koloidie L., Ambroise-Thomas P. and Santoro F. (1992). Monocyte tissue factor expression induced by *P. falciparum* infected erythrocytes. Thrombosis and Hemostasis, **68**:111-4.

Prasad R.N. and Virk K.J. (1993). Malaria as a cause of diarrhoea-a review. Malaria Research Centre, Shahjahanpur, India. Papua New Guinea Medical Journal, **36**:337-41.

Prato M., Giribaldi G., polimeni M., Gallo V. and Arese P. (2005). Phagocytosis of hemozoin enhances matrix metalloproteinase in the pathogenesis of falciparum Malaria. Journal of Immunology, **175**:6436-6442.

Premaratna R., Gunatilake A.K., de Silva N.R., Tilakaratne Y., Fonseka M.M. and de Silva H.J. (2001). Severe hepatic dysfunction associated with falciparum malaria. Southeast Asian Journal of Tropical Medicine and Public Health, **32**:70-72.

Pukrittayakama S., White N. and Clemens R. (1989). Activation of the coagulation cascade in falciparum Malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene, **83**:726-66.

Punyagupta S., Srichaikul T., Nitiyanant P. and Petchelia B. (1974). Acute pulmonary insufficiency in falciparum Malaria: summary of 12 cases with evidence of disseminated intravascular coagulations. American Journal of Tropical Medicine and Hygiene, **23**:551-9.

Redd S., Kazembe P., Luby S., Nwanyanwu O., Hightower A., Ziba C., Wirima J., Chitsulo L., Franco C. and Olivar M. (2006). "Clinical algorithm for treatment of *Plasmodium falciparum* Malaria in children". Lancet, **347** (8996): 80.

Reeve P.A, Toaliu H., Kaneko A., Hall J.J. and Ganczakowski M. (1992). Acute intravascular hemolysis in Vanuatu following a single dose of primaquine in individuals with glucose-6-phosphate dehydrogenase deficiency. Journal of Tropical Medicine and Hygiene, **95**:349-54.

Rey L. (1991). Parasitologia. Rio de Janeiro, RJ: Editora Guanabara Koogan S.A. pp. 351-62.

Ribeiro de Jesus, A., Magalhães A., Miranda D. G., Miranda R. G., Araujo M. I., de Jesus A. A., Silva A., Santana L. B., Pearce E. and Carvalho E. M. (2004). Association of type 2 cytokines with hepatic fibrosis in human *Schistosoma mansoni* infection. Infection and Immunity, **72**:3391-3397.

Rockett K., Awburn M., Rockett E. and Clark I. (1994). TNF and IL1 synergy in the context of Malaria pathology. American Journal of Tropical Medicine and Hygiene, **50**:735-42.

Rojkind M. and Dunn M. A. (1979). Hepatic fibrosis. Gastroenterology, **76**:849-63.

Romero A., Matos C., González M.M., Nuñez N., Bermudez L. and de Castro G. (1993). Changes in gastric mucosa in acute Malaria. Gastroenterology, **47**:123-8.

Ruppel A., Shi Y.E. and Moloney N.A (1990). *Schistosoma mansoni* and *S. japonicum*: comparison of levels of ultraviolet irradiation for vaccination of mice with cercariae. Parasitology, **101**:23.

Sarkar S., Prakash D., Marwaha R.K., Garewal G., Kumar L., Singhi S., (1993). Acute intravascular hemolysis in Glucose-6-Phosphate Dehydrogenase deficiency. Annals of Tropical Pediatrics, **13**:391-4.

Schnitzer B., Sodeman T., Mead M. and Contacos P. (1973). An ultrastructural study of the red pulp of the spleen in Malaria. Blood, **41**: 207-18.

Senaldi-G, Vesin C., Chang R., Grau G. and Piguet P.F. (1994). Role of polymorphonuclear neutrophil leukocytes and their integrin CD 11a (LFA 1) in the pathogenesis of severe murine Malaria. Infection and Immunity, **62**:1144-9.

Sher A., Coffman R. L., Hieny S., Scott P. and Cheever A. W. (1990). Interleukin 5 is required for the blood and tissue eosinophilia but not granuloma formation induced by infection with *Schistosoma mansoni*. Proceedings of the National Academy of Sciences of the United States of America, **87**:61-65.

Silamut and White. (1993). Relation of the stage of parasite development in the peripheral blood to prognosis in severe falciparum Malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene, **87**:436-443.

Singh B., Kim Sung L., Matusop A., Radhakrina A., Shamsul S.S. and Cox-Singh J. (2004). "A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings". Lancet, **363**:1017-24.

Sitprija V. (1998). Nephropathy in falciparurn malaria. Kidney International, **34**:867-867

Smith D.H., Warren K.S. and Mahmoud A.A. (1979). Morbidity in *Schistosomiasis mansoni* in relation to intensity of infection: study of a community in Kisumu, Kenya. American Journal of Tropical Medicine and Hygiene, 28:220-229.

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

Snow R.W., Guerra C.A., Noor A.M., Myint F.l.Y. and Hay S.I. (2005). The global distribution of clinical episodes of *Plasmodium falciparum* Malaria. Nature, **434**:214-217.

Sokhna C., Le Hesran J.Y., Mbaye P.A., Akiana J., Camara P., Diop M., Ly A. and Druilhe P. (2004). Increase of Malaria attacks among children presenting concomitant infection by *Schistosoma mansoni* in Senegal. Malaria Journal, **3**:43.

Sosman J.A., Verma A., Moss S., Sorokin P., Blend M., Bradlow B., Cnachlani N., Cutler D., Sabo R., Nelson M., Bruno E., Gustin D., Viana M. and Hoffman R. (2000). Interleukin 10-induced thrombocytopenia in normal healthy adult volunteers: Evidence for decreased platelet production. British Journal of Haematology, **111**:104-111.

Spiegel A., Tall A., Raphenon G., Trape J.F. and Druilhe P. (2003). Increased frequency of Malaria attacks in subjects co-infected by intestinal worms and *Plasmodium falciparum* Malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene, **97**:198-199.

Srichaikul T., Panikbutr N. and Jeumtrakul P. (1967). Bone marrow changes in human malaria. Annals of Tropical Medicine and Parasitology, **61**:40-50.

Stevenson M.M. and Tam M.F. (1993). Differential induction of helper Tcell subsets during blood-stage *Plasmodium chabaudi* AS infection in resistant and susceptible mice. Clinical and Experimental Immunology, **92**:77-83.

Strickland G.T. (2000): Schistosomiasis In: Hunter's Tropical Medicine. 8th ed. Philadelphia, W. B Sunders Company. Pp 804.

Sturm A., Amino R., van de Sand C., Regen T., Retzlaff S., Rennenberg A., Krueger A., Pollok JM., Menard R. and Heussler VT. (2006). "Manipulation of host hepatocytes by the Malaria parasite for delivery into liver sinusoids". Science, **313**:1287-1490.

Sturrock R.F., Butterworth A.E. and Houba V. (1976). *Schistosoma mansoni* in the baboon (Papioanubis): Parasitological responses of Kenyan baboons to different exposures of a local parasite strain. Parasitology, **73**:239-253.

Stvrtinová V., Jakubovský J. and Ivan H. (1995). Inflammation and fever from Pathophysiology: Principles of Disease. Computing Centre, Slovak Academy of Sciences: Academic Electronic Press. ISBN 80-967366-1-2.

http://web.archive.org/web/20010711220523/nic.savba.sk/logos/books/scientific/Inffever.html

Symmers W.C. (1904): Note on a new form of liver cirrhosis due to the presence of the eggs of Bilharzia haematobia. Journal of Pathology and Bacteriology, **9**:237.

Tacchini-Cottier F., Vesin C., Redard M., Buurman W. and Piguet P.F. (1998). Role of TNFR1 and TNFR2 in TNF-induced platelet consumption in mice. Journal of Immunology, **160**:6182-6186.

Talman A., Domarle O., McKenzie F., Ariey F. and Robert V. (2004). "Gametocytogenesis. the puberty of *Plasmodium falciparum*". Malaria Journal, **3**:24.

Tatke M. and Malik G.B. (1990). Pulmonary pathology in severe Malaria infection in health and protein deprivation. Journal of Tropical Medicine and Hygiene, **93**:377-82.

Taylor W.R., Widjaja H., Basri H., Ohrt C., Taufik T., Tjitra E., Baso S., Fryauff D., `Hoffman S.L. and Richie T.L. (2008). Changes in the total leukocyte and platelet counts in Papuan and non Papuan adults from northeast Papua infected with acute Plasmodium vivax or uncomplicated Plasmodium falciparum Malaria. Malaria Journal, **18**:7:259.

Tran T.M.T., Nguyen H.P., Ha V., Tran T.H., Bui M.C., Tran T.H.C., Nguyen T.H.M., Deborah J.W. and Nicholas J.W. (1992). Acute renal failure in patients with severe *falciparum* Malaria. Clinical Infectious Diseases, **4**:874-80.

Tong M. J., David B. Y. and Charles L. C. (1972). Acute Pneumonia in Tropical Infections American Journal of Tropical Medicine and Hygiene, **21**:50-57.

Turner G. D. H., Jones H. M. M., Davis T. M. E., Looareesuwan S., Buley I. D., Gatter K., Newbold C. I., Pukritajakamee S., Nagachinta B., White N. J. and Berendt A. R. (1994). An immunohistochemical study of the pathology of fatal Malaria. Evidence for widespread endothelial activation and a potential role for intercellular adhesion molecule-1 in cerebral sequestration. American Journal of Pathology, **145**:1057-1069.

Udomsangmetch R., Wahlin B., Carlson J., Berzins K., Torii M., Aikawa M., Perlman P. and Wahlgren M. (1989). *Plasmodium falciparum-infected* erythrocytes form spontaneous erythrocyte rosettes. Journal of Experimental Medicine, **169**:1835-40.

United Kingdom Health Protection Agency. (2007).

Von Lichtenberg F. (1987). Consequences of Infection with Schistosomes. In: The Biology of Schistosomes, Rollinson, D. and A.J.G. Simpson (Eds.). Academic Press, London and New York.

Walker O., Salako L.A., Sowunmi A., Thomas J.O., Sodeine O. and Bondi F.S. (1992). Prognostic risk factors and post mortem findings in cerebral malaria in children. Transactions of the Royal Society of Tropical Medicine and Hygiene, **86**:491-493.

Walters J.H. and McGregor I.A. (1960). The mechanism of Malarial hepatomegaly and its relationship to hepatic fibrosis. Transactions of the Royal Society of Tropical Medicine and Hygiene, 54:135-45.

Warhurst D.C. and Williams J.E. (1996). "Laboratory diagnosis of Malaria". Journal of Clinical Patholology, **49**:533-38.

Warren K.S., Reboucas G. and Baptista A.G. (1965). Ammonia metabolism and hepatic coma in hepatosplenic Schistosomiasis; Patients studied before and after portocaval shunt. Annals of Internal Medicine, **62**:1113-6.

Weinstock J.V. (1992). The pathogenesis of granulomatous inflammation and organ injury in Schistosomiasis: interaction between the schistosome ova and the host. Immunological Investigations, **21**:455-475.

White N.J. and Ho M. (1992). The pathophysiology of Malaria. Advanced Parasitology, **31**:83-173.

Whittle H., Gelfand M., Sampson E., Purvis A and Weber M. (1969). Enlarged livers and spleens in an area endemic for Malaria and Schistosomiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene, **63**:353-361.

Wickramasinghe S., Looareesuwan S., Nagahinta B. and White N. (1989). Dyserythropoiesis and ineffective erythropoiesis in *P.vivax* Malaria. British Journal of Haematology, **72**:91-9.

Williams M. E., Montenegro S., Domingues A. L., Wynn T.A., Teixeira K., Mahanty S., Coutinho A. and Sher A. (1994). Leukocytes of patients with *Schistosoma mansoni* respond with a Th2 pattern of cytokine production to mitogen or egg antigens but with a Th1 pattern to worm antigens. Journal of Infectious Diseases, **170**:946-954.

Wilson S., Jones F.M., Mwatha J.K., Kimani G., Booth M., Kariuki H.C., Vennervald B.J., Ouma J.H., Muchiri E. and Dunne D.W. (2008). Childhood Schistosomiasis and Malaria co-infection: hepatosplenomegaly is associated with low regulatory and Th2 responses to schistosome antigens. Infection and Immunity, **76**:2212-2218.

Wilson S., Vennervald B.J., Kadzo H., Ireri E., Amaganga C., Booth M., Kariuki H.C., Mwatha J.K., Kimani G.,Ouma J.H., Muchiri E. and Dunne D.W. (2007). Hepatosplenomegaly in Kenyan schoolchildren: exacerbation by concurrent chronic exposure to Malaria and *Schistosoma mansoni* infection. Tropical Medicine and International Health, **12**:1442-1449.

Wilson R.A. and Coulson P.S. (2009). "Immune effector mechanisms against Schistosomiasis: looking for a chink in the parasite's armour". Trends in Parasitology, **25**:423-31.

Woodruff A., Amsdell V. and Pettiti L. (1979). Cause of anemia in Malaria. Lancet, **I**: 1055-7.

World Health Organization, Division of Control of Tropical Diseases. (1990). Severe and complicated Malaria. Second edition. Transactions of the Royal Society of Tropical Medicine and Hygiene, **84**:1-65.

World Health Organization. (2001a). The use of antiMalarial drugs. Report of a WHO Informal Consultation. Geneva.

World Health Organization. (2001b). AntiMalarial drug combination therapy: Report of WHO technical consultation Geneva.

Wynn T.A. and Cheever A.W. (1995). Cytokine regulation of granuloma formation in Schistosomiasis. Current opinions in immunology, **7**:505-511.

Yan Y., Inuo G., Akao N., Tsukidate S. and Fujita K. (1997). Down-regulation of murine susceptibility to cerebral Malaria by inoculation with third-stage larvae of the filarial nematode *Brugia pahangi.* Parasitology, **114**:333-338.

Yole D.S., Shamala K.T., Kithome K., and Gicheru M. (2007). Studies on the interaction of *Schistosoma mansoni* and *Leishmania major* in experimentally infected Balb/c mice. African Journal of Health Sciences, **14**:80-85.

Yoshida A., Maruyama H., Kumagai T., Amano T., Kobayashi F., Zhang M., Himeno K. and Ohta N. (2000). *Schistosoma mansoni* infection cancels the susceptibility to Plasmodium chabaudi through induction of type 1 immune responses in A/J mice. Introduction to Immunology, **12**:1117-1125.