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IMMUNOLOGICAL STUDIES ON SCHISTOSOMIASIS
(*Schistosoma haematobium*) RESISTANT INDIVIDUALS
IN AN ENDEMIC RURAL COMMUNITY IN GHANA

This Thesis is submitted to the
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In Partial fulfilment of
the Requirement for the award of
Master of Philosophy (M. Phil.) degree in
Animal Science

By

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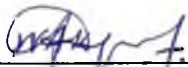
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DECLARATION

I do hereby declare that except for references to other people's work which I have duly acknowledged, this exercise is a result of my own original research, and this thesis, either in whole, or in part, has not been presented for another degree elsewhere.



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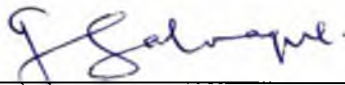
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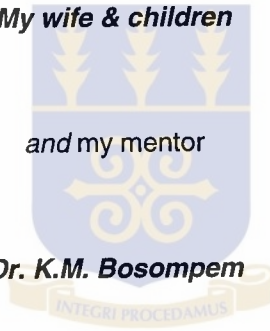
DEDICATION

To

My wife & children

and my mentor

Dr. K.M. Bosompem



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I sincerely wish to express my indebtedness to all individuals who, in diverse ways, contributed to the formulation, execution and submission of this thesis work.

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ABBREVIATIONS

NMIMR	-Noguchi Memorial Institute for Medical Research
PBS	-Phosphate buffered saline
PEG	-Polyethylene glycol
pH	-Negative logarithm base of hydrogen ion concentration
SDS	-Sodium dodecyl sulphate
SDS-PAGE	-Sodium dodecyl sulphate polyacrilamide gel electrophoresis
TBS	-Tris buffered saline
TEMED	-N,N,N',N'-tetramethylethylenediamine
WHO	-World Health Organization
Xg	-Times gravitational force

SUMMARY

Schistosoma haematobium is the least studied amongst the three major human schistosomes (*S. haematobium*, *S. mansoni* and *S. japonicum*). This is so because of the ease with which *S. mansoni* and *S. japonicum* are maintained in the laboratory as against the difficulty in maintaining the life cycle of *S. haematobium* in the laboratory. Therefore, work on identification of potential protective antigens, which could serve as, vaccine candidates continues to lag behind.

The work reported in this thesis was aimed at identifying *S. haematobium* resistant individuals from an endemic community, and to determine whether they produce certain immunoglobulin classes against specific schistosome parasite antigen(s) that have the capacity to protect against disease or serve as vaccine candidate(s).

From studies reported in this thesis, following water contact activity index (WCAI ≥ 300), eleven putatively resistant individuals were identified in the endemic community. Fifteen (15) water contact sites were identified in the study community. A total of 346 individuals responded to having water contact, at three major sites; the Ayensu river 95.4% (330/346), the irrigation dam 74.9% and the irrigation fields 70.5% (244/346). None of the responded indicated having water contact at the remaining twelve minor well and ponds. Snail survey showed 10 *B. truncatus* snails and 25 *B. pfeifferi* at the irrigation dam, 52 *B. pfeifferi* snails at the irrigation fields.

Out of 346 individuals, 115 subjects (33.2%), were found have *S. haematobium* eggs by microscopy using the standard 10ml urine filtration,

although 10 (5.7%) of them could only be revealed by sediment obtained from excess urine. Prevalence of *S. haematobium* was low among females of all ages compared to the males. The disease prevalence was highest at age groups (10-14yrs) for both sexes, and decreased continuously with age. The highest intensity of infections were also found in the age groups with the highest prevalence as demonstrated by the egg range and mean egg counts.

A high percentage (53.1%) of individuals in the *S. haematobium* egg positive group had high water contact (WCAI ≥ 300), whilst a smaller percentage (44.9%) of the *S. haematobium* egg negatives were in this high risk category.

Repeated screening of 96 *S. haematobium* egg negative individuals for antigen by monoclonal antibody dipstick to confirm their uninfected status revealed that, 14 out of 25 who were able to provide urine on three or more occasions tested positive at least once for antigen. Eleven individuals (11/25) 44.0% remained negative for *S. haematobium* antigen and were selected as putative *S. haematobium* resistant candidates.

Resistant, susceptible and control serum samples were used in Western immunoblot analysis to reveal anti-schistosome IgG, IgA, IgE and IgM against crude adult worm and egg antigens and to detect protein bands which could have potential of being use as a vaccine candidate.

SDS-PAGE analysis of *S. haematobium* worm and egg antigens showed more than 14 polypeptide bands with molecular weight ranging from 115kDa to 16KDa for crude worm protein extract, where egg antigen revealed only four polypeptide bands ranging from (105 – 52). Two polypeptide bands (105 and 78) kDa were common to both *S. haematobium* worm and egg antigens.

Sera from *S. haematobium* resistant and susceptible individuals analysed in the western immunoblot revealed 2 bands (102 and 104) kDa that were detected by both *S. haematobium* susceptible and resistant sera analyzed, as well as the normal controls. These two protein bands were revealed by all the different classes of immunoglobulins (anti-schistosome IgG, IgA, IgE and IgM) that were tested. Immunoglobulins IgA, IgE and IgM in each of the sera analyzed reacted with a 32kDa antigen band, which was not detected by IgG antibodies. However, anti-schistosome IgG was the only immunoglobulin isotype that detected two higher molecular weight antigens (112 and 110). Comparatively, anti-schistosome IgG detected distinct and more protein bands in the sera analysed.

A 100 kDa was detected in all the resistant sera except one by only anti-schistosome IgG. From egg antigen, anti-schistosome schistosome IgA and anti-schistosome IgG detected only one band each 42kDa and 58kDa. Respectively. IgE and IgM failed to detect any antigens in the egg extracts using sera of resistant, susceptible and controls.

The extensive reactivity of anti-schistosome IgG with worm antigens, suggests that it may be important in immunity during infection. However, IgG alone may not confer complete protection. The identification of a unique band detected by resistant individual sera may also suggest possible involvement of the antigens in the band in protection against the disease. However, more work is needed to determine the potential of this protein band as vaccine candidate in schistosomiasis.

In conclusion, therefore, this study shows that anti- schistosome IgG may be involved in protection against *S. haematobium* infection.

CHAPTER 1

INTRODUCTION

Schistosomiasis is a chronic, debilitating and socio-economically important disease of man and livestock, caused by blood-dwelling digenetic trematodes of the genus *Schistosoma*. It is a major public health problem with increasing concern in developing countries. The disease is common in most parts of Africa and some countries in Western Asia, South America and the Carribeans (Rollinson and Southgate, 1987). Schistosomes are widespread because of the presence of intermediate fresh water snail hosts (family- *Planorbidae*) in the endemic areas. Throughout the world, schistosomes infect 200 million people, and another 600 million are at risk of infection, making it the second largest parasitic disease after malaria (WHO, 1985; 1993, Manson-Bahr and Bell, 1991). Bergquist (1987), documented that the highest schistosomiasis infection rates are found in Brazil, Egypt and Ghana.

Schistosomiasis may be acute or chronic. The acute disease is characterized by Katayama fever, anaemia, or fluid /electrolyte problems in severely toxæmic victims. There is a real threat of Central Nervous System involvement during early infections (von Lichtenberg, 1987), which may result in myelitis or cranial palsies. It is therefore important to administer anti-schistosomal drugs in early infections to help ameliorate the course of the disease. Chronic urinary schistosomiasis is marked by haematuria, resulting

from penetration of the bladder wall by schistosome eggs, frequent micturation and occasional dysuria. Late complications of *S. haematobium* may include calcification and carcinoma of the bladder, hydronephrosis and hydro-ureter. It is also known that a few parasite egg patches at or near the ureterovesical junction can cause obstructive uropathy. However, in chronic *S. mansoni* and *S. japonicum* infections, there is marked enlargement of the liver and spleen (splenohepatomegaly), diarrhoea with blood and mucus in the stool resulting from ulcerative and polypoidal lesions in the colon, granulomatous gut and liver that could result in pipestem fibrosis and cirrhosis of the liver and ascites. In 1990 the disease was estimated to be responsible for the loss of 1.5 million Disability Adjusted Life Years (DALYs), and mortality exceeded 100,000 per year (WHO, 1993). In endemic rural areas of many developing nations, schistosomiasis is an important occupational hazard (Doumenge *et al.*, 1987).

Three principal species of schistosomes pathogenic to man are *S. haematobium*, which causes urinary schistosomiasis, and *S. mansoni* and *S. japonicum* that cause intestinal schistosomiasis (Boothroyd and Komuneicki, 1995). Factors responsible for increasing transmission of schistosomiasis include; 1) creation of favourable environment for the growth of water plants like *Ceratophyllum*, *Pistia*, and *Commelina* spp. that provide a good habitat for proliferation of the schistosomiasis host snails, 2) migration of infected humans from endemic to non endemic areas, and 3) introduction of host snails into water bodies and contamination of the water with infected human urine and/ or stool. Generally, transmission of schistosomiasis has been

facilitated by the construction of dams for hydroelectric, agricultural and domestic purposes.

In Africa, urinary schistosomiasis is endemic in 38 countries, whilst intestinal schistosomiasis is found in 36 countries, however, the distribution overlaps in 34 countries (Wurapa *et al.*, 1989). Furu (1987), reported that both urinary and intestinal schistosomiasis were widely distributed in Ghana. Schistosomiasis haematobia is present in all regions of the country whilst schistosomiasis mansoni that was concentrated in the northern and southern quarters (McCullough and Ali, 1965; Odei, 1975) is now more widespread.

The standard method of diagnosis of schistosomiasis is by microscopic demonstration of schistosome eggs in the excreta (urine and stool). This method though specific is not sensitive enough (Savioli and Mott, 1989), partly due to great fluctuation of egg output and/ or by the small number of eggs excreted. On the other hand, the use of haematuria as a symptom of *S. haematobium* infection can be misleading since bloody urine can be caused by other conditions such as prostatic disease, genito-urinary tract infection and carcinoma of the genito-urinary tract (Goldsmith, 1985). Similarly, traces of blood in stool may not necessarily be due to *S. mansoni* infections, but other conditions including gastro-intestinal tract infections such as dysentery and ancylostomiasis (Savioli and Mott, 1989). The limitations resulting from the use of standard diagnostic methods have led to significant progress in the development of improved tools for detection of schistosomes in infected hosts. Kremsner *et al.* (1994), reported a MoAb-based ELISA for detecting *Schistosoma* genus-specific Circulatory Anodic Antigen (CAA) and Circulatory Cathodic Antigen (CCA) (Deelder *et al.*, 1989; 1990). Over the years,

significant progress was made in the use of CAA and CCA detection diagnostic probes (Deelder *et al.* 1989; Deelder *et al.* 1990). However, the tests were not successfully adopted for routine diagnosis in the field (Kremsner *et al.*, 1994). Bosompem *et al.* (1996c), therefore, developed a highly sensitive and specific-field applicable MoAb-dipstick assay for diagnosis of urinary schistosomiasis. It was communicated that the increasing rate of mixed schistosome infections in hyperendemic communities in Africa, and particularly in Ghana necessitates differential diagnoses. This is especially so, because specific identification of infecting schistosome species has several advantages, which include the influence of schistosome species on disease prevalence, incidence and intensity of infection in hyperendemic areas, and drug efficacy and the possible emergence of drug-resistant schistosome species or strains (Bosompem *et al.*, 1996a).

Many developing countries continue to battle with schistosomiasis because there is lack of proper sanitary facilities leading to improper disposal of infected human waste and contamination of water bodies, which serve rural communities. Individuals are exposed to the risk of infection as they carry out routine water contact activities such as washing, swimming, crossing and fetching water for drinking, cooking or bathing.

Efforts to control schistosomiasis include the use of molluscides, biological and mechanical agents as well as environmental modification aimed at the host snails (Jobin, 1979), chemotherapy, health education and provision of sanitary facilities (WHO, 1993). However, these have so far been either temporal, difficult and/ or expensive. In many of the affected countries the entire health care budget can hardly meet the cost of health education, diagnosis and

chemotherapy. Interestingly in areas of high transmission, chemotherapy is rapidly followed by high re-infection rates, which require prolonged expensive surveillance and treatment effort that may be unsustainable in endemic countries. In view of these difficulties, vaccination-based control of schistosomiasis is being vigorously pursued.

Following chemotherapy, some schistosomiasis infected individuals become resistant to re-infection, whilst few individuals show natural resistance to infection in endemic communities. For example, Walker *et al.*,(1970), Viana *et al.* (1994, 1995) reported the absence of schistosome infection over a number of years in people who used water from known transmission sites. Studies involving *S. haematobium* and *S. mansoni* in endemic areas have revealed that older individuals show less infection or intensity of infection as compared to younger children (Butterworth *et al.*, 1984 and 1985; Wilkins *et al.*, 1987). This age-dependent resistance to re-infection is not dependent on chemotherapeutic history but is more likely a manifestation of an acquired immunity based on previous experience rather than age related physiological change. Correa-Oliveira *et al.* (2000) found significant difference in naturally acquired resistant individuals in endemic communities (endemic normals) and infected individuals using *S. mansoni* antigens. Butterworth *et al.* (1987); Dessien *et al.* (1988); Correa- Oliveira *et al.* (1989) and Grzych *et al.* (1993) demonstrated that several antigenic preparations show promising role in the induction of protective immune responses and could be explored for vaccines. Some progress on schistosome vaccine development has been made with *S. mansoni* because of the relative ease with which this species is adapted to the laboratory. Extensive work on *S. japonicum* has resulted in a long list of cloned antigens (Waine *et al.*, 1993)

thereby considerably improving the chances of developing a vaccine against this parasite. Unfortunately, studies on *S. haematobium* antigens continue to lag behind (reviewed by, Bergquist, 1995) even though the widespread nature of urinary schistosomiasis and high frequency of reinfection calls for more work towards identification of protective antigens. It was for these reasons that this research work was carried out to identify *S. haematobium* resistant individuals in an endemic community and explore the immunological differences between them and susceptible groups so as to identify putative protective antigens.

This approach of utilizing schistosome resistant individuals in endemic communities is feasible because the presence of protective immune mechanism including the phenomenon of concomitant immunity which has been reported (Wilson *et al.*, 1983 and Smithers and Gammage, 1980). This work focused on identification of putative schistosomiasis resistant individuals and immunological analysis of their sera, utilizing parasite antigens in the western immunoblot assay.

1.2 *The objectives of the study*

1.2.1 To identify schistosomiasis infected and resistant individuals in an endemic community, using microscopy and water contact activity index by questionnaire.

1.2.2 To confirm *S. haematobium* resistant groups through screening for urinary antigens using monoclonal antibody based (MoAb) dipstick assay.

1.2.3 To cross-analyse sera from *S. haematobium* infected and putative-resistant individuals, using schistosome antigens by the Western immunoblot assay.

1.3 *Justification*

Identification of *S. haematobium* resistant individuals in an endemic community would provide epidemiological information on schistosomiasis in endemic communities. Furthermore, investigation into reactivity of sera from putative resistant subjects against *S. haematobium* antigens will facilitate identification of unique polypeptide antigens, which may have protective properties.

CHAPTER 2

LITERATURE REVIEW

2.1 SCHISTOSOMES AND SCHISTOSOMIASIS

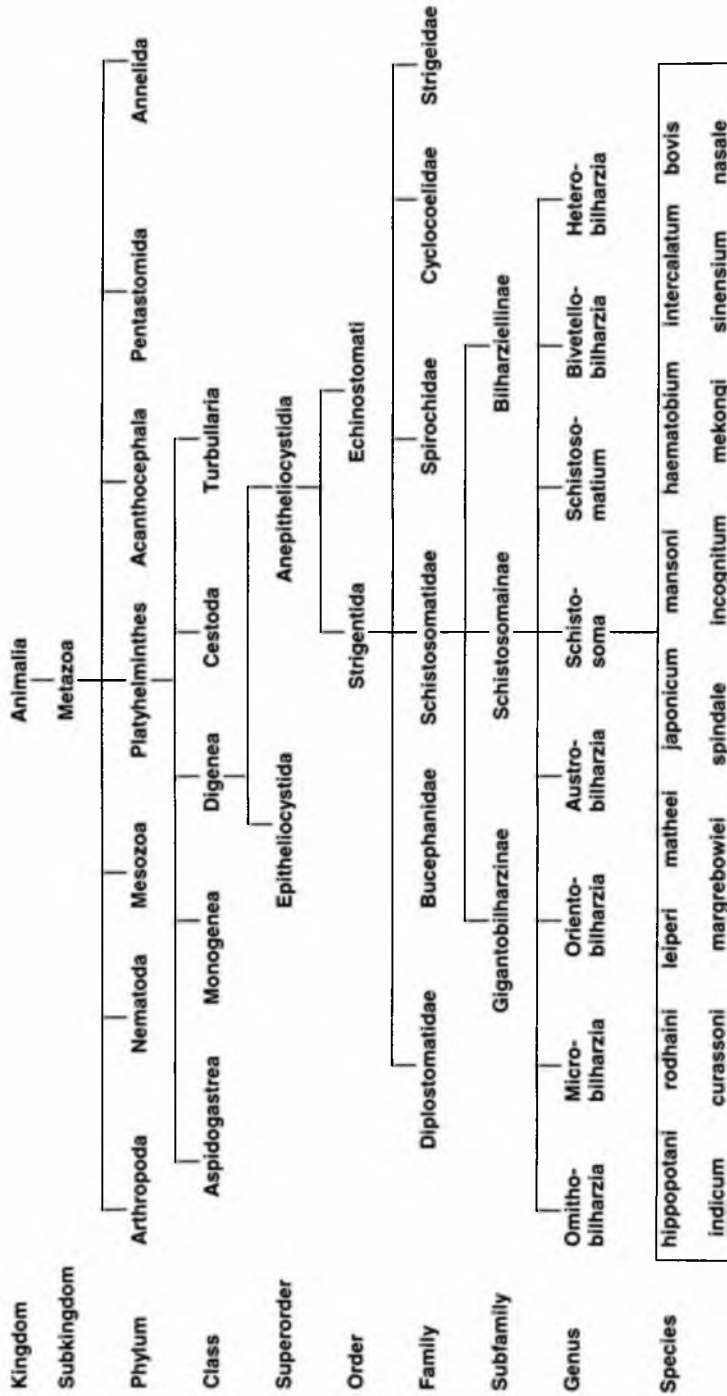
Human schistosomiasis is a parasitic disease that indicates a complex of acute and chronic infections, caused by mammalian blood flukes of the genus *Schistosoma*. Schistosomes are transmitted by specific amphibious and aquatic snails in a wide variety of fresh water bodies (Sturrock, 1987).

The schistosomes belong to the family *Schistosomatidae*, which are dioecious digenea parasites of vertebrates. The family is divided into three subfamilies; the *Schistosominae*, *Bilharziellinae* and *Gigantobilharziinae* (Table 1), and contain twelve genera, of which seven are confined to birds and the remaining five are associated with mammals. Of all the mammalian flukes, it is only the genus *Schistosoma* that is known to be associated with man. The *Schistosoma* has achieved the greatest geographical distribution and diversification, considering the number of recognised species and different host parasites (Rollinson and Southgate, 1987). As summarized in Table 1, the genus *Schistosoma* comprises eighteen (18) species and are placed in four (4) different groups also known as species complexes based on; 1) the genera of intermediate snail in which the parasite develops in nature, 2) zoo geographical distribution pattern of the parasite, and 3) egg morphology of the parasite species (Kuntz, 1955). The species complexes are the *S. haematobium*, *S.*

mansoni, *S. japonicum* and *S. indicum* groups. Of the different species of schistosomes infective to humans, only five are responsible for the overwhelming proportion of infection. These are *S. haematobium*, *S. mansoni*, *S. japonicum*, *S. intercalatum*, and *S. mekongi*, which have a localised distribution.

Schistosome species in the same species complex may be genetically compatible with sexual interchanges between them leading to the creation of hybrid parasites that could present different immunological challenges to individuals in endemic populations.

Table 2.1: Classification of Schistosomes (After Manson, 1989; Rollinson and Southgate, 1987)



2.1.1 The *S. haematobium* group

The species of schistosomes in the *S. haematobium* group are endemic in 52 countries; distributed in the African continent and adjacent islands of Zanzibar and Madagascar; the Eastern Mediterranean including the Arabian peninsula, on many of the Indian Ocean Islands and Western Asia (Doumenge *et al.*, 1987). The *S. haematobium* group consist of seven species, namely, *S. haematobium*, *S. intercalatum*, *S. matheei*, *S. bovis*, *S. curassoni*, *S. margrebowiei* and *S. leiperi*. Although they belong to the same group, the members show marked differences in the shape of their eggs morphology and kinds of definitive hosts. In Africa, except for Rwanda and Burundi some members of this group occur in all endemic countries, where the different parasite species are often found in loci corresponding with the pattern of distribution of the intermediate snail hosts (Wilkins and Gilles 1987). There is enough evidence for geographical variation in many characters associated with the *S. haematobium* group of species. For example, Wright and Knowles (1972), demonstrated that laboratory studies with hamsters revealed differences between strains of the parasites that differ in many biological features such as intermediate host specificity, infectivity of cercariae, growth rates and maturation times of adult worm, egg productivity and the distribution of eggs in infected host organs.

A comprehensive review by Brown (1980), indicated that *S. haematobium* in the tropical regions of Africa is generally transmitted by snails of the *Bulinus africanus* group. In the Mediterranean area and South West Asia, transmission is mainly by the tetraploid members of the *B. truncatus/tropicus*

complex and in Arabia and Mauritius by members of the *forskalii* group of snails. In West Africa, all three of these snail groups are known to act as intermediate hosts and in Arabia the *B. truncatus* group is also implicated. Of particular significance is the observation that *S. haematobium* from North Africa and the Middle East develops in *B. truncatus* and the parasite from tropical Africa develops in snails of the *B. africanus* group, but with few exceptions, neither of these forms can develop in the intermediate host of the other. Notwithstanding the marked differences between members of the *S. haematobium* group of parasites, natural hybridization between female *S. intercalatum* and male *S. haematobium* has been described in Cameroun (Wright *et al.*, 1974; Burchard and Kern 1985 and Southgate *et al.*, 1976), and between female *S. mattheei* and male *S. haematobium* in parts of Southern Africa (Wright and Ross, 1980; Sturrock, 1987). Also, Chu, Kpo and Klumpp (1978) reported possible hybridization between the two biologically different strains of *S. haematobium* (*globosus* and *rohlfsi*) in parts of Ghana. These observations may suggest antigenic polymorphism among this group of schistosomes.

2.1.2 The *S. mansoni* group

The *S. mansoni* group comprises four species; *S. rhodaini*, which infects rodents and dogs (Pitchford, 1977), *S. edwardiense* and *S. hippopotami*, which infects hippopotamus (Pitchford and Viser, 1981), and *S. mansoni*, which infects man.

S. mansoni is responsible for intestinal schistosomiasis in 53 countries in both the New and Old Worlds (WHO, 1985). The parasite occurs in the Arabian peninsula, many countries on the African continent- particularly in the Nile valley, neighbouring Sudan and Egypt. In South America, intestinal schistosomiasis, exists in Brazil, Surinam and Venezuela, as well as seven islands in the Carribean (Rollinson and Southgate, 1987).

Of the seventeen well-defined species of *Biomphalaria* in the Americas, only *B. glabrata*, *B. straminea* and *B. tenagophila* exhibit natural susceptibility for *S. mansoni* (Rollinson and Southgate, 1987). Snails from different geographical areas tend to show variation in levels of susceptibility to different strains of *S. mansoni* (Basch, 1976; Michelson and Dubois, 1978) and populations of snails from a given area may vary in their susceptibility to allopatric strains of the parasites. In Africa, twelve species of *Biomphalaria* are recognized (Brown, 1980) and all those that have been tested seem to show some compatibility with at least certain strains of *S. mansoni*. *B. pfeifferi* reveals a broad compatibility and is, therefore, regarded as an important intermediate host, where as species such as *B. alexandria* appear to be susceptible only to the local *S. mansoni* from Egypt (Frandsen, 1979). *B. pfeifferi* are naturally infected with *S. rhodaini* and also supports mixed infection of *S. mansoni* and *S. rhodaini* (Schwetz, 1953b). Le Roux (1954) and Taylor (1970), reported successful hybridization of *S. mansoni* and *S. rhodaini* in the laboratory. Also, Khalil and Mansour (1990) produced a hybrid using *S. mansoni* and *S. haematobium* in the laboratory and a year after, a natural focus of this hybrid was reported (Ratard and Greer, 1991).

2.1.3 The *S. japonicum* group

Members of the *S. japonicum* group are: *S. japonicum* transmitted by populations of polytypic *Oncomelania hupensis*, which are amphibious snails; *S. mekongi* transmitted by aquatic snail *Tricula aperta* (Voge, Bruckner and Bruce, 1978) and *S. sinensium* which is also transmitted by species of *Tricula aperta* (Pao, 1959). There are six subspecies of *Oncomelania hupensis* namely, *O. hupensis chiui* and *O. hupensis formosana* in Taiwan, *O. hupensis hupensis* in mainland China, *O. hupensis lindoensis* in Sulawesi, *O. hupensis quadrasii* in Philippines and *O. h. nosophora* in Japan (Davis, 1980). *S. mekongi* is responsible for human schistosomiasis, and is endemic on Khong Island in the Mekong River in southern Laos and in many parts of northern and central Cambodia, whereas *S. sinensium* is endemic in the Szechuan Province of China, where it infects only field rats (Pao, 1959). However the use of the same snail as intermediate host by *S. mekongi* and *S. sinensium* (Kruatrachue *et al.*, 1984) could possibly result in hybridization between the two biologically different species that could either infect man or the field rat or both.

S. japonicum is responsible for a grave, debilitating and chronic form of intestinal schistosomiasis which affects both man and domestic animals. The disease is widespread in the Far East where it is endemic in parts of China, Taiwan, Indonesia and the Philippine Islands of Leyete, Samar, Mindanao, Bohol, Mindoro and Luzon (Olveda and Domingo, 1987 and Rollinson and Southgate, 1987). *S. japonicum* is a true zoonosis and occurs as a natural parasite of a large number of mammalian species which play an important role in the epidemiology of the disease. On mainland China, 31 species of wild

mammals including carnivores, rodents, primates, insectivores and artiodactyls have been found with natural infections (Mao and Shao, 1982). However, domestic animals, especially cattle, pigs and dogs may probably play the most important role in the epidemiology of the disease. Hybridization has so far not been seen in any of the Asian schistosomes (Sturrock, 1987).

2.2 *Schistosomiasis In Ghana*

Urinary and intestinal schistosomiasis caused by *S. haematobium* and *S. mansoni*, respectively, are widely distributed throughout Ghana (Wen and Chu, 1984; Paperna, 1969; McCullough and Ali, 1965; Onori *et al.* 1963). The intermediate snail hosts of the two parasites in the country are; *Bulinus* spp. for *S. haematobium* and *Biomphalaria* spp. for *S. mansoni* (McCullough and Ali, 1965; Paperna, 1969; Wen and Chu, 1984). Urinary schistosomiasis was identified in 1895 whereas the intestinal schistosomiasis was identified in 1920 (Furu, 1987). Research workers studying the diseases in Ghana, observed that the construction of dams for hydroelectric power (Volta dam); for domestic and agricultural purposes (Kpong, Weija, Bui, Barekesi and Tono) have immensely enhanced the growth of water plants which serve as suitable habitats that induced proliferation of the intermediate snails responsible for the spread of the disease. Consequently, high prevalence rates of over 60% were registered in most regions of Ghana between the years 1970-1980. Today, schistosomiasis is reported to occur in all the regions of Ghana, especially, within the riparian communities, some of which have recorded prevalence rates as high as 100% for urinary schistosomiasis

(Liese, 1986; Okoh, 1994). This supports an earlier report, which documented that both *S. haematobium* and *S. mansoni* were distributed throughout the country, Furu (1987).

2.2.1 S. haematobium infection in Ghana

Urinary schistosomiasis caused by *S. haematobium* was first identified in Ghana in 1895 (Furu, 1987). By 1963, an estimated number of 15 to 20% of the population of Ghana were infected with *S. haematobium* (Furu, 1987). The number of identified endemic areas was noted to increase proportionately after 1965 (Furu, 1987). This increase in incidence and prevalence was associated with increasing socio-economic hydrological projects coupled with excessive human migration. The construction of the Akosombo dam in 1964 led to the migration of inhabitants to other communities, and the creation of the Volta Lake caused a change in the snail host ecology and these subsequently increased schistosomiasis transmission rate in the country (Odei, 1975; Derban, 1984; Liese, 1986; Okoh, 1994). The growth of aquatic plants, especially *Pistia stratiotes*, *Spirodela polurhiza* and *Ceratophyllum demersum* in the Volta Lake promoted the extensive proliferation of *B truncatus rohlfsi* (Paperna, 1969) and hence the spread of *S. haematobium*.

There are two species of the bulinid snails- *Bulinus globosus* and *Bulinus truncatus* that transmit two strains of *S. haematobium*. The *Bulinus truncatus* occur frequently around the Volta lake, where as *Bulinus globosus* are found in other parts of the country. According to Onori *et al.* (1963), a survey carried at the Medical Field Unit in 1959, before the construction of the Volta lake indicated that urinary schistosomiasis was confined to only certain

areas of the Volta river basin. After the construction of the Volta dam, a report by Paperna (1969), described a high prevalence of 99% parasite infection in some communities along the shores of the lake by January, 1968. It is now estimated that over 90% of the children living around the Volta lake are infected by urinary schistosomiasis. Studies carried out by Bosompem *et al.* (1998) in the Ga district of Greater Accra region where intermediate snail is *B. globosus* recorded prevalence of 43.8% at Kojo Ashong, 70.2% at Amarmoley and Odomase. A report by McCullough (1957), described a prevalence of 75% for urinary schistosomiasis at Pokoase and Mayera in the North eastern part of Accra; while 30% *S. haematobium* prevalence was also described in the neighbouring communities of Agbogba, Ashongman and Abladjai. Further studies in Cape Coast, and Abodom in the Agona district revealed prevalence of 60.3% and 72.8% respectively (Bosompem *et al.*, 1999). Aryeetey *et al.* (1999) established that *B. globosus* was responsible for the transmission of urinary schistosomiasis in some parts of southern Ghana. The snails were confined to the Densu river, its tributaries and ponds. Their distribution was also observed to be focal. Epidemiological studies on urinary schistosomiasis in eight communities drained by the Densu river and its tributaries, showed that the prevalence of *S. haematobium* ranged between 54.8% and 60.0%. In another community, Ayikai Doblo, a similar observation was made. These studies showed that the infection rates increased with age, peaking at 10-19 years and decreased thereafter (Aryeetey *et al.*, 2000).

2.2.2 S. mansoni infection in Ghana

The prevalence of intestinal schistosomiasis caused by *S. mansoni* in Ghana is far less than that of urinary schistosomiasis. Intestinal schistosomiasis was first described in 1920, and by 1955 the north-eastern sector of the country was identified as the only known intestinal schistosomiasis endemic zone, with a total prevalence of 2.4%. McCullough and Ali (1965), recorded prevalence rates mostly between 5 and 7% in two foci in the Western region, two in the Volta region and one in the Ashanti region of Ghana. Although work was ongoing, it was not until 1984 that Wen and Chu reported the discovery of four intestinal schistosomiasis endemic foci in localities between the mouth of the Volta River and the Akosombo dam. *Biomphalaria pfeifferi* was the intermediate host snail responsible for transmitting the intestinal schistosomiasis (*S. mansoni*). On account of the short life cycle of the parasite and adaptability of the host snail to different environment *S. mansoni* have continued to spread rapidly and compete with *S. haematobium* in areas previously known to be endemic for urinary schistosomiasis.

Amankwa *et al.* (1994) working in the Tono irrigation area reported a prevalence ranging from 55.1% to 100% for *S. mansoni* among school children (5-20 years), with the peak of infection in the 15-20 age group. There was mixed infection with both *S. haematobium* and *S. mansoni*, with a prevalence of 47.7%. In a pilot study carried out in 1999 in school children living in communities around the Weija dam in Accra, it was established that the area is hyperendemic for both *S. haematobium* and *S. mansoni* due to the

prevalence of *B. truncatus* and *B. pfeifferi* host snails (Bosompem *et al.*, unpublished). So far, only *B. pfeifferi* has been reported to transmit *S. mansoni* in Ghana.

2.3 The Life Cycle Of Schistosomes

Rollinson and Southgate (1987) and Sturrock (1987) comprehensively reviewed the life cycle of all the species of schistosomes infecting man. The schistosomes were shown to have a common pathway from sexual generation of adult worms within the vascular system of man to asexual phase in the intermediate snail host. The parasites return to man via cercarial penetration of the skin or mucosa following exposure to infested water. Within the capillary blood vessels of the host the adult worms pair up, slender female held in the gynaecophorical groove of the male, and copulate. The female worms lay varying numbers of eggs (depending on species), which are partly mature at oviposition. The eggs penetrate the blood vessel endothelium and other tissues into the lumen of the urinogenital tract (*S. haematobium*) or the bowel (*S. mansoni* and *S. japonicum*) and finally leave the definite host in excreta (urine and/or faeces). Viable eggs on encountering freshwater in a suitable environment of warmth and light will hatch into free-swimming larva, miracidia (Rollinson and Southgate, 1987; Manson, 1989). The miracidia are active parasites that seek and find compatible intermediate snail hosts, which they penetrate via soft tissues. Chernin (1970), reported that *S. mansoni* miracidia are attracted to *B. glabrata* by a complex of water-soluble substances (miraxone) secreted by the snails. Shiff and Kriel (1970), working

with *S. haematobium* miracidia and *B. globosus* snails, also confirmed this observation.

In the intermediate snail, asexual reproduction begins with miracidium being transformed into a sacculate mother sporocyst. The mother sporocyst then divide asexually to give rise to a large number of daughter sporocysts, each of which in turn produces a large number of fork-tailed free swimming larvae termed cercariae. After a period of incubation within the snail, depending on schistosome species and the surrounding physical environment like temperature, light and pH cercariae emerges from the snail into the surrounding water. The miracidium to cercaria development, and its release ranges from less than three weeks at temperatures approaching 30°C to several months at temperatures below 18°C. As indicated above it is the free-swimming cercariae, which are capable of penetrating the intact skin or mucosa of the host.

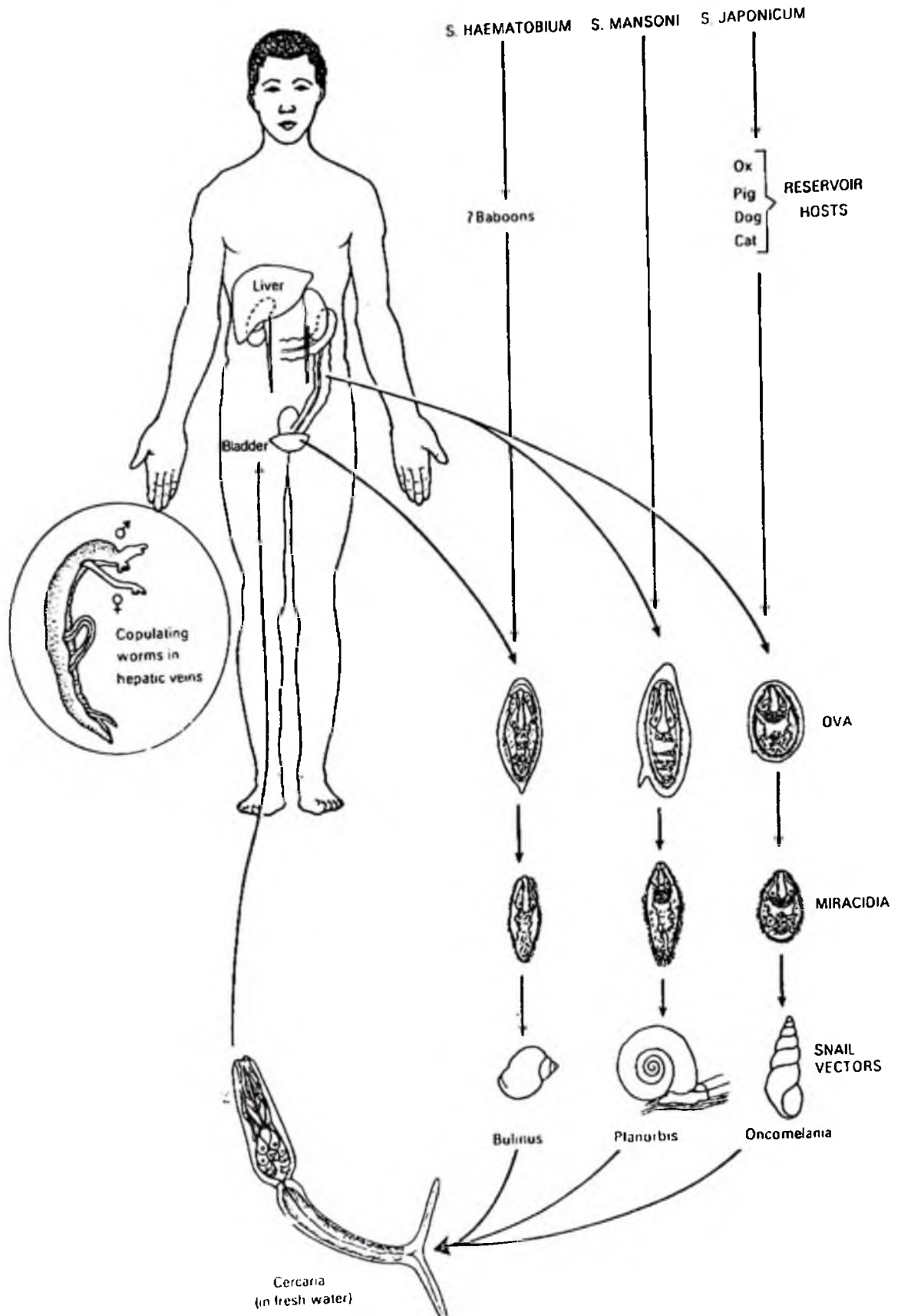


Figure 2.3 The Schistosome Life Cycle

2.3.1 Morphology and ultrastructure of the schistosome egg

The microscopic appearance of schistosome eggs is of great importance in the diagnosis of the different schistosomiasis species. Generally the eggs are oval in shape and non-operculate. The schistosome egg possess an outgrowth spine, which is an extension of the eggshell, and this is a characteristic feature which is very important in the identification of the different schistosome species. For instance, a laterally placed spine characterizes both *S. mansoni* and *S. japonicum* eggs, but the spine is rudimentary in the latter. On the other hand, *S. haematobium* possess a terminal spine. Each schistosome egg contains an embryo "miracidium", which matures within a period of 16 days. Microscopically, the embryo is seen to be viable by observation of flame cells, ciliary or whole body movement.

The schistosome egg possess sub-microscopic eggshell pores via which various egg antigens and enzymes are released (Inatomi, 1962). Kusel (1970), described *S. mansoni* eggs and showed that the eggshell is scleroproteic and lined internally with vitelline membrane, which is held in place by four or more vacuoles pressing on the embryo anteriorly and posteriorly. The number of eggs excreted daily by schistosome is estimated as 50% of the total number of eggs per worm pair. This estimate differs greatly between different species of the parasite. The adult *S. haematobium* pair produces 20-200 terminal spine eggs per day, *S. mansoni* produces 100-300 or more lateral spine per worm pair per day and *S. japonicum* produces 500-3500 ovoid eggs with rudimentary lateral knob per worm pair per day (WHO, 1985). The remaining 50% are trapped within tissues and die about twenty-one days after oviposition.

2.3.2 Morphology and ultrastructure of the miracidium

The miracidium is the first free larval stage in the life cycle of schistosomes. Electron microscopy studies by several workers (Pan, 1965; Schutte, 1974a; Eklun-Natey *et al.*, 1985), have revealed more information on the morphology and ultrastructure of the miracidium, whose size differs according to the parasite species. A newly released miracidium is generally pyriform, with body size ranging from 150 – 180 μm in length and 70 – 80 μm in width. The body of this larval stage is covered with 21 or 22 ciliated, anucleated cells, responsible for its swift movement in water. The anterior end of the miracidium has a hemispherical structure called tetraborium, which is made of anastomosed membrane folds that carry at least 12 ciliated sensory organelles (Eklun-Natey *et al.*, 1985). The secretory ducts of the apical and lateral glands are situated on the membrane folds of the sensory organelles. Located directly under the ciliated cells is a smooth, well-developed musculature, composed of an external layer of circular fibres and an internal layer of longitudinal fibres. Within the intercellular space of the miracidium are cytoplasmic extensions of interstitial cells, reminiscent of the fibroblasts of higher animals. A quadrinucleate gland cell, flanked by two uninucleate lateral glands occupies the mid anterior region of the miracidium. The parasite has a nervous system comprising of a neural mass, which innervates numerous peripheral sensory papillae and muscular fibres.

The miracidium has an excretory system, which comprises an anterior and posterior pair of flame cells and a common duct that opens laterally into the

posterior third of the body. It is likely that a large amount of soluble egg antigens found in the urine of infected persons are secreted by the miracidium via this route. Generally, it is accepted that penetration of miracidia into snail tissues occurs by a mechanical action, however, the role of histolytic secretions of the glandular cells of the miracidia could be implicated (Jourdane and Theron, 1987).

2.3.3 Morphology and ultrastructure of the Cercarial

The cercariae, is the second free larval stage in the life cycle of the schistosome. All cercariae emanating from one miracidium are of the same sex. The cercaria is furcocercous and is less than 1mm in length, consisting of two parts namely the head or main body and the bifurcated tail. There is a muscular oral sucker (Cousin and Dorsey, 1991), but no pharynx and eyespot. The ventral sucker or acetabulum is prominent and muscular. The digestive system has a mouth that is located in the oral sucker, and this leads to a Y- shaped gut, anterior to the acetabulum, comprising an oesophagus and a pair of dorsally placed caeca. The excretory system consists of flame cells connected by tubules to a posterior excretory bladder, and a pair of flame cells, at the base of the tail. Cephalic glands are the most prominent internal features. There are two pairs of gland in front of the acetabulum and four pairs behind it with ducts running forward to open on the edge of the oral sucker. Externally, a tegument bounded by a lipid bilayer covers the body, which appear trilaminar in electron micrographs, usually covered by glycocalyx. Minute hairs and spines are found on the teguments, and are dense especially towards the anterior and posterior

margin of the body. Cercariae released from snail exhibit a pronounced diurnal periodicity with one burst of shedding each 24 hours, however, a twin peak has been reported for *S. japonicum* (Nojima *et al.*, 1980). The pattern of release differs somewhat between the three main human schistosomes. *S. mansoni* shedding normally starts within 1 – 2 hours of infected snail exposure to light, and entire daily output completed within 5 hours. With *S. haematobium* cercariae first appear 2 – 4 hours after exposure and are produced for a further 4- 6 hours. Although Pesigan *et al.* (1958) reported a much lower response to shedding, laboratory studies with both Chinese and Philippine *S. japonicum* showed a course time similar to *S. mansoni* (Moloney and Webbe, 1983). After emergence from infected snails, cercaria can remain infective for approximately 20 hours, even though they are capable of penetrating the unbroken skin of man and other mammals within minutes (Cousin, Stirewalt and Dorsey, 1981).

2.3.4 Morphology and ultrastructure of the Schistosomula

The schistosomulum is the stage where after cercariae penetration of an unbroken skin, the cercaria loses its tail and external layer, and changes from a fresh water organism into one which can survive only in a salty environment. The external morphology of schistosomula is similar to that of cercaria except that the body is devoid of ciliated sensory nerve endings (Crabtree and Wilson, 1980). The cercarial glycocalyx is lost at this stage and the single bi-lipid epithelial membrane surrounding the body is replaced by a double bi-layer, which appears as a heptalaminated membrane in electron micrographs. The initial source of the second bi-layer may be secretion from

the post-acetabular glands but new material are subsequently produced within the deeper layers of the syncytium beneath the tegumental membrane (Wilson and Barnes, 1977; McLaren 1980; Wilson, 1987). A fully transformed schistosomulum shows an obvious oral and ventral sucker and is elongated posteriorly (Basch and Samuelson, 1990). The Y-shaped gut is clearly visible (Stirewalt, 1973). The schistosomulum moves through the tissues into the lymph and blood vessels and ultimately reaches the lungs where they remain for several days. They then migrate to the liver via the bloodstream or directly through the tissues (Wilson, 1987). In the liver, they grow into adult male and female schistosomes, depending on the sex of the cercariae.

2.3.5 Morphology and ultrastructure of the adult worm

Clinicians normally rely on egg detection and immunodiagnosis criteria because the adult schistosomes are not likely to be seen, unless, perhaps in biopsy material.

The adult worm tegument appears smooth and lacks any tuberculation, but is covered with minute, acuminate spines visible ultra-microscopically. The tegument is made of a unique double lipid bi-layer heptalamine similar to that in schistosomula (Hoffman and Strand, 1996). Structurally the schistosome tegument conforms to the basic platyhelminth tegument reviewed by Threadgold (1984). The two lipid bi-layers as compared to the single layer found in other parasites is highly significant in the ability of the genus *Schistosoma* to withstand destructive immunological attack by its host (McLaren and Hockley, 1977). Each adult worm has an oral sucker, which

opens into the gut and a posteriorly positioned ventral sucker for attachment to the luminal lining of the blood vessel. The worm's alimentary canal develops directly from the cercarial gut. It may be divided into two clear regions namely foregut comprising of the oral sucker and the oesophagus, and the hind gut which comprises of the two posterior caeca. The foregut is lined by tegument reminiscent of the body wall; but at the posterior section of the oesophagus the tegumental surface is deeply invaginated. The invaginated region has electron dense granules within which ingested host erythrocytes are disrupted. In contrast, the hind gut which is lined with syncytial layer of cytoplasm, projects into numerous villi-like lamellae on its luminal surface, where absorption of host nutrient is presumed to occur. A most conspicuous feature is the large ventral groove, the gynaecorphical canal in which the female is retained. The reproductive system comprises 4 to 5 pairs of dorsally placed testes connected to a vas deferens and seminal vesicle, that opens to the exterior through a highly invaginated spongy cirrus located just posterior to the ventral sucker (Faust *et al.*, 1934).

2.4 *Schistosome antigens*

During a normal schistosomal infection, a complex array of antigens are presented to the host. These include soluble secretory/excretory antigens, and structural antigens on the surface of the different developmental stages of the parasites that occur in the host. However, it is reported that the major antigenic stimuli are from the adult worms and eggs (Kelly, 1987). Even though these antigens play a major role in host immune responses to schistosomes, it is the

surface membranes of cercariae and the newly transformed schistosomula that present the major targets of protective host immune responses (Dissous *et al.*, 1987).

The young schistosomulum has been found to be susceptible to a wide variety of cytotoxic mechanisms involving specific antibodies and effector cells (Butterworth *et al.*, 1982; Capron *et al.*, 1982). Antigens exposed at the surface of schistosomula have therefore been extensively studied. Research workers including Aronstein and Strand (1983), Norden and Strand (1984) and Kelly *et al.* (1987) identified about 20 – 30 antigenic polypeptides of adult worm and about 20 glycoprotein egg antigens from each of the three major human schistosome species *S. mansoni*, *S. haematobium* and *S. japonicum*. Extensive cross-reaction was observed in the case of the glycoproteins. For example, sera from patients infected with *S. haematobium* precipitated all but three of *S. mansoni* adult worm glycoprotein antigens recognized by *S. mansoni* infected sera. However, a slightly lower degree of cross-reaction was shown using *S. japonicum* infected sera. There is now accumulating evidence that both polypeptides and carbohydrate epitopes are involved in the expression of immune response to schistosomes and are responsible for both cellular and humoral responses. Nonetheless, glycoproteins obtained from eggs of each of the schistosome species, using sera from patients infected with *S. mansoni* and *S. haematobium* could not be distinguished on the basis of their reactivity. Infected sera from *S. japonicum* patients though showed some differences in immune response. Analysis of antigens prepared from eggs of the three species of schistosomes using two dimensional gel electrophoresis, revealed marked differences between the species (Norden and Strand, 1984).

2.4.1 Schistosomula polypeptide surface antigens

Radioiodination of live transformed schistosomula, with subsequent immunoprecipitation utilising a variety of antisera, identified numerous polypeptide surface antigens of the schistosomula (Dissous *et al.* 1981). However, Dissous, Grzych and Capron (1982), and earlier work by Dissous *et al.* (1981), revealed that infected rat sera recognized only a subset of the polypeptides available for surface labelling. Studies by Ramasay (1979), and Simpson and Smithers (1985), showed polypeptides of Mr < 100,000 using lactoperoxidase-catalysed iodination labelling. Further studies by Simpson and Smithers (1985), utilizing iodogen-catalysed radioiodination followed by immunoprecipitation with infected human sera reveal major *S. haematobium* schistosomula surface antigens of Mr. 17,000 and a complex of 24–30,000. These antigens appeared to be species-specific, since human anti-*S. mansoni* sera failed to recognise them. The use of other labelling techniques like the diazonium salt of ¹²⁵I iodosulphanilic acid made possible the identification of three major schistosomula surface antigens of Mr 105,000, 69,000 and 28,000 (Taylor, Haguya and Vannier, 1981).

Sera from vaccinated mice or rabbits have been successfully used to precipitate labelled *S. haematobium* antigens of Mr 94,000; 38,000; 26,000 and 10,500. Furthermore, immunoprecipitation techniques involving the use of either lactoperoxidase or iodogen-catalysed radioiodination revealed that sera from chronically infected mice recognized a slightly different profile of antigens compared with sera from mice immunized by exposure to radiation-attenuated

cercariae. Collectively, however, both sera were found to precipitate antigens of $M_r > 200,000$, 92-94000, 32-38000, 20000, 17000 and 15000. In addition both sets of sera precipitated an antigen of M_r 22,000 following lactoperoxidase but not iodogen-catalysed radioiodination. Significant progress in the identification of new schistosomula surface antigens has, however, been made through the use of monoclonal antibodies as determined by immunofluorescence (Kelly, 1987). Ham *et al.* (1985a and 1985b), produced a monoclonal antibody, which bound to schistosomula surface and precipitated an antigen of M_r 22,000 and 28,000 that corresponded to the same M_r antigens identified by lactoperoxidase-catalysed iodination.

Three *S. mansoni* proteins have been identified in the 27-28 kDa range. Immunofluorescence studies demonstrated that P28 was mainly located in the parenchyma of schistosomula and adult worms, including the dorsal spines of the parasite. However, immunoprecipitation of a 28 kDa labelled molecule from surface-radioiodinated schistosomula using anti-P28 antibodies, indicated that the antigen could be exposed at the surface of schistosomula and may represent an excreted or secreted metabolite. Western blot analysis revealed that this antigen is present in four schistosome species: *S. mansoni*, *S. japonicum*, *S. haematobium* and *S. bovis* (Balloul *et al.*, 1987a). In similar studies using monoclonal antibodies Harn *et al.* (1985a), identified a major surface antigen of *S. mansoni* analogous to the 28kDa antigen actively synthesized by developing schistosomulum. However, this antigen was found to be present in extracts of all four stages of the life cycle. Functional properties of the anti-P28 antibodies were first demonstrated *in vitro* by their ability to mediate killing of schistosomula in the presence of eosinophils.

2.4.2 Schistosomula Carbohydrate surface antigens

Studies on schistosome antigens focused on protein or glycoprotein antigens. This was because of the possibility of synthesizing unique protein antigens in large quantities in micro-organisms using recombinant DNA technology to provide the basis of an anti-schistosome vaccine. Nevertheless, a number of studies have shown that carbohydrates contribute immensely to the antigenicity of the schistosomula surface, and play significant role in the development of immunity (Simpson, James and Sher, 1983). Treatment of schistosomula with reagents such as trifluoromethanesulfonic (TFMS) and sodium periodate which selectively remove or modify carbohydrate residues showed that most (approximately 90%) of the binding serum antibodies from chronic infections was specific to carbohydrate epitopes. The predominant antigens involved were of Mr >200,000; 38,000 and 17,000 which were not recognized by sera from vaccinated animals.

In studies with monoclonal antibodies that bound carbohydrate antigenic epitopes in glycoprotein antigens Yi, *et al.*, (1986) suggested that carbohydrate epitopes might be responsible for inducing non-species-specific concomitant immunity, whereas polypeptide epitopes were more likely to be involved in species-specific immunity. The immunogenic stimuli for concomitant immunity could, therefore, come from either eggs or adult worms, since both share carbohydrate epitopes with the schistosomulum (Hamburger, Lustigman, Arap Siongok, Ouma and Mahmoud, 1982; Omer-Ali *et al.*, 1986; Yi *et al.*, 1986). Likewise, several workers have demonstrated the presence of IgM blocking

antibodies in the sera of chronically infected rats and humans that are directed against carbohydrate epitopes (Grzych, Capron, Lambert, Dissous, Torres and Dissous, 1985; Yi *et al.*, 1986; Capron, Pearce, Balloul, Grzych, Dissous, Sondermyer and Lecocq, 1987). The role of these blocking antibodies may explain in part the susceptibility to re-infection by *S. mansoni* in humans whilst the loss of the antibodies may ultimately lead to the acquisition of immunity (Capron *et al.*, 1987).

A group of 30-40 kDa antigens were also demonstrated during infection of various animal species including rats, mice, monkeys and humans (Dissous *et al.*, 1981; Dissous and Capron, 1982; Simpson *et al.*, 1983). One of these antigens (38 kDa) was selectively precipitated by a rat monoclonal IgG2a (IPLSm1) that induced *in vitro* eosinophil-mediated cytotoxicity and protected naive rats by passive transfer (Dissous *et al.*, 1982; Grzych, Capron, Bazin and Capron, 1982). The protective epitope was characterized as a 115 kDa molecule in adult worm metabolic products (Dissous and Capron, 1983), and also identified in high molecular weight antigens of cercariae (Dissous *et al.*, 1982) and miracidia (Dissous, Grzych and Capron, 1986) surfaces. These observations suggested that the epitope could involve carbohydrate chains linked to different proteic or proteolipidic structures. Moreover, the sensitivity of the epitope to periodate treatment and the inability of IPLSm1 to precipitate *in vitro* translation products from parasite messenger RNA (mRNA) confirmed the hypothesis of a carbohydrate nature of the epitope. The major immunogenicity of the 38kDa carbohydrate moieties was confirmed in mouse and human schistosomiasis (Omer-Ali *et al.*, 1986; Khalife, Capron, Capron, Grzych, Butterworth, Dunne and Ouma, 1986). This in part explains recent interest in

the prospect of developing parasite-derived glycan conjugates as vaccine for schistosomiasis (Nyame *et al.*, 2003 and 2004). These works have raised the possibility of using carbohydrate antigens as putative vaccine candidate.

2.4.3 Schistosome egg antigens

The main pathologic feature of hepatosplenic schistosomiasis involves the formation of granulomatous inflammation around parasite eggs entrapped in the tissues and portal vasculature. The immune response also leads to fibrosis, which affects the architecture and circulation in the affected organs (Warren 1972; Kelly, 1987). The granulomatous reaction around the eggs is essentially a cell-mediated immune response to antigens normally secreted by mature viable eggs (Boros and Warren, 1970; Hang, Warren and Boros, 1974). Warren (1972), showed that soluble egg antigens (SEA) in the supernatant of ultracentrifuged egg homogenate could elicit granulomatous hypersensitivity and other immunologic reactions characteristic of intact eggs. The SEA preparation was shown to contain multiple glycoproteins and non-glycoconjugated proteins (Carter and Colley, 1978, 1979; Pelley, Hamburger, Peters and Warren, 1976). Also, studies with SEA glycoproteins (purified from SEA by affinity chromatography on immobilized Concanavalin A) suggested their possible importance in the induction of granulomatous hypersensitivity as well as the elicitation of other delayed type responses (Pelley *et al.*, 1976; Carter and Colley, 1979). Furthermore, Carter and Colley (1979) showed that only the glycoprotein fraction of SEA was capable of eliciting T-cell responses.

Investigations by Pelley *et al.* (1976), showed that sera from mice with light chronic *S. mansoni* infections identified three major serological antigens which formed the bulk of the glycoprotein fraction obtained with ion exchange chromatography on Concanavalin A. These antigens were designated MSA₁, MSA₂ and MSA₃ and their molecular weights estimated by gel filtration to be of Mr. 50, 450 and 80 respectively. Hamburger, Pelley and Warren (1976), investigated the specificity of these antigens using antigen-binding radioimmunoassay, and showed that antibodies that reacted with MSA₂ and MSA₃ cross-reacted with cercaria, but not adult worm antigens of *S. mansoni*. Nevertheless, they both cross-reacted with SEA from *S. japonicum* and *S. haematobium*. In contrast, MSA₁ appeared to be both egg stage- and species-specific to *S. mansoni*. Further investigations with MSA₁ suggested that it might be a major immunopathological egg antigen (Kelly, 1987).

Three glycoprotein antigens of *S. japonicum* were also identified by Boros and Warren (1970), who demonstrated the sensitizing activity in mice of Concanavalin A binding fraction of *S. japonicum* SEA. The molecular weights of the antigens were determined by gel filtration to be 590, 245 and 46 kDa. The 46 kDa antigen was shown to possess less sensitizing activity compared with the others. Boros and Warren (1970) and Warren *et al.* (1975) studied *S. japonicum* and *S. mansoni* SEA and reported that SEA from *S. japonicum* elicited an immediate (antibody-mediated) inflammatory response when injected into the footpads of *S. japonicum* infected mice, whereas *S. mansoni* SEA elicited a delayed type (cell-mediated) response. An enhanced granulomatous reaction was only observed when mice were pre-sensitized with eggs or SEA of *S. japonicum* injected subcutaneously as against intraperitoneal injection in the

case of *S. mansoni*. Owhashi and Ishii (1982) also purified a glycoprotein antigen of approximate M_r 90 from SEA of *S. japonicum*. The antigen showed eosinophilic chemotactic activity, which appeared to be dependent on the integrity of the carbohydrate moiety.

Even though schistosome egg antigens are reported to be mainly involved in pathological manifestations of the disease, anti-egg monoclonal antibodies which passively transferred immunity to mice have been produced (Harn *et al.*, 1984). Furthermore, studies by van der Kleij *et al.* (2002) showing that egg glycolipids stimulated the production of IL-10, IL-6 and TNF suggests carbohydrate potential as useful vaccines.

2.4.4 Hepatotoxic egg antigens

It has been observed that T-cell deprived mice, when infected with *S. mansoni* do not develop granulomatous reactions around eggs deposited in tissues within seven days of infection, however, they suffer an acute hepatotoxicity reaction (Byram, Doenhoeff, Musallam, Brink and von Lichtenberg, 1979). Injection of such mice with serum from chronically infected mice prevents the liver damage (Doenhoeff, Musallam, Bain and McGregor, 1979). Using chronic sera, Dunne *et al.*, (1981); identified 12 of the *S. mansoni* egg antigens by immunoelectrophoresis. Also, work with a series of sera having partially overlapping specificities, showed that recognition of one particular antigen was required for sera to protect against liver damage. Subsequently, Dunne and Doenhoff (1983), purified and characterized this antigen, coded w_1

as a non-glycosylated polypeptide of approximate M_r 22-26. This antigen appeared to be stage-specific and has proved a valuable immunodiagnostic reagent (Kelly, 1987).

2.4.5 Circulating schistosome antigens

Several studies have been conducted to detect circulating antigens in schistosome-infected mammals. These studies are found necessary because existing assays utilizing crude or partially purified schistosome antigens to detect anti-schistosome antibodies do not give information suitable for the estimation of worm burden (Mott, 1982), or for identification of active infection. However, similar to the egg detection method, detection of circulatory antigens in body fluids of the host would provide a basis for the identification of active infection and give a better correlation to infection intensity (Deelder, Kornelis, Marck, Van Eveliegh and Egmond, 1980).

Berggren and Weller (1967), described a circulating schistosome antigen in the serum of mice and hamsters heavily infected with *S. mansoni*. Gold, Rosen and Weller (1969), characterized the antigen and also demonstrated its presence in the urine of infected hamsters. They found that the antigen is anodic (on the basis of its mobility in immunoelectrophoresis) and is heat-stable and dialysable. Nash (1974), reported that the circulating antigen was a large molecular weight substance, most likely a polysaccharide. It was demonstrated in *S. mansoni*, *S. haematobium* and *S. japonicum* homogenates and in the serum of mice and hamsters heavily infected with *S. mansoni* and *S. japonicum*

(Bawden and Weller, 1974). Nash (1974) and von Lichtenberg, Bawden and Shealy (1974), also showed that the same antigen was present in the epithelial cells of the schistosome gut. Deelder, Klappe, Van den Aardweg and Van Meerbeke (1976), confirmed the presence of circulating anodic antigens (CAA) in *S. mansoni* infected hamsters and also demonstrated the occurrence of a lower molecular weight circulatory cathodic antigen (CCA). Deelder *et al.* (1976), demonstrated both the CAA and CCA in adult worms extract, as well as in the excretory and secretory products of the worms. Analysis of circulating antigens (CAA and CCA), as a direct measure of worm burden and/or faecal egg count in determining the intensity dependent fecundity in human schistosome infection was reported by Polman *et al.* (2001).

2.5 *Electrophoretic separation of proteins*

Polyacrylamide gel electrophoresis has proven to be one of the most useful methods for separation of proteins. One and two-dimensional gel electrophoresis are normally performed to determine whether a protein is pure, or has subunits, and to reveal its isoelectric point (pI) and molecular weights. Both electrophoreses methods have high-resolution. Polyvinylidene difluoride (PVDF) membrane filter may be used for blotting the protein from the gels and antibodies used to detect antigens of interest. Furthermore, separated proteins can be electroeluted from polyacrylamide gels and utilized in diverse experiments including those aimed at improved diagnosis or in the search for protective antigens. When a protein is demonstrated to be pure, an estimate of the molecular size of the units can be determined by

electrophoresis under denaturing conditions in the presence of the anionic detergent “sodium dodecyl sulphate” (SDS) and by comparison to the molecular sizes of standard proteins.

2.5.1 Polyacrylamide gel electrophoresis

Polyacrylamide gels are formed following polymerization of monomeric acrylamide into polymeric acrylamide chains and the cross-linking of these chains by N, N'-methylene-bisacrylamide. This polymerization occurs at room temperature and is initiated by addition of ammonium persulphate and catalysed by N,N,N',N'-tetramethylethylenediamine (TEMED). A total of 3-20% (w/v) acrylamide is required in a gel. Any gel containing less than the required acrylamide will disintegrate easily, whilst too much of the reagent may cause the gel to be excessively brittle. Degassing of the gel solution before addition of TEMED is very crucial since dissolved oxygen may inhibit the polymerization process. Polyacrylamide gel electrophoresis has an added advantage of high resolution and sensitivity, as well as simplicity. Also, it is robust and relatively inert compared with the available alternatives like agar and starch gels. Both acrylamide and bisacrylamide are neurotoxic and therefore great care is required in their use. Stock preparations of acrylamide and bisacrylamide solutions may polymerize explosively if heated, therefore it is necessary to store at 4°C. However, if the solution crystallizes, a temperature of 40°C and not higher is required to warm until it dissolves.

In polyacrylamide gel electrophoresis, it is advisable to use the discontinuous system in which the gel buffer is different from the electrode buffer (Ornstein, 1964; Davis, 1964). The system consists of a resolution gel made with a buffer of relatively high molarity (0.37M) tris- HCl, pH 8.8; and a stacking gel also with low molarity tris HCl buffer, pH 6.8 formed above the resolution gel. The electrode has a low molarity tris- glycine buffer, pH 8.3. The proteins under the prevailing buffer conditions are virtually negatively charged and rapidly migrate into the stacking gel that has a minimum sieving effect. The proteins become concentrated into a narrow band in this neutral stacking gel buffer, which subsequently reduces their electrophoretic mobility. The concentrated bands of protein migrate through the stacking and enter the separating gel. Once in the separating gel, which has smaller pore sizes, the highly charged proteins separate based on molecular size. The samples are always loaded together with a dye (bromophenol blue) and this helps monitor the progress of electrophoresis as the dye travels faster than the proteins in the sample. However, if albumin is present in the sample, its rate of migration is usually as fast as the dye, which binds to it and shows the position of albumin. For slow moving proteins it is necessary to continue electrophoresis for 1--2 hours after the dye has run off the bottom of the gel.

2.5.1.1 *Denaturing conditions*

The robust nature of polyacrylamide gel makes it an ideal support for electrophoresis in the presence of denaturing agents. For instance if SDS which is a strong anionic detergent is used, then proteins are denatured and combine with a constant ratio (w/v) of the SDS. The overwhelming negative

charge provided by SDS coating makes any charge contributed by the protein negligible, and therefore separation of proteins is almost entirely based on the gel pore sizes and dependent on the molecular size of protein. The molecular weight of most proteins can be accurately determined by comparison of their electrophoresis mobility with that of a standard protein with known molecular weight.

2.5.1.2 *Sample concentration*

It is necessary to concentrate the sample to be electrophoresed when it is too dilute to be loaded directly. For dilute samples containing less than 10µg/ml, a carrier protein like albumin or haemoglobin of 20µg/ml can be added to facilitate precipitation. To concentrate, 1 volume of prepared sample is added with 9 volumes of ethanol and 1/50 volumes of sodium acetate, mixed and incubated at –20°C for 48 hours. The sample is then centrifuged at 1800g for 30 minutes at –10°C. By inverting the tube, the excess ethanol is allowed to drain off and the pellete re-dissolve in appropriate sample buffer for analysis. Alternatively, the pellet may be stored at –20°C until required.

2.5.1.3 *Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE)*

SDS PAGE is one of the most widely used electrophoresis techniques. Following the formation of the polyacrylamide gel, sample proteins are

denatured or solubilized by heating in the presence of an anionic detergent (SDS) and 2-mercaptoethanol. Any disulphide bonds in the sample proteins are splitted by the 2-mercaptoethanol, which is a reducing agent. The SDS wraps around the protein and masks the intrinsic electrical charge of the sample proteins, thereby forming an anionic complex with constant negative charge per unit. It is for this reason that protein separation in SDS PAGE is dependent on molecular weight. Despite the fact that proteins bind a constant amount of SDS, there are exceptions leading to anomalous migration. For example, carbohydrates, which may also be present as residues do not bind SDS. Therefore, heavily glycosylated proteins may migrate more slowly than non-glycosylated proteins of the same molecular weight. Also some proteins are not fully unfolded by the denaturing condition of SDS-PAGE and hence slower migration is expected. The denatured sample proteins are loaded onto a stacking gel which has large pores and is made with buffer containing chloride ions (called the leading ions) whose electrophoretic mobility are greater than the mobility of the proteins in the sample. On the other hand the electrophoresis buffer contains glycine ions (called the trailing ions) whose electrophoretic mobility are less than the mobility of the proteins in the sample. The net result is that the faster migrating ions leave a zone of lower conductivity between itself and the migrating protein. The higher voltage gradient in this zone allows the proteins to move faster and to stack. After leaving the stacking gel the protein enters a separating gel, which has smaller pores and is made with a buffer of higher salt concentration and higher pH. In the separating gel, therefore, the glycine ions migrate past the proteins and

the proteins are separated according to molecular size in denaturing gel containing 0.1% SDS.

2.5.2 Non-denaturing polyacrylamide gel electrophoresis

In nondenaturing polyacrylamide gel electrophoresis, the protein subunits are not dissociated and the biological activity of the protein is maintained. This type of electrophoretic separation is used for assessing the purity of a protein complex or a multimeric protein, which will be identified as a single protein band. In this case, however, a true estimate of the molecular size (M_r) of the native protein cannot be made, because the separation is based on molecular size, shape and charge. Such gels are only used to confirm the purity of the non-denatured form of a protein.

2.5.3 Common problems encountered in polyacrylamide gel electrophoresis

If an electrophoretic separated protein will be electro eluted or electro blotted, then the highest purity reagents available should be used. After a separating gel is poured, it may be stored with an overlay of the same buffer used in preparing the gel. Stacking gel should be poured immediately prior to use; otherwise, there will be a gradual diffusion-drive mixing of buffers between the two gels that will cause a loss of resolution. It is recommended that the unknown protein of interest should be present in 0.2-10 μ g in a

complex mixture of protein if the gel is to be stained by Coomassie Brilliant Blue, however, up to 100 μ g of a complex protein mixture can be loaded onto a slab gel. Some important problems likely to be encountered in SDS-PAGE analysis of proteins are as follows.

In situations when the protein bands curve upwards at both sides of the gel (called "smiling"), it is recommended to increase heat transfer from the gel by adding more buffer to the lower buffer chamber up to the level of the sample wells or by cooling the lower buffer below ambient temperature, and by stirring it. Another problem involves the spread of protein bands laterally from gel lanes. This spreading, which is due to diffusion of sample out of the wells may be corrected by reducing the time interval between sample application and running of the gel. Alternatively, the acrylamide percentage in the stacking gel should be increased from 4% to 4.5% or 5% or the operating current in the stacking gel should be increased by 25%. On the other hand, the protein bands may be uneven because the stacking gel is not adequately polymerized or the bands may be distorted because of the presence of salt in the protein sample. This problem may be corrected by degassing the stacking gel solution thoroughly or by increasing the ammonium sulphate and TEMED concentrations by 25%. Also, excess salt in the sample may be removed by dialysis, gel filtration or precipitation. Another cause of skewed protein bands is an uneven interface between the stacking and separating gels, which can be prevented by not disturbing the separating gel surface when overlaying it with absolute methanol.

Vertical streaking of protein bands may be corrected by decreasing the amount of sample loaded onto the gel, or by further purifying the protein so as to reduce the amount of contaminating protein applied or by reducing the operating current by 25%. Where streaking is caused by precipitation in the sample, it may be corrected by centrifugation of the sample or by reduction of the percentage of acrylamide. When the protein bands are diffuse, it is advisable to increase the operating current by 25 to 50% or use a higher percentage of acrylamide. The use of gradient gels may also improve the sharpness of the bands. Double protein bands are observed when the protein is partially oxidized. Such oxidation can be reduced by increasing the concentration of 2-mercaptoethanol in the sample buffer or by preparing fresh protein samples.

In situations where the tracking dye band is diffuse, it is advisable to prepare new buffers and acrylamide monomer stocks. If the run takes too long the buffers may be too concentrated or the operating current too low, while if the run is too short, the buffers may be too dilute or the operating current too high.

2.5.4 Interactions between schistosome antigens and host immune system

Interactions of schistosome parasites with host immune system begin with penetration of the skin by cercariae. The cercariae transform into schistosomula, which migrate to the lung and finally reside in the veins of the vesical plexus for *S. haematobium* or the mesenteric vessels for *S. mansoni* and *S. japonicum*. They develop into adult worms (male and female), pair

and engage in permanent copulation and egg laying for many years. The lifespan of the adult worm in humans has been estimated to average 3.5-12 years with some surviving for 30 years or longer (Arnon, 1990; Harris *et al.*, 1984). Although very little immune response is produced when cercariae penetrate the skin (von Lichtenberg, 1987), exposure of the host to excretory and secretory products from the tegument and intestines of the adult worm, and enzymes from the eggs induce immunological reactions that result in the development of chronic disease. Nevertheless, sudden exposure to an upsurge of these antigens may lead to acute infections (von Lichtenberg, 1987). These potent immunogens induce strong cell-mediated responses and often result in circulatory immune complexes (Hiatt *et al.*, 1979; Lunde *et al.*, 1979; Nash *et al.*, 1983 and Gazzinelli *et al.*, 1985). Erasmus (1987), demonstrated that most pathology is due to host immunological responses to the accumulated parasite eggs in the host tissues.

Interestingly, schistosome worms have developed various adaptive mechanisms to evade host immune system. These include rapid turnover of antigenic membrane, enzymatic cleavage of antibodies on the parasite's surface, and masquerading of the parasite's surface with host blood group antigens, serum components and histocompatibility antigens. These mechanisms obviously help to promote the chronicity of the disease (Colley and Colley, 1989; Bloom, 1979).

2.5.5 Immunity to schistosomes

Animal models have been used by researchers to greatly facilitate investigation into the mechanism involved in immune response in schistosomiasis. The lack of specificity shown by schistosomes for their definitive hosts has made possible the use of different experimental animals to study the three principal schistosome species, which infect man. However, *S. haematobium* has been less extensively studied because of more restricted host specificity, as compared to *S. mansoni* and *S. japonicum*. *S. haematobium*, also takes a longer time (12 weeks) to reach maturity compared to 4-6 weeks in the case of *S. mansoni* and *S. japonicum*. Furthermore, only a small percentage of *S. haematobium* parasites used to infect experimental animals actually survive to the adult stage (McLaren and Smithers, 1987). All these have culminated in the difficulty in maintaining the life cycle of this parasite under laboratory conditions, thereby slowing down work with *S. haematobium*. For *S. mansoni* and *S. japonicum*, the mouse model has been used extensively in the study of pathology and acquired resistance. This is because they show the full spectrum of egg-induced hepatic portal fibrosis (Warren, 1973) and develop partial immunity to re-infection (Dean, 1983).

Immunity to schistosomes can be induced in two different ways. Following exposure to normal cercariae, resistance to infection develops at about the time of egg laying and immunity reaches its peak some 4-6 weeks later (Smithers and Terry, 1967; Dean, 1983). By intrahepatic transfer of adult worms into naïve Rhesus monkeys or mice/baboon, Smithers and Terry, (1967) Webbe *et al.* (1976) and Peresan and Cioli, (1980), demonstrated that protective immunity

could be induced without prior exposure to cercariae/ schistosomula. This immunity was termed concomitant immunity because in this situation, resistance to re-infection occurs in the presence of active infection with adult worms (Smithers and Terry, 1969). Smithers, (1986) and Rogers, (1986), were able to induce immunity in Rhesus monkey and Guinea pig after exposure to unisexual schistosome infection in which case there was no egg associated pathology. In contrast, following exposure to irradiated cercariae, an immunity termed "vaccine immunity" rapidly develops, plateaus at week five and remains high indefinitely (Dean, 1983). It is clear that this type of immunity is not associated with the adult worm of the parasite, nor with the egg- induced pathology, rather is induced by radiation-damaged schistosomula during their incomplete migration. Similar to the concomitant immunity, the migrating larvae or immature juvenile of challenge infection are the target of vaccine resistance (McLaren, Pearce and Smithers, 1985). Although concomitant immunity and "vaccine immunity seem to exhibit common mechanism of immunity, there appears to be crucial differences in their relative specificities. It is long known that concomitant immunity can cross-react with the different species (Smithers and Doenhoff, 1982) whereas Bickle *et al.* (1979), Moloney and Webbe (1987), demonstrated the species specificity of vaccine immunity. Sher *et al.* (1982), demonstrated that vaccine immunity is based on lymphocyte associated immunity that is dependent on T- and B- cells. On the other hand, Dean *et al.* (1981), Harrison, Bickle and Doenhoff (1982), Wilson, Coulson and Metting (1983), suggested that concomitant immunity is dependent on non-immunological factors. These perhaps involve vascular changes in the liver, which affect the circulatory patterns of the challenged schistosomula. However,

in a further study, James and Cheever, (1985), observed that a major part of the resistance associated with infected mice was immunologically based. Also, studies with a monoclonal antibody NIMP/R.41 which binds and destroys mouse neutrophils have shown that depletion of these cells abrogate dermal inflammatory response to schistosomula, leading to the suppression of both vaccine and concomitant immunity.

Immunity studies so far documented involves experimental animals *in vivo*, and *in vitro* cytotoxic assays. Smithers *et al.* (1977), using *in vivo* studies, reported that the skin stage schistosomulum is the major site of protective immune response. However, the recovery of parasite from host tissue (Smithers and Miller, 1980), site elimination of parasites injected intravenously or intrahepatically to bypass the skin and the lung (McLaren *et al.*, 1985; Mangold and Dean, 1986), tracking of migrating radiolabelled parasites by organ autoradiography (Dean *et al.*, 1984; Knopf *et al.*, 1986; Wilson *et al.*, 1986; Kamiya *et al.*, 1987) and administration of protective or inhibitory serum at various times of post challenge (Mangold and Knopf 1981; Ford *et al.*, 1984; McLaren and Smithers, 1985; McLaren *et al.*, 1987) revealed the susceptibility of adult worms to immune response. Experiments involving passive transfer, site elimination and autoradiography tracking all indicate that little or no immune attrition occurs in the cutaneous tissues of either a vaccinated or an infected host (Dean *et al.*, 1984, Mangold and Dean 1986; and Wilson *et al.*, 1986). The lung has been reported to be a major site of immune dependent elimination in both vaccinated and infected rats (Ford *et al.*, 1984; McLaren *et al.*, 1985; Knopf *et al.*, 1986).

In vitro assays have been used primarily to investigate and compare the efficiency of potential effector mechanisms and to reveal differential susceptibility of developing schistosome larvae. For example, 3-hour old schistosomula were susceptible to killing when 4-day old lung stage parasites were totally refractory (Clegg and Smithers, 1972; McLaren *et al.*, 1975). Furthermore, McLaren and Incani (1982) clearly demonstrated that mechanically transformed schistosomula were more susceptible to cellular cytotoxicity, than the schistosomula that have penetrated an isolated skin prepared *in vitro*. Schistosomula that has experienced the skin of a live host were found to be least susceptible. This phenomenon could be attributed to the uptake of host red blood cell glycolipids (Goldring *et al.*, 1977), intracellular substance of the skin (Smith and Kusel, 1979) and histocompatibility antigens (Sher *et al.*, 1978), which afford an immunological disguise. However, in mice there is evidence that immunity involves inflammatory responses that depend upon humoral and cellular mechanisms. It is though not yet certain which parameter of immune response is the key to protection. The stimulation of cell-mediated immunity leading to recruitment of activated macrophages may be sufficient to promote protection before antibody levels become elevated, but antibody-dependent mechanisms could play an important role once significant levels are achieved.

Antibody-opsonized schistosomula are efficiently killed in the presence of complement mediated cytotoxic activity, which is directed against the parasite surface (Sher *et al.*, 1974). In humans and a variety of rodents, eosinophils have been shown to adhere complement, which kill young schistosomula *in vitro*, in the presence of antibodies (McLaren, 1982 and 1985; Butterworth *et al.*, 1975; McLaren and Ramalho-Pinto, 1979). Of all the potential effector cells

tested, the eosinophil is the most effective cytotoxic leucocytes for skin-stage parasites (McLaren, 1980).

2.5.6 Protective antigens

Identification and characterization of antigens for induction of protective responses in the host has tremendously advanced in the immunology of schistosomiasis since the introduction of hybridoma and gene cloning technology (Kelly *et al.*, 1987). Some monoclonal antibodies transfer passive protection to naïve recipients, or kill schistosomula in vitro by recognising antigens on the surface of the newly transformed larva. These antigens play a major role in protection (Kelly *et al.*, 1987). However, vaccination studies have demonstrated that a somatic antigen – Paramyosin may contribute to the development of immunity.

Carbohydrate epitopes are known to be responsible for most antigenicity of schistosomula surface. Omer–Ali *et al.* (1986) showed that infection sera bind to whole schistosomula 3–4 times more efficiently than to sera of vaccinated animals. Several protective antibodies analysed recognised either carbohydrate or polypeptide surface epitope, and therefore may have a role in the induction of protective immunity. Available data indicates that for laboratory mice and guinea pigs carbohydrate molecules constitute the primary target of concomitant immunity, whereas the less abundant polypeptide antigens form the basis of vaccine immunity. Nevertheless, polypeptide antigens play a significant role in human immunity to schistosomes. This has been shown in analysis of antigens

recognised by sera collected from patients in an endemic community for *S. haematobium* and *S. mansoni*. It was demonstrated that in younger or actively infected people, there was high IgG antibodies, which could recognise carbohydrate epitope on schistosomula surface. However in older infected patients there were increased levels of antibodies directed more to polypeptide epitopes with a low level of antibodies binding to schistosomula surface carbohydrates. Carbohydrate antigens are also present on the surface of miracidia (Yi *et al.*, 1986) however their similarities with schistosomula surface antigens have not been well studied.

Studies on blocking antibodies, which modulate the efficiency of immune effector mechanisms, have been documented. Grzych *et al.* (1984) demonstrated that rat IgG2c, monoclonal antibody which failed to promote schistosomula killing in vitro, could inhibit eosinophil-mediated killing promoted by an IgG2a monoclonal antibody recognising the same determinant. Khalife *et al.* (1986) and Yi *et al.* (1986), showed that infection sera harvested from both mice and patients contained IgM antibodies which blocked the protective capacity of the serum in vitro. The IgM antibodies identified cross-reacting carbohydrate epitopes associated with schistosomula surface and egg antigens, thereby effectively inhibiting the lethal activity of antibodies recognising other schistosomula surface antigens. Presumably, blocking antibodies might explain why concomitant immunity has long proven difficult to transfer passively.

2.5.7 Immunity in Humans

Different hosts vary in the nature of the protective responses they mount against schistosome infections, and therefore it is not likely that any one experimental animal model can reflect the situation in humans. However, schistosome infections in animal models have provided the background in immune studies in man (McLaren, 1980; Smithers and Doenhoff, 1982; Butterworth *et al.*, 1982; Capron *et al.*, 1982). Human schistosomiasis is usually a well-tolerated infection, however, there is enough evidence for immunity (Colley, 1987). In chronic infections, adult worms may survive for as long as 12 years during which time they produce large numbers of eggs and, excretory/secretory antigens (Smyth and Halton, 1983). The exposure to these antigens leads to a wide variety of complex immunoregulatory interactions between the parasite and host (Colley, 1981 and 1987), with evidence supporting the view that immunity plays a role in determining the levels of infection and reinfection. For example, Walker *et al.* (1970), reported the absence of *S. haematobium* infection over a number of years in children who played in pools which served as a source of infection in other school children. Also, the constant egg output in urine of *S. haematobium* infected children over a three- year period suggested the lack of super-infection and was interpreted to be due to concomitant immunity. In another, Wilkins and Scott (1978), reported that egg output in children are higher than in adults and there was a continuous turnover in infection with worms constantly being lost or acquired in a dynamic equilibrium. This was in part explained by the possibility of children having more water contact than the adults (Warren, 1973; Dalton and Pole, 1978). However, Butterworth *et al.* (1984; 1985) and Hagan *et al.* (1985b) found a group of children who had relatively high contact with infested water but

low levels of reinfection. Also, Wilkins *et al.*,(1987), demonstrated that adult women had low levels of reinfection after chemotherapy than did children with the same level of exposure. The expected relationship between exposure and reinfection was present in the 5 – 9yr and 10 – 14yr group, but absent in adults who resisted reinfection despite exposure levels which were as high as those of the children that were heavily reinfected. All these are clear evidence in favour of acquired immunity in man.

2.6 *Diagnostic methods for schistosomiasis*

In schistosomiasis control, diagnosis is very important (WHO, 1983a, b, c). Simple, rapid, sensitive and reproducible diagnostic tests that allow better decision making regarding individual and community treatment have been emphasized by several authors (Hoffman, Lehman, Stott, Warren and Webbe, 1979; Peters and Kazura, 1987; Kremsner, Enyong, Krijger, De Jonge, Zotter, Thalhammer, Muhlschlegel, Bienzle, Feldmeier and Deelder, 1994; Bosompem *et al.*, 1996d; Bosompem *et al.*, 1998). Indeed, sensitive diagnostic tools are important for the evaluation of the outcome of chemotherapy and other control measures. Presently, there are three main categories of diagnosis. First, there is the indirect method which is based on clinical symptoms such as painful micturation, haematuria, haemoglobinurias, cancer of the urinogenital tract and proteinuria in urinary schistosomiasis; or diarrhoea with blood and mucus, hepatomegaly and splenomegaly in intestinal schistosomiasis (Peters and Kazura, 1987). The second is the direct parasitological methods based on demonstration of parasite eggs in urine, stool and specimens from rectal

biopsies, liver or surgically removed tissues in which schistosomula, adultworms or eggs can be demonstrated by histological methods. Thirdly, there are immunological methods for measuring the immune response to certain schistosome antigens or concentration of parasite-derived antigens in blood or urine.

In hospitals where diagnosis is mostly centered on individuals, the methods usually selected require the highest possible combination of sensitivity, specificity and predictive value, whereas in community diagnosis, other criteria such as cost of equipment and disposable materials, skills and time required are usually more important. Therefore, the choice for a technique will depend on its suitability to the clinician or the epidemiologist responsible for the individual and community diagnosis.

2.6.1 The Parasitological methods

2.6.1.1 *Demonstration of S. haematobium eggs in urine*

A definitive diagnosis may be made by direct detection of parasite eggs in urine by microscopy. Urine filtration, which has been widely used (WHO, 1983a), provides an enormous advantage over other techniques because of its suitability for determination of intensity of infection with maximal specificity. To increase the sensitivity of this method, a large volume of urine is filtered through a membrane with the aid of a suction pump, or is centrifuged and the entire sediment examined for the presence of parasite ova (Peters and Kazura, 1987).

An improved filtration method developed by Peters (1976) and adopted by the WHO involved passing 10ml of urine through a suitable membrane made of polycarbonate, polyamide or synthetic fibres of pore size 1 – 45um (Bradley 1965; Peters *et al.* 1976; Mott, 1982). The membrane is secured in a swinnex support chamber and traps parasite eggs during filtration. When ready for microscopy the membrane (filter) is laid face down on a microscope slide for examination, and a drop of saline is placed on the filter to improve the refractive index (Peters, Warren and Mahmoud, 1976). Even though the technique allows for determination of intensity of infection, the time consuming nature of the procedure limits its field applicability. Furthermore, egg detection in urine may be difficult in women of childbearing age (Feldmeier *et al.*, 1993). This is because the epithelia cells frequently present in female urine have the capacity to clog membrane filters when urine volume is greater than 5 ml. Also, urine may be contaminated with menstrual blood, and this requires 10% hydrochloric acid to lyse erythrocytes to reveal eggs in sediments or in filter membranes. Different types of crystals in urine may also, be dissolved by adding a few drops of 2% acetic acid, 15% hydrochloric acid or 1M sodium hydroxide respectively.

2.6.1.2 *Faecal examination for schistosome eggs*

In infection with *S. mansoni*, *S. japonicum* and *S. intercalatum*, parasite eggs are mostly excreted in the faeces. The simplest method which is the direct faecal smear technique is however not sensitive enough. This is due to the small size of specimen examined and the presence of large amounts of fibres, which interfere with visualization of the parasite eggs. Using the technique,

approximately 2mg of stool is emulsified following the addition of 1–2 drops of 0.9% sodium chloride solution on a glass slide. The emulsified stool is spread by placing a coverslip on it prior to examination by microscopy. The less sensitive nature of the technique makes it mainly useful for identification of heavily infected persons (Peters and Kazura, 1987).

Many improved methods based on concentration techniques have been described, and all involve the removal of fat, faecal debris and mucus. Garcia and Shimuzi (1981) reported on an improved method of stool examination called the formal-ether method. The technique involves the preservation of specimen, and is also simple to perform. By this technique, approximately half a teaspoonful of fresh stool is placed in 10ml of 10% formalin and allowed to "fix" for at least 30 minutes. The preserved specimen is then passed through two layers of gauze into a centrifuge tube. The filtered stool is sedimented and washed twice by centrifugation at 290 Xg in 0.9% NaCl and the final pellet resuspended in 7ml of 10% formalin. The mixture is then added to 3ml of ether and shaken for 30 seconds in a capped tube before centrifugation again at 290 Xg for 2-3 min. This separates the mixture into four layers consisting of small sediments containing schistosome eggs at the bottom with a layer of formalin above it followed by faecal debris and ether on top. The sediments containing parasite eggs is recovered and examined by microscopy. Even though the method is very sensitive, it is semi-quantitative and, therefore, not suitable for accurate determination of intensity of infection (Peters and Kazura, 1987).

The "Bell method" (Bell, 1963), which is also a concentration technique was the first to be extensively used in field studies (Teesdale and Amin 1976).

In this method, a sufficient volume of stool is added to 90ml of a formalin-glycerol solution to bring the final volume to 100ml. The preparation is then mixed at medium speed in a blender for 15-20 seconds to give a creamy suspension. An aliquot of 1ml (representing 0.1ml of stool) is placed on a 7 cm diameter piece of filter paper that is fixed on a suction apparatus and subjected to negative pressure to spread the specimen. The paper is then sprayed with ninhydrin, which stains the egg blue. Before counting the eggs by microscopic examination, the paper is wetted with distilled water to improve its refractive index.

To date, the Kato method or one of its numerous modifications (Kato and Miura, 1954; Kato, 1960; Komiya and Kobayashi, 1966; Martin and Beaver, 1968 and Katz, Chaves and Pellgrino, 1972), has been widely accepted as a standard diagnostic tool in both clinical and epidemiological studies. In this method, only a small amount of faecal specimen (usually between 20–50mg) is examined, and the technique compares favourably with the filtration and sedimentation method where the concentration of eggs is ≥ 100 eggs per gram of stool (Teesdale and Amin, 1976; Sleight *et al.*, 1982). Kato and Miura (1954) and Kato (1960), described that a sample of stool is taken with a wooden spatula and forced through a stainless steel mesh to remove particulate and fibrous material. The specimen is then applied to fill a hole in a template that is set on a glass microscope slide. The template is carefully lifted off the slide to leave an intact plug of faeces. The stool specimen is then covered by a 25 x 35mm cellophane coverslip, previously impregnated with 50% (v/v) glycerol in water containing 3% malachite green. The slide is subsequently turned face down on a flat surface, and pressed gently but firmly to spread the stool

specimen evenly. The prepared slide is left for about 30min in the light to clear before it is examined microscopically for parasite eggs. This technique is rapid and suitable for large epidemiological surveys in which the sensitivity needed to detect light infections is not crucial.

2.6.1.3 *Demonstration of schistosome eggs in host tissue*

The absence of schistosome eggs in faecal or urine specimen, does not necessarily mean that there is no infection (Peters and Kazura, 1987). Therefore, Badran *et al.* (1955); Cancado *et al.* (1965) developed the biopsy technique that has been used for decades as a simple direct diagnostic tool in individuals at the clinic level. Rectal biopsy provides an alternative way of visualizing parasite eggs, which may not be present in urine or stool (da Cunha, 1982; Bardan *et al.*, 1955). In the case of intestinal schistosomiasis, this method appears useful in the assessment of cure rates after chemotherapy. Viable eggs can be distinguished from non-viable eggs under the microscope by observation of the flame cells or miracidium movement in egg shell. In the technique, rectal snips may be taken from a rectal valve using a crocodile forceps. Alternatively, a more sophisticated procedure requiring a curette and rectoscope may be employed. The biopsy specimen (1–2 mm snip of mucosa) is soaked with water on a microscopic slide, and a second slide is placed on it to give a crush preparation of tissue. Addition of a few drops of glycerol-malachite solution before placing the cover slip, enables a better differentiation of non-viable and viable eggs. Cancado *et al.* (1965), van Wijk (1969), advocated the use of rectal swab with less risk for patients in tropical countries. However, this technique is

more time consuming, expensive and requires skilled medical personnel like gastroenterologists.

2.6.2 Indirect Methods

Quite a number of indirect indicators for both urinary and intestinal schistosomiasis exist (Gryseels, 1989; Zwingenberger *et al*, 1989). However, no single (indirect) pathological process is sufficiently sensitive and specific to be used as a substitute for parasitological methods in individual diagnosis.

The availability of urine analysis reagent strips for determining; haematuria, proteinuria and leucocyturia has facilitated studies on the suitability of these indicators for diagnosis (Wilkins *et al.*, 1979; Pugh *et al.*, 1980, Sellin *et al.*, 1982; Savioli *et al.*, 1990; Taylor *et al.*, 1990). Accumulated evidence show that micro-haematuria by the reagent strip or in combination with proteinuria is a valuable tool in determining prevalence, and assessing the effectiveness of an intervention. Haematuria correlates with egg excretion in an intricate, non-linear manner. Doehring *et al.* (1985a) showed that haematuria follows a circadian pattern with peak around 18 hours, which is almost 6 hours later than the maximum egg excretion. However, the use of haematuria alone as an indirect method may be severely biased in women of childbearing age. For instance, menstrual blood may be contaminated with urine and thereby give false positive reagent strip reading. In communities where female circumcision is widely practiced, false positives are expected and this would interfere with specificity of this method or test. In intestinal schistosomiasis, HemoQuant, which is a sensitive test for occult blood in stool, is more appropriate (Ahlquist *et al.*, 1985). Proietti and Antunes (1989) and Ruiz-Peres *et al.* (1990) demonstrated that the

occult blood in stool is an indication of high intensity of infection. However, the overall sensitivity of the test is usually less than 20% (Feldmeier *et al.*, 1993). A study by the WHO, (1985) in Philippines showed no correlation between the presence of occult blood and intensity of infection. Therefore occult blood may bias sensitivity of the test.

2.6.3 Immunodiagnosis of schistosomiasis

Diagnosis of schistosomiasis by parasitological methods has extensively been used in the identification of infected individuals (WHO, 1992). However, the technique is labour intensive and relatively insensitive as it is affected by daily fluctuation in the rate of egg excretion, and the trapping of eggs in the host tissue. Microscopy is therefore unreliable in areas with low intensities of infection.

Immunodiagnostic techniques involve the detection of either specific antibodies or identification of schistosome antigens. Several procedures, including enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), indirect immunofluorescence test (IFAT), gel precipitation technique (GPT), indirect haemagglutination assay (IHA), latex agglutination test (LAT), circumoval precipitin test (COPT) and complement fixation test (CFT) have been developed for detection of antibodies to adult worms, schistosomula, cercariae, or egg antigens (Kagan, 1968). However, very few of these tests have been recommended for large-scale diagnosis. This is because they have limited field applicability and inadequate sensitivity in most cases. For example, complement

fixation tests and immunofluorescence, all involving the use of adult worms showed comparative specificity and each could detect antibodies in only 70% of infected cases investigated (Reviewed by Smithers and Doenhoff, 1982).

Smithers and Doenhoff (1982), reported that both the ELISA and radioimmunoassay have improved the sensitivity of serodiagnostic procedures for schistosomiasis. However, ELISA has several advantages over radioimmunoassay. These advantages include the stability of antigen antibody complexes and the use of enzymes in place of radioactive labels. In different studies with ELISA, McLaren *et al.*, (1987) found that schistosome egg antigens were more reactive than worm antigens and was better for detecting antibodies in acute or early infections. Using antibody detection in ELISA, it was revealed that 82-100% of intestinal schistosomiasis patients in St. Lucia could be diagnosed even under circumstances where up to three different stool examinations were required to reveal some infections (McLaren, Long, Goodgame and Lilleywhite 1979). Although Kelley (1987), showed that antibody detection is the most sensitive serodiagnostic approach, its field applicability is limited by the inability to differentiate active infections. The technique is also limited by extensive cross-reactivity between *S. mansoni*, *S. japonicum* and *S. haematobium* especially when crude parasite antigens were used (McLaren *et al.*, 1987). It is widely accepted that problems associated with antibody detection assays could be overcome by detecting parasite antigens instead (Gold, Rosen and Weller, 1969; Bawden and Weller, 1974).

Nash (1974) and Deelder *et al.* (1980) studied and reported on circulatory schistosome antigens, which could be exploited in the diagnosis of

schistosomiasis. Two important circulatory proteoglycan antigens, both associated with the gut of adult schistosomes have been identified and named; circulatory anodic antigen (CAA) and circulatory cathodic antigen (CCA) on the basis of their electrophoretic mobilities (Deelder *et al.*, 1976). Although both antigens are not strong immunogens, Feldmeier, Nogueira-Queiros, Doehring, Dessaint, de Alencar, Daffalla and Capron (1986), used polyclonal antisera in ELISA to detect CCA at very low concentrations in a two-site radioimmunoassay. More recently, several investigators including De Jonge, Fillie, Hilberath, Krijger, Lengeler, De savigny, van Vliet and Deelder (1989) and Kreamsner *et al.* (1994) used MoAbs produced against the CAA and CCA and showed that they could be used to detect the antigens in both urine and sera of patients. The advantages of antigen detection in the management of schistosomiasis have since been clearly communicated by Kreamsner *et al.* (1994) who reported that quantitative measurement of schistosomal antigens in human specimen can be used to :(1) diagnose infection; (2) measure the severity of pathological manifestations; (3) monitor impact of chemotherapy and (4) investigate ongoing transmission in endemic areas.

Even though neither the CAA nor CCA are specific to any of the schistosome species, it is generally accepted that *Schistosoma* genus-specific assays are desirable because of the common drug (praziquantel) treatment. However, it may be argued that schistosome species-specific assays are necessary, for example, to differentiate between urinary and intestinal schistosomiasis in hyperendemic areas of Africa (Bosompem *et al.*, 1996a). A recent advance in the production of *Schistosoma* species-specific MoAbs with

diagnostic potential (Amanor *et al.*, 1996) further demonstrates the applicability of MoAbs in the diagnosis of schistosomiasis.

2.6.4 Monoclonal antibodies (MoAbs)

Immune sera are products of several antigenic determinants and are made up of many different antibodies. Consequently, immune sera are non-specific to any particular antigenic epitopes and extensively cross-react with antigens from different sources (Campbell, 1984). Polyclonal antibodies are therefore unsuitable for assays in which antibody specificity is very important (Lopes and Alves, 1984). The introduction of monoclonal antibody production based on single antigenic epitope detection (Billing, 1985) has overcome this limitation and greatly improved the performance of immunological assays (Sikora and Smedley, 1984). Furthermore, they have played an indispensable role in the isolation and characterization of specific parasite antigens leading to identification of diagnostic, immunoregulation and protective antigens (Sikora and Smedley, 1984; Kremsner *et al.*, 1994; Amanor *et al.*, 1996). Kelly (1987), utilized protective monoclonal antibodies in immunoaffinity chromatography to purify adequate amounts of antigens for vaccination exercise. As a result, MoAbs are now being used in immunoparasitology; (1) as probes for the detection and localization of antigen, and analysis of its organization and availability; (2) for studies of antigenic heterogeneity (variability) in parasite populations; (3) for detection of cloned DNA in various vectors; and (4) for parasite typing and parasite detection (Mitchell, 1984; Sikora and Smedley, 1984; and Goding, 1986). This considerable potential of MoAbs has already

been widely exploited in the study of the most common parasitic diseases such as malaria (Yoshida, Nussenweig, Potocnjak, Nussenzweig and Aikawa, 1980; Rener, Carter, Rosenberg and Miller, 1980), leishmaniasis (McMahon-Pratt, Bennet and David, 1982) and trypanosomiasis (Nantulya, Musoke, Rurangirwa, Saigar and Minja, 1987; Bosompem, Assoku and Nantulya, 1996b). In schistosomiasis, MoAbs have become useful tools in the development of standardized immunodiagnostic reagents of high specificity and for the identification and characterization of immunoregulatory, and protective antigens (Taylor and Butterworth, 1982; Smith *et al.*, 1982; and Amanor *et al.* 1996).

In vitro culturing of MoAb secreting hybridoma cells or *in vivo* passage of these cells can result in large quantities of monoclonal antibodies in culture supernatant or ascitic fluid, respectively (Clark and Waldmann, 1986). Impurities in antibody containing supernatant or ascites may interfere with specificity and sensitivity of monoclonal antibody-based immunoassays. It is therefore crucial to purify MoAbs from culture supernatant and ascites, as standard reagents in immunoassays or for the characterization of the MoAbs themselves (Goding, 1986). Purification of MoAbs involves precipitation using lower concentrations of ammonium sulphate. This technique freely separates immunoglobulins from contaminating proteins such as albumin (Campbell, 1984). Knowing antibody class and subclass is very necessary for further purification of the reagent (Goding, 1986), which may be achieved by high capacity ion exchange and column gels depending on MoAb class. Double immunodiffusion method (Ouchterlony, 1976) is the simplest known method of determining antibody class, although there are alternatives like

radioimmunoassay, enzyme-linked immunosorbent assay or indirect immunofluorescence utilizing class-specific antibodies.

2.6.5 Vaccination against schistosomiasis

There is still no vaccine available for schistosomiasis because development in this area has proved very difficult (reviewed by Bergquist, 1995). The search for protective antigens against the disease has been lengthy partly because *S. mansoni* is the only schistosome species that can adapt with ease to the laboratory (Bergquist, 1995). For this reason, most schistosome antigens were initially identified in *S. mansoni*, even though some specific and polymorphic antigens in other species have been described. For example, variants of glutathione S-transferase (Sm28GST) from all three major schistosome species affecting humans, as well as, *Schistosoma bovis*, have been identified and cloned. Recent advances in immunology and molecular biology have further increased the chances of identifying putative antigens, which may provide effective immune prophylaxis against schistosomiasis (Simpson, Chandler, Kelly, Walder, Knight and Smithers, 1987; Bergquist, 1995). These new developments have already resulted in a long list of cloned *S. japonicum* antigens, considerably improving the prospects for developing a vaccine against this parasite in parallel with one against *S. mansoni* (Brindley, Ramirez, Tiu, Wu, Wu and Yi, 1995). Recently, MoAbs were generated against *S. haematobium* antigens (Bosompem *et al.*, 1996a) and characterized (Amanor *et al.*, 1996). Even though the monoclonal antibodies were screened for diagnostic properties and some bound *S. haematobium* species-specific antigens, work on identification of protective antigens continues to lag behind.

The search for protective schistosome antigens has focused on the less protected larval (schistosomula) stage, partly because of the elaborate defence mechanisms employed by adult schistosome worms (Dissous *et al.*, 1987; Kelly, 1987). Several investigators (Bickle *et al.*, 1979; Bergquist, 1995) have shown that the injection of live schistosomula attenuated by sub-lethal doses of radiation into experimental animals results in high levels of resistance in a subsequent challenge. This observation was attributed to the presence of particular antigens on the surface membranes of schistosomula. As a result, both carbohydrate and polypeptide epitopes expressed on the surfaces of newly transformed schistosomula have been tried and found to induce protective immunity (Kelly, 1987). However, the carbohydrate epitopes are reported to be unsuitable as candidate antigens for vaccine production because of their similarity with schistosome egg glycoprotein antigens in terms of their ability to induce pathological responses (Warren, 1987). In spite of this, Dissous *et al.* (1982) identified a carbohydrate epitope on a major 38-kDa schistosomulum surface antigen, as defined by a protective rat monoclonal antibody was found to be capable of inducing a high level of protection against *S. mansoni* infections.

Polypeptide epitopes on the other hand, appear to be more suitable for use in vaccine production. This is because they do not cross-react with the pathology-inducing egg antigens (Hackett, Simpson, Knight, Ali, Payeres and Smithers, 1986). For this reason, a range of schistosomulum surface peptides have now been cloned and expressed in *E. coli* (reviewed by Simpson and Cioli, 1987). These include several major polypeptide antigens of Mr 97, 32, 28, 25 and 20 kDa which have also been reported to be expressed by adult worms and

are presented to host immune systems during natural infections (Simpson, Hackett, Kelly, Knight, Payeres, Ali, Lilleywhite, Fleck and Smithers, 1986). According to Simpson *et al.* (1987) more schistosomula surface polypeptide antigens are becoming available from several laboratories.

Lanar, Pearce, James and Sher (1986), identified and cloned a 97-kDa antigen from *S. mansoni* adult worm parasites. This internally localized antigen known as paramyosin was found to resemble the alpha-helical protein, which forms the core for myosin filaments in invertebrate muscle. This molecule is important not only because of its immunological potential as a vaccine immunogen, but also because of its probable physiological function for the parasite. As a core structure for myosin filaments, paramyosin is suspected to be an important molecular component in a "catch" mechanism aiding adult schistosomes in continuously maintaining themselves against the venule wall and thus avoiding being dislodged by the blood flow (Lanar *et al.*, 1986). Several other antigens have been discovered and are undergoing confirmatory tests and subsequent vaccine trials. These include SRP and gp68 from *S. mansoni* schistosomula and adult worms, respectively, and Sj26 from *S. japonicum*. JM8-36, an anti-idiotypic antibody and Fh(SmIII) from *Fasciola hepatica* adult worms have also been found to be useful in protection against schistosomiasis (Colley and Colley, 1989).

At present, the strongest candidate antigens for the production of vaccines for schistosomiasis are Glutathione-S-transferase, a 28 kDa molecule (Sm28GST) that is found in parenchymal cells but expressed transiently on schistosomulum and adult worm surfaces, Paramyosin (Sm97) and an

irradiation-associated vaccine antigen (IrV-5) (Bergquist, 1995). Sm28GST, Sm97 and IrV-5 have been found to induce 30-60%, 30% and 50-70% protection in mice respectively (Pearce *et al.*, 1988; Bergquist, 1995).

CHAPTER 3

IDENTIFICATION OF *SCHISTOSOMA* *HAEMATOBIIUM* RESISTANT INDIVIDUALS

3.1 INTRODUCTION

Urinary schistosomiasis caused by *Schistosoma haematobium*, is estimated to affect almost 130 million people in Sub Sahara Africa (Chitsulo *et al.*, 2000). In Ghana *S. haematobium* is present in almost every region. The development of water projects for irrigation and dams have immensely contributed to the disease and its spread. In endemic rural settings of many developing countries schistosomiasis is an important occupational hazard (Doumenge *et al.*, 1987).

Several efforts have been made to control schistosomiasis through elimination of snail hosts and health education to interrupt parasite life cycle, however, this has not yielded appreciable success (Manson, 1989). The use of drugs, which has so far shown some promise (Mott 1987; Bergquist 1987), is also burdened with re-infection. The development of vaccines is therefore highly desirable.

Several approaches to vaccine development have been identified. These include the study of resistant individuals in endemic areas to identify potential vaccine candidate molecules. In this endeavour it is necessary to accurately

diagnose the disease and to determine the infection status of individuals. This together with their water contact indices enable the identification of resistant individuals.

This study therefore sort to identify *S. haematobium* resistant individuals in an endemic community by first determining their water contact sites and activity indices. Secondly, to repeat screening of *S. haematobium* egg negative individuals by microscopy and further test for urinary antigens by monoclonal antibody (MoAb) based dipstick assay to confirm infection status. This could facilitate the identification of *S. haematobium* resistant individuals in furtherance of the search for potential vaccine candidates. The objective of the work described in this Chapter, therefore, was to identify *S. haematobium* resistant individuals in an endemic community.

3.2 MATERIALS AND METHODS

3.2.1 **Study area:**

Gomoa Okyereko is a village located in the Gomoa District of the Central region of Ghana. It lies about 1.5 km off the Accra- Winneba road, and is approximately 20 km from Winneba. This village is in the dry equatorial climate region of Ghana with daily temperatures within 26 – 30°C. There are two rainfall maxima with the main rainy season lasting from May to July and the minor season between September and October. Average rainfall is between 700-1000 milimetres annually, and therefore exhibits a coastal savannah type of vegetation. The estimated population is 2,500 and the settlement itself is between an earth dam constructed across a minor tributary of the river Ayensu

and an irrigation facility (approximately 40 hectares) installed in 1973- 74. The main source of water supply to the village is the Ayensu river. Farming is the major occupation of the people, and the irrigation project is targeted toward increases in the production of cereals, mainly rice and vegetables during the dry periods. Irrigable land surrounding the village is also used for the cultivation of maize. A few inhabitants also engage in fishing in the lake/ river.

3.2.2 Study design and ethical considerations

Both cross sectional and longitudinal design were utilized in this study. Briefly, a durbar involving the Chief, elders, opinion leaders and the people of Gomoa Okyereko was organized to inform the community about the presence of urinary schistosomiasis, and seek their consent to be involved in the study. Community members were encouraged to ask questions in order to prepare them for a questionnaire aimed at obtaining information on transmission and control of schistosomiasis. Data collected included, sex, age, occupation, sources of water contact, frequency of contact and activities at the contact site that could influence transmission of the disease. After discussions and consultation with opinion leaders from the community, dates for questionnaire administration and urine collection were decided. Consent approval in writing, was sought from the parents and/or guardians of children aged 10 years and above before their inclusion in a longitudinal study. Following questionnaire administration study subjects age 10 – 96 years were recruited. Urine specimen were collected from each individual and examined for *S. haematobium* eggs by microscopy. The intensity of urinary schistosomiasis infection was determined by the *S. haematobium* egg count in a fixed (10ml)

volume of urine. Individuals who provided urine were also requested to produce a stool specimen that were analysed by Kato-Katz technique for *S. mansoni* and other intestinal parasite ova. Subsequently, study subjects who tested *S. haematobium* egg negative by microscopy were made to provide at least three urine specimens on different days for a two months period. On each occasion, 10ml of each sample was filtered and any excess centrifuged for examination of the sediment by microscopy. Each urine specimen was also tested for *S. haematobium* urinary antigen by monoclonal antibody dipstick ELISA. This was done in addition to the repeated microscopical examinations so as to determine putative resistant individuals. Final selection of patently infected and putative resistant subjects was based on their water contact activity (WCA) index. Only individuals with substantial water contact activity (WCA ≥ 300) were considered. Three individuals who have not stayed in a schistosomiasis endemic area, and have no history of the infection were also screened and selected as control subjects. Sera were obtained from blood collected from each individual and utilized in Western immunoblot assay that were designed to detect differences and similarities between the resistant and susceptible groups. Protein antigens from schistosome eggs and adult worms were used in the analysis.

3.2.3.1 *Ethical consideration*

This project formed part of the Noguchi-JICA infectious diseases project, which involved studies on HIV, STDs, Tuberculosis, Measles and The Schistosomiasis. The project was approved by the ethics committee of the Ministry of Health, Ghana.

3.2.3 Urine collection and analysis

Twenty millilitres (20ml) to 100ml of urine was collected from each individual within the period of peak schistosome egg excretion (11:00- 14:00 hours). The samples were transported on ice to the laboratory and analysed within 12 hours. 10ml of each urine sample was filtered through a 25mm nuclepore filter (Polycarbonate or polyamide membrane) of pore size 12µm secured in a Swinnex support chamber, which trapped schistosome eggs. With a forceps each filter was removed from the chamber and the side that received the urine laid face down on a microscope slide. The remaining urine from each specimen was centrifuge at 400Xg for 3 minutes at a temperature of 4°C. The filters and entire sediment from each specimen was examined separately for *S. haematobium* eggs, which were counted to estimate the intensity by microscopy.

3.2.4 Stool collection and analysis

Approximately 5 of faecal specimen were collected from each individual and analyzed using the Kato- Katz method (Kato, 1960). 1gm of stool was taken with a wooden spatula and forced through a stainless steel mesh sieve (105µm pore size) to remove particulate and fibrous material. The specimen was then applied to fill a hole in a card template set on a glass microscope slide. The template was carefully lifted off the slide, and the stool specimen remaining on the slide was then covered with a 25 x 35mm cellophane cover slip that has been impregnated with 50% (v/v) glycerol in water containing 3% malachite green. The slide was subsequently turned upside down on a flat surface, and pressed gently but firmly to spread the stool specimen evenly under the

cellophane. The slide was left for at least 30min in the open at room temperature to clear before it was examined microscopically for *S. mansoni*, and other intestinal parasite ova.

3.2.5 Dipstick ELISA procedure

The dipstick ELISA was performed as described by Bosompem *et al.* (1996c). Briefly, Polyvinylidene difluoride (PVDF) membrane strips (3mm by 25mm) were immersed in absolute methanol up to 1/3 their length and stored in distilled water. The membrane strips were each incubated in test urine for 30 minutes, at room temperature (22 – 25 °C). The test strips were retrieved from urine specimen and rinsed with Tris Buffered Saline (TBS) consisting of 50mM Tris and 200mM NaCl, at pH 7, and then blocked in 35% skimmed milk for 1 minute. The strips were once again rinsed with TBS and transferred into a combined reagent mix containing 1:100 dilution of *S. haematobium* species-specific monoclonal antibody and 1:500 dilution of goat anti- mouse IgG horse raddish peroxidase (HRPO) in TBS with 0.1% skimmed milk for 60 minutes. Finally the strips were washed three times (10 minutes/wash), followed by incubation in substrate solution [0.025g of 3,3'- diaminobenzidene, 30ul of 35% H₂O₂ and 300ul of 5mM Cobalt II Nitrite Hexahydrate (CoNO.6H₂O) in 50ml of TBS]. Positive results were bluish black whilst negative results remained unstained.

3.2.6 Water contact activity index (WCAI)

Data on water contact activity levels were obtained by questionnaire. Each subject provided information about the site of water contact, reason for

contact and frequency of contact. The Water Contact Activity Index (WCAI) was calculated using the formula below:

$$\text{WCAI} = R \times F$$

where; R= reason for contact
and F= frequency of contact

The table below describes the two factors (R and F) and various scores that were assigned (Table 3.1).

Table 3.1: Criteria used to determine scores for water contact activities

Factor	Score	Definition
R (reason for contact)	2	Fishing or crossing the streams (F/CS)
	3	collecting water for the household or dish washing (CW/DW)
	4	laundering, or watering agricultural fields (L/W)
	5	bathing, swimming or playing in water (B/S/P)
F (frequency of contact)	0	No contact at all
	1	less than two contacts per month
	2	at least two contacts per month
	4	at least one contact per week
	28	at least one contact per day

All individuals who had WCAI less than three hundred (<300) were excluded from the analysis. Putative resistant individuals were therefore selected from the highest risk group (WCAI \geq 300).

3.2.7 Blood sample collection and processing

From individuals who were selected as either susceptible or resistant to *S. haematobium* infection and control cases (with no history of residing in a schistosomiasis endemic area) up to 10 ml of venous blood was collected in heparinised vacutainer and then placed on cool container and transported to

the laboratory for processing. The blood was diluted with 2vol. of PBS. 30ml of the diluted blood was slowly layered onto 10ml of Ficoll-Hypaque and centrifuged for 20min at 2000rpm, at room temperature. Mononuclear cells (lymphocytes and monocytes) were concentrated at the intermediate layer. The upper layer contained the plasma and with the aid of a sterile pipette was transferred into eppendorf tubes and stored at -20°C . The mononuclear cells (ca. 3-4ml) was collected with pasteur pipette into 15ml tube. PBS was added up to 12ml (3-4 times dilution) and centrifuged for 10min at 1200rpm. The supernatant was removed and the pelleted cells washed twice with 2% FCS/RPMI for 5min at 1200rpm. Erythrocytes in the mononuclear cell pellet was haemolyzed by addition of 2ml ACT. The cells were washed twice with 2% FCS/RPMI for 5min at 1200rpm and suspended in 10%FCS/RPMI for immunodetection assays. Viable cells which excluded trypan blue were countered by haemocytometer. The cell were kept at -20°C to be use in another study.

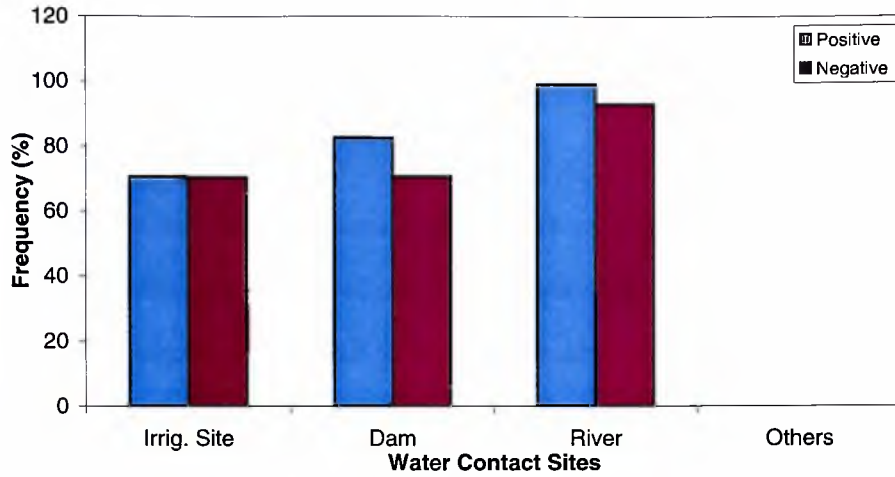
3.3 RESULTS

3.3.1 **Identification of Water contact sites and Snail survey**

A total of 15 water contact sites were identified in the study community. These were the Ayensu river, the irrigation dam, the irrigation fields and twelve minor water bodies which comprised of old ponds, fish ponds and drainage channels. A total of 346 individuals responded to the questionnaires. The questionnaire results revealed that the major sites of contact were at the Ayensu river which involved 95.4% (330/346) of the interviewed subjects (Figure 3.3.1). This was followed by the irrigation dam 74.9% (259/346) and the irrigation fields 70.5% (244/346). None of the responded indicated having water contact at the remaining twelve minor wells and ponds.

From the snail survey carried out at all the water contact sites, 10 *B. truncatus* snails and 25 *B. pfeifferi* were found at the irrigation dam. Also, 52 *B. pfeifferi* snails were collected at 3 contact points at the irrigation fields. No schistosomiasis host snail was found in the Ayensu river and the minor sites. Large numbers of *B. forskali* snails were also found in the dam.

Fig 3.3.1 Proportion of infected and uninfected subjects visiting water contact sites



3.3.2 Prevalence of various parasitic infections as revealed by stool examination

Out of a total of 346 individuals, 324 (93.6%) provided stool for analysis. Table 3.3.2, summarizes the data on parasitic infections among the subjects as revealed by stool examination. In all, 100 (30.9%) of the subjects were diagnosed to be infected with intestinal parasites, which involved *Necator americanus* (hookworms), *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), and *S. mansoni*. More males were infected 58/100 (58.0%). Ninety one (91) individuals comprising 37 females and 54 males were diagnosed with hookworms, from which 36 (3 females and 33 males) were also *S. haematobium* positive. A 24 year-old male was the only person diagnosed with whipworm and he was also infected with *S. haematobium*. Two (2) subjects, a 12 year old female and a 22 year old male were found to have *S. mansoni*, and they were both positive for *S. haematobium*.

Table 3.3.2 Number of subjects infected with various intestinal parasites

Parasites	Positive subjects		
	Male	Female	Overall
<i>Shistosoma mansoni</i>	1 (1)*	1 (1)	2 (2)
<i>Necator americanus</i>	54 (33)	37 (3)	91 (36)
<i>Trichuris trichiura</i>	1 (1)	0	1 (1)
<i>Ascaris lumbricoides</i>	2 (1)	4 (1)	6 (2)
Total	58 (36)	42 (5)	100 (41)

* The number of subjects who were also infected with *S. haematobium*.

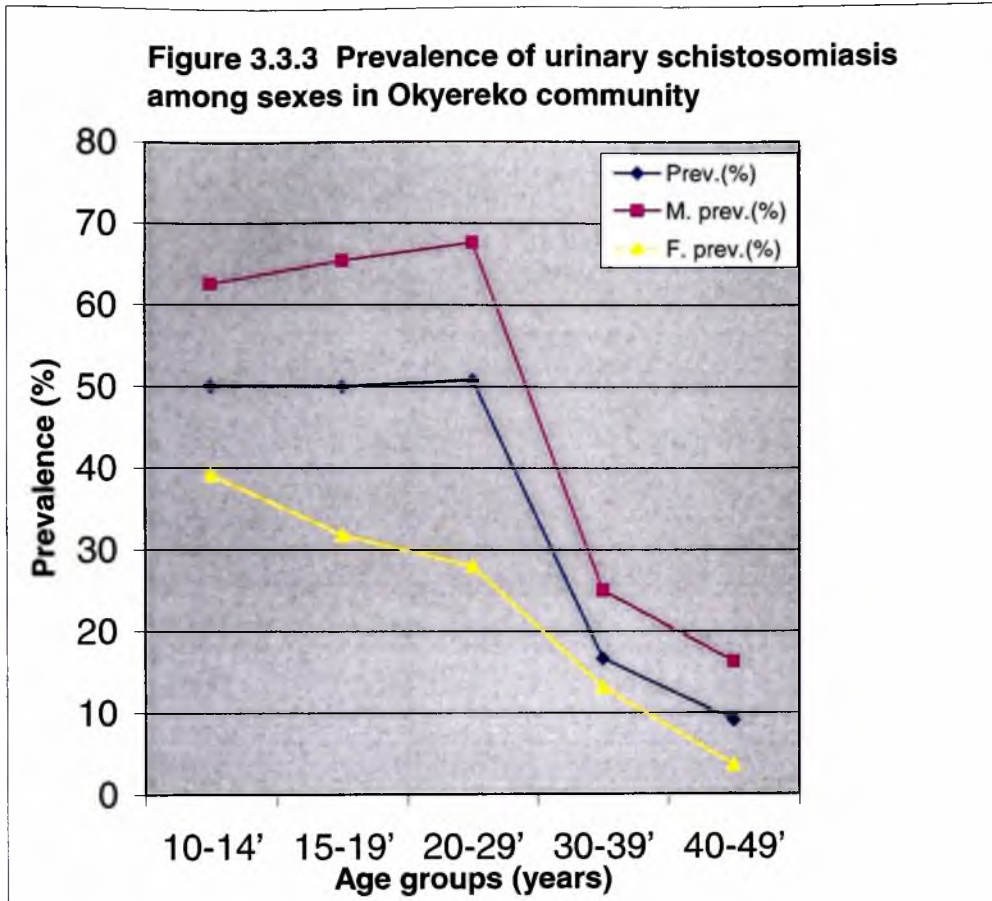
3.3.3 Identification of urinary schistosomiasis infected individuals

Table 3.3.3 shows the prevalence and intensity of *S. haematobium* infection in different age groups. A total of 346 individuals consisting of 159 males and 187 females were screened for *S. haematobium* infection by microscopic demonstration of the parasite's eggs. One hundred and fifteen (115) subjects (33.2%), made up of 76 males (47.8%) and 39 females (20.9%), were found to be infected with *S. haematobium* (Table 3.3.3). Interestingly, *S. haematobium* infection in 10 individuals (5.7%) could not be revealed by the standard 10ml urine egg count. These infections were detected by microscopy of sediment obtained from excess urine.

Table 3.3.3 Prevalence and intensity of *S. haematobium* infection as stratified by age

Age group (years)	Total Number Tested	Egg Positive (%)	Males		Females		Egg count (eggs/10ml urine) for both sexes	Mean egg
			Number tested	Egg Positive (%)	Number tested	Egg positive (%)		
10-14'	86	43 (50)	40	25 (62.5)	46	18 (39.1)	1-3846	369.6
15-19'	48	24 (50)	26	17 (65.4)	22	7 (31.8)	1-2114	371.2
20-29'	59	30 (50.8)	34	23 (67.6)	25	7 (28.0)	1-376	37.2
30-39'	54	9 (16.7)	16	4 (25.0)	38	5 (13.2)	2-230	35.6
40-49'	99	9 (9.1)	43	7 (16.3)	56	2 (3.6)	1-29	13.3
Total	346	115 (33.2)	159	76 (47.8)	187	39 (20.9)	1-3846	165.4

The infection profile in males and females is illustrated by Figure 3.3.3. *S. haematobium* prevalence was shown to be lower among females of all ages compared to the males. In the males, the disease prevalence was high at age groups (10-14yrs) and (15-19yrs), and peaked at age group (20-29yrs) after which there was a rapid decline. However, in the females the prevalence was highest in the age group (10-14yrs), and decreased continuously with age. The highest egg counts were also found in the age groups with the highest prevalence as demonstrated by the egg range and mean egg counts.



3.3.3 Effect of water contact activity (WCA) on prevalence and intensity of infection

A total of 295 individuals (85.3%) of the study subjects responded to the questionnaire (Table 3.3.4a). This comprised of 81 (27.5%) *S. haematobium* infected and 214 (85.3%) uninfected individuals. A high percentage (53.1%) of individuals in the *S. haematobium* egg positive group had high water contact (WCAI \geq 300), whilst a smaller percentage (44.9%) of the *S. haematobium* egg negatives were in this high-risk category. As shown, a higher percentage (60.7%) of uninfected females had low water contact compared to males (44.6%).

Table 3.3.4a Water contact activity index (WCAI) of study subjects as determined by questionnaire

Subjects	<i>S. haematobium</i> egg positive subjects			<i>S. haematobium</i> egg negative subjects		
	Number Involved	WCAI < 300 No. (%)	WCAI \geq 300 No. (%)	Number involved	WCAI < 300 No. (%)	WCAI \geq 300 No. (%)
Males	52	24 (46.2)	28 (53.8)	74	33 (44.6)	41 (55.4)
Females	29	14 (48.3)	15 (51.7)	140	85 (60.7)	55 (39.3)
Total	81	38 (46.9)	43 (53.1)	214	118 (55.1)	96 (44.9)

WCAI < 300 indicates low water contact or exposure to *S. haematobium* infection

WCAI \geq 300 indicates high water contact or exposure to *S. haematobium* infection

As shown in Table 3.3.4b, most *S. haematobium* infected individuals had water contact at the identified sites. The percentage of infected individuals was more in higher water contact activity index categories. The highest risk group (WCAI \geq 300) had the highest percentage, followed by (WCAI =101-299) with the least being (WCAI =0). Generally, the intensity of infection was higher in the lower age groups (10-14 yrs) compared to the rest and decreased with age. There was negative correlation of egg intensity with respect to age, however, the differences recorded for the various age groupings was not significant ($P>0.05$). The only individual who reported to have no water contact (WCAI=0), but tested positive for *S. haematobium* was in the older age group (\geq 40 yrs).

Table 3.3.4b Intensity of *S. haematobium* infection in individuals with different Water Contact Activity Indices (WCAI)

Age group (years)	WCAI and <i>S. haematobium</i> egg count (egg/10ml urine) of infected individuals*							
	WCAI = 0		WCAI=1-100		WCAI=101-299		WCAI \geq 300	
	number involved	egg range	Number involved	egg range	number involved	egg range	number involved	egg range
10-14	0		2	18-39	14	0-299	16	0-779
15-19	0		0		4	0-6	5	0-503
20-29	0		2	0-89	6	0-24	12	0-74
30-39	0		1	11	3	11-230	6	0-44
\geq 40	1	0	0	-	5	0-17	4	1-29
Total	1	0	5	0-89	32	0-299	43	0-779

* Infected individuals with no *S. haematobium* eggs (egg/10ml urine) were identified by sedimentation of total urine.

3.3.5 Selection of putative *S. haematobium* resistant individuals

Individuals who were *S. haematobium* egg negative were screened for *S. haematobium* antigen by monoclonal antibody dipstick assay to confirm their uninfected status. To improve the chances of identifying putative resistant individuals, only egg negative individuals with high Water Contact Activity Index (WCAI ≥ 300) were further examined by the monoclonal antibody dipstick assay. Out of a total of 96 individuals found to be *S. haematobium* egg antigen negative, only 25 were able to provide urine on three or more occasions when urine was collected once every week for a period of two months. Fourteen (14) out of 25 tested positive at least once for antigen, and were excluded from further analysis on the basis of being possibly infected. Eleven individuals (11/25) 44.0% remained negative for *S. haematobium* antigen and were selected as putative *S. haematobium* resistant candidates.

3.4 SUMMARY

A total of 346 individuals responded to the questionnaire and fifteen (15) water contact sites were identified in the study area. The major sites of water contact were the Ayensu river 95.4% (330/346), followed by the irrigation dam 74.9% and the irrigation fields 70.5% (244/346). None of the respondents indicated having water contact at the remaining twelve minor wells and ponds found in the area. Snail survey at all the water contact sites

revealed 10 *B. truncatus* snails and 25 *B. pfeifferi* at the irrigation dam, and 52 *B. pfeifferi* snails at the irrigation fields.

Out of 346 individuals that were screened for *S. haematobium* infection through microscopic demonstration of the parasite's eggs using standard 10ml urine egg count, 115 subjects (33.2%), were found to be infected. However, 10 (5.7%) of the infections were detected following microscopy of sediment obtained from excess urine. The prevalence of *S. haematobium* was shown to be low among females of all ages compared to the males. The disease prevalence was highest in the (10-14yrs) age groups for both sexes, and decreased with age. The highest intensity of infections were also found in the age groups with the highest prevalence as demonstrated by the egg range and mean egg counts.

From 324 subjects (93.6%) who provided stools for analysis, 100 (30.9%) were diagnosed to be infected with intestinal parasites. These parasites included *Necator americaus* (hookworms) (91) subjects from which 36 were also *S. haematobium* positive. Other parasites found were *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm) and *S. mansoni*.

Two hundred and ninety five (295) individuals (85.3%) of the study subjects, comprising of 81 (27.5%) *S. haematobium* infected and 214 (85.3%) uninfected individuals responded to the questionnaire on water contact activity index (WCAI). A high percentage (53.1%) of individuals in the *S. haematobium* egg positive group had high water contact (WCAI \geq 300), whilst a smaller percentage (44.9%) of the *S. haematobium* egg negatives were in

this high-risk category. Most of the *S. haematobium* infected individuals had water contact at the identified sites.

The *S. haematobium* egg negatives were screened for antigen by monoclonal antibody dipstick to confirm their uninfected status. Out of a total of 96 individuals found to be *S. haematobium* egg antigen negative, only 25 provided additional urine on three or more occasions. Fourteen (14) out of 25 tested positive at least once for antigen, and were excluded from further analysis. Eleven individuals (11/25) 44.0% remained negative for *S. haematobium* antigen and were selected as putative *S. haematobium* resistant candidates.

3.5 DISCUSSIONS

The aim of this study was to identify *S. haematobium* infected and uninfected individuals in an endemic community by microscopic demonstration of eggs, and to further select putative resistant individuals based on water contact activity and antigen detection by monoclonal antibody dipstick.

Bosompem *et al.* (1998), showed that in diagnosis of urinary schistosomiasis, the sensitivity of the microscopic technique was greatly enhanced by repeated examination of suspected individuals on different days. They also showed that the urinary schistosomiasis monoclonal antibody dipstick had superior ability to detect low intensity infections. In this study, putative *S. haematobium* resistant individuals were selected based on their water contact

activity index (WCAI) and repeated microscopy alongside the monoclonal antibody dipstick assay.

The inability of the 10ml urine filtration method to detect some infections as compared to sedimentation of larger volumes of urine was not surprising since it had been reported (Bosompem *et al.*, 1998). Earlier, Erasmus, (1987) and Wilkins *et al.* (1987), indicated that the 10ml urine filtration method as used in epidemiological studies has some limitations, due to fluctuation in egg output, and small number of eggs sometimes excreted.

The urinary schistosomiasis prevalence curve obtained in this study followed similar trends as reported in earlier studies (Mott, 1987; Bosompem *et al.*, 1996c, d; 1997) in endemic communities. However, there was a shift in peak prevalence (Woolhouse *et al.*, 1991 and 1998; Anderson *et al.*, 1987) towards the younger adults (20-29yrs), and that may be attributable to the occupation related exposure (mainly irrigation farming) prevailing in the study community. However, by the third decade of life, both prevalence and intensity of infection rapidly dropped and remained low as communicated by Jordan and Webbe (1989).

With respect to water contact activity, the irrigation dam, irrigation fields and the Ayensu river were all associated with *S. haematobium* infection. The presence of schistosomiasis host snails in the dam and irrigation fields therefore suggest their involvement in transmission, whilst the absence of snails in the Ayensu river raises questions about the role of the river in the transmission of schistosomiasis in the area. The presence of *B. truncatus* and *B. pfeifferi* in the dam suggests mixed infections as evident in two subjects who had both *S. haematobium* and *S. mansoni*. Iramaya *et al.*

(1995) pointed out that endemic normals (putative resistant individuals in schistosomiasis endemic areas) could be egg negative for several reasons. These include abortion of infection before worm maturation occurs, light infections which are self cured or single sex infections. Furthermore, such individuals may actually be infected but express strong anti-fecundity effects (Xu *et al.*, 1991; Grzych *et al.*, 1993). Nevertheless, there is evidence that individuals could develop resistance to schistosome infection, especially in adults and following chemotherapy (Butterworth *et al.*, 1988). It is likely that the inability to demonstrate schistosome eggs and/or antigen in the group of individuals with high water contact (WCAI>300) in this study is due to resistance to infection. The high prevalence and intensity of infection in the younger adults (10–29 yrs), with WCAI \geq 300 found in this study may be due to the frequent water contact. Such individuals would at best have low level of immunity (Butterworth *et al.*, 1988). Again, Butterworth *et al.* (1987, 1992, 1998), Khalife *et al.* (1986); Hagan *et al.* (1991) and Iskander *et al.* (1981), reported that the high levels of inappropriate or blocking antibodies (IgM, IgG2, IgG4) responses mounted during early age may interfere with protective responses. On the other hand, the low prevalence and intensity of *S. haematobium* infection in the older people (30 yrs and above) in this study is most likely due to their less contact with water. Nevertheless, the possibility that some degree of immunity develops and therefore plays a significant role in lowering the intensity and prevalence of infection in the adults cannot be underestimated, since there is no knowledge of their water contact in the first and second decade of life. Woolhouse and Hagan (1999), suggested that older people may acquire natural resistance, which could be due to immune

responses from earlier infections. Indeed, the spontaneous death of adult worms may make available more antigens for the host to develop immune responses (Kloos *et al.*, 1990).

In studies on immunity to schistosome infections, several authors have identified putative resistant subjects in endemic communities (Viana *et al.*, 1994 & 1995; Correa- Oliveira *et al.*, 2000). In these studies, resistant individuals were identified mainly through intensive microscopy examination for eggs and the degree of contact with potentially infested water. In the present study putative resistant individuals were further screened for schistosome antigens using a highly sensitive monoclonal antibody dipstick (Bosompem *et al.*, 1998). Indeed, the exclusion of potentially resistant subjects, due to antigen positivity strengthens the basis for classification of resistant and susceptible individuals in the study.

CHAPTER 4

ANTIBODY-ANTIGEN REACTIVITY PROFILE IN WESTERN IMMUNOBLOT

4.1 *Introduction*

Western blotting is a powerful tool in that electrophoretically fractionated protein can be immobilized onto a solid phase. This technique has therefore been used widely to characterize antibodies to parasite antigens (Ham *et al.*, 1985a; Capron *et al.*, 1987). The advantages of such characterization include the possibility of purification and sequencing of blotted protein antigens.

The search for protective antigens in schistosomiasis has mainly focused on two major human schistosomes (*S. mansoni* and *S. japonicum*), which has resulted in a long list of cloned antigens (Davern, Wright, Herrman and Mitchell, 1991). Work on *S. haematobium* continues to lag behind, partly because of the difficulty in maintaining this species in the laboratory as against the relative ease with which (*S. mansoni* and *S. japonicum*) species adapt to the laboratory (Bergquist, 1995). Identification of any *S. haematobium* antigens with protective ability will, therefore, greatly enhance work towards control of schistosomiasis.

Human related studies on immune response and resistance to infection with schistosomes have described both positive and negative response with

various antibody isotypes reacting with different antigenic preparations. (Viana, *et al.*, 1995). Further studies have involved antibody- dependent cell mediated killing of various parasite stages (Butterworth *et al.*, 1985; 1987; Gounni *et al.*, 1994).

Various schistosomal antigens and antigenic preparations have shown putative roles in the induction of protective immune response. (Butterworth *et al.*, 1987; Dessien *et al.*, 1988; Correa- Oliveira *et al.*, 1989; Grzych *et al.*, 1993) The aim of the experiments conducted in this chapter was to examine the reactivity of the different antibody isotypes against adult worm and egg polypeptides, utilizing sera from *S. heamatobium* infected, *S. heamatobium* resistant and normal individuals. And also to determine the molecular weights of any potentially protective antigen(s) using the western immunoblot technique.

4.2 MATERIALS AND METHODS

4.2.1 Generation of Parasite stages

4.2.1.1 Isolation and hatching of *S haematobium* eggs

Urine samples from *Schistosoma haematobium* infected human were pooled into a large conical flask (1000-2000ml) and allowed to stand for more than 30 minutes at room temperature to allow the sedimentation of the parasite eggs. A large portion of the supernatant was sucked by means of a suction pump without disturbing the sediment at the bottom of the flask. The sediment was transferred into 50ml tubes and centrifuged at 400 Xg for 5 minutes. Pellets obtained were re-suspended in 0.9% normal saline and spun at 400 Xg for 5 minutes to obtain a nearly clean pellet of eggs. The pelleted eggs were re-suspended in conditioned water (tap water allowed to age for two weeks). A 500ml volumetric flask was filled with conditioned tap water up to 2cm below the neck region and the egg suspension gently transferred to the bottom of the flask by means of a 10ml pipette. More water was added to the flask along the walls to avoid disturbing the suspension at the bottom. The flask was covered with a black polythene bag, leaving only the upper third of the neck region exposed to light. Hatched miracidia were attracted to light in the exposed upper part of the volumetric flask and harvested with a pipette into a clean conical flask. More water was added to the volumetric flask for continuous harvesting of the emerging miracidia.

4.2.1.2 Infection of *Bulinus truncatus* snails with miracidium to generate cercariae

Snails used in this study were obtained from the breeding colony at the Parasitology Unit of the Noguchi Memorial Institute for Medical Research. The snails were maintained in acrylic tanks fitted with aerators for air circulation.

With the aid of a dissecting microscope, fifty *Bulinus truncatus* snails were placed separately into the wells of 24 well tissue culture plate (FALCON, Becton Dickinson and Co. Lincoln Park, New Jersey). Each snail was then exposed to 5 miracidia for 4-10 hours at room temperature (25-27°C) before they were transferred into a big plastic bowl filled with conditioned tap water. The snails were maintained at room temperature (25-27°C) and fed oven-dried lettuce. The breeding medium was changed every 3 days and the colony transferred into a dark environment from day 28 post exposure to miracidia. From the 30th day post exposure, the snails were prepared for cercariae shedding by transferring each snail into a separate test tube containing 2-3ml of conditioned water. Cercariae shedding was done under day light exposure for a period of 30-90 minutes. Snails shedding cercariae were put together in the same container and kept in the dark. The infected snails were exposed to light every other day and the cercariae harvested. The cercariae collected were used to infect Balb/c mice and hamsters to generate adult worms. To ensure environmental safety, dead snails were disposed off by first treating them with hot boiling water.

4.2.1.3 Infection of laboratory animals with *S. haematobium* cercariae

Ten (9 weeks old) hamsters and four ICR mice (8weeks old) were used for the generation of adult *S. haematobium* worms. Mice and hamsters were anaesthetized by peritoneal injection of pentorbabitol sodium (nembutal) (50mg/kg body weight) diluted 1 in 10 with normal saline before they were exposed to infective cercariae. Each ICR mouse was placed in a restrainer and the tail immersed in a suspension of 500 cercariae in a test tube. In the case of hamsters, the abdominal fur was shaved and the skin exposed to 1000 live cercariae. The animals were kept for one hour to ensure maximum penetration by the larvae and then transferred into cages where they regained consciousness after 2–6 hours. All the animals used in these experiments were obtained from the NMIMR colonies.

4.2.2 Perfusion for *S. haematobium* adult worms

Twelve-week old *S. haematobium* infected ICR mice and hamsters were each anaesthetized with diethyl ether and dissected to open up the thoracic and abdominal cavities. Each animal was secured on a special holding board and the hepatic portal vein cut open with scissors. A 23-gauge hypodermic needle attached to a connecting tube fitted to a pump apparatus was inserted carefully into the renal artery along the vertebral column and perfusion carried out using sodium citrate and sodium chloride each at 0.85% concentration in distilled water. Pumping of the perfusion solution through the vascular system resulted in the collection of adult worms onto a sieve. Worms that were firmly attached to the walls of the mesenteric vessels were

dislodged with the aid of a pair of forceps and flushed out with the perfusion solution. The worms were washed from the sieve and stored at -20°C until required.

4.2.3 Preparation of *S. haematobium* parasite crude antigen extracts

S. haematobium eggs from infected human urine and adult worms used to prepare crude parasite antigen extracts were obtained from Ghanaian strains of the parasite.

S. haematobium adult worm and egg antigens extracts were obtained using Dulbeccos PBS buffer. 2mls of the buffer was added to each 1 ml of frozen pellet of parasites. Each specimen was disrupted for 5 minutes by ultrasonication at 70% output, 0.9sec sonication and 0.1sec rest to release the soluble antigens. The resulting suspension was transferred into 1.5 ml eppendorf tubes and centrifuged at 8500xg for 5 minutes at 4°C . The supernatant (first extract) was pipetted into new eppendorf tubes and stored at -20°C . Fresh Dulbecco's PBS was added to the pellet and the extraction process repeated, for a second and third extract, respectively. The concentration of protein in the various antigen extracts was estimated using Biorad protein assay reagent (Biorad laboratory 2000, CA 94547) following the manufacturers instructions.

4.2.4 Estimation of Protein Concentration of Adult worm and egg antigens

Different concentrations (1000µg/ml, 500 µg/ml, 50 µg/ml, 10 µg/ml, 5 µg/ml and 1 µg/ml) of Bovine Serum Albumin (BSA) standard protein solutions were prepared using Dulbecco's PBS. Ten microlitres each of the BSA Standards was carefully pipetted into different wells in an ELISA plate. Similar volumes (10ul) of titrated *S. haematobium* adult worm and egg antigens was also pipetted into other wells of the ELISA plate. Hundred microlitres (100ul) of Bio Rad reagent (Cat. 500-0006, Bio-Rad Laboratories) diluted with distilled water prepared as described by the manufacturer was then dispensed into the used wells of the ELISA plate. The ELISA plate was shaken to mix the contents and the optical density (O.D) recorded after 5 minutes using 570nm filter. A standard protein curve was plotted from the ODs of the BSA and utilized to estimate the concentration of test samples by extrapolation.

4.2.5 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

Electrophoresis of schistosome proteins was performed with the ATTO Slab Gel Apparatus (Bunkyo- Ku, Tokyo, Japan), following the SDS-Tris-glycine discontinuous buffer system (Laemmli, 1970).

4.2.5.1 Gel apparatus, materials and reagents/chemicals for preparation of resolution and stacking gels

The gel casting apparatus was composed of, a pair of glass plates (one smooth and one notched –1.5mm thickness), 4 clamps, a comb and a rubber gasket or spacer.

Chemicals and reagents used are indicated in appendix A.

To prepare the gel for electrophoresis, the glass plates were wiped clean with absolute methanol. The gels were cast in the mould formed by the two glass plates put together and sealed with the U-shaped spacer. The assembled plates were kept vertically upright by 4 clamps. The gel consisted of two parts, a resolution gel below which was first formed and a stacking portion on top where the sample wells are created using the comb. Resolution acrylamide gels (10%) and the stacking gel (3%) were prepared utilizing the reagents in Appendix B

Solutions A and B were prepared without TEMED in separate beakers and degassed for 30 minutes in a chamber. Solution A was completed by adding the required volume of TEMED and swirled to mix. It was then poured into the opening between the assembled plates, kept vertically upright by 4 spring clamps and allowed to polymerize within 30 – 60 minutes. The surface of the poured gel was then layered with absolute methanol. On polymerisation of the gel, the absolute methanol was drained off using tissue paper and the gel surface rinsed with distilled water, which was also drained off with the aid of a tissue paper. Solution B with the required TEMED added was poured on the resolution gel. The comb was carefully inserted into the solution B, and on polymerizing the comb was removed, thus casting the desired wells for sample application. The clamps and the spacer were carefully removed and the polymerised gel in the glass plates were assembled in an ATTO Cell Electrophoresis chamber. The upper electrophoretic chamber, at the cathode,

was filled with 0.4 litres of running buffer (24.8mM Tris, 191.8mM Glycine and 3.47mM SDS) and the lower chamber, at the anode, filled with 0.6 litres of the running buffer.

4.2.5.2 Preparation of samples and electrophoretic run

S. haematobium egg and worm crude antigen extracts were diluted with sample buffer [25mM Tris, 192mM Glycine, 0.1%(w/v) SDS and 20%(v/v) 2-mecaptoethanol] at a ratio of 2:1 (sample to buffer) to give 1µg/µl of protein. The samples were boiled for 5 min at 100°C and centrifuged briefly at 10000Xg to remove any particulate matter before they were loaded into the stacking gel. Standard molecular weight markers (Sigma, St. Louis Mo, USA) already prepared was loaded, 5ul per well. The electrophoresis set-up was connected to a power supply (ATTO Corporation, Japan) and constant current of 30mA supplied. When the tracking dye reached the interface between the stacking and resolution gel, the current was increased to 50mA. The power supply was interrupted when tracking dye had barely run out of the separating gel.

4.2.6 Immunoblotting (Western blotting)

Materials involved in the experiment are indicated in appendix C.

4.2.6.1 Antigen transfer

Separated proteins from worm and egg antigens were transferred electrophoretically from unstained gels onto Immobilon-P transfer membrane as described by Towbin, Staechelin and Gordon (1979) and Burnette (1981). Briefly, Immobilon-P transfer membrane (0.45mm pore size Millipore

Corporation, Bedford, U.K.) was wetted in absolute methanol for 10 seconds and immediately transferred into distilled water for 5 minutes. Also, six pieces of Whatman 3MM filter paper (Whatman, Maidstone, England) were cut to the size of the gel. First, two filter papers were soaked with the Anode transfer buffer (1) pH 10.4 and placed on top of the anode electrode plate of a semi-dry blotter unit (MiliBlot™-Graphite Electroblotter 1 Milipore Company, Incorporated, Seattle, USA). A third filter paper was soaked with the Anode buffer (2) and placed on top of the first two filter papers. The Immobilon-P transfer membrane was equilibrated in anode buffer (2) for a few minutes, and placed on top of the third filter paper. The unstained gel with the antigens was carefully placed in contact with the membrane without trapping air bubbles. A third batch of 3 filter papers was soaked in cathode buffer pH 9.4 and placed on top of the gel. The cathode electrode plate was then placed on top of the sandwiched gel and electrophoretic transfer was conducted at a constant current of 168mA for 90 minutes.

4.2.6.2 *Staining and destaining*

The region of the blotted Immobilon-P transfer membrane with the protein molecular weight standard markers together with a small portion of the region with sample antigens was cut with a sharp razor. The blotted membrane was stained with 0.1 % coomassie blue in 50% methanol and 7%(v/v) acetic acid for 2 minutes. The stained, blotted membrane was transferred into a destaining solution containing 50%(v/v) methanol and 7%(v/v) acetic acid in a plastic bowl. The destaining was done for 10 minutes, on a platform shaker (Platform Shaker STR6, Stuart Scientific – U. K.) at 10 rev/min. Destaining was repeated a

second time under the same conditions and the protein bands were seen on a clear background (Weber and Osborn, 1969).

4.2.6.3 *Immunodetection*

The blotted Immobilon-P transfer membrane with either worm or egg antigens was cut into smaller strips. The strips were placed in a trough and blocked with 5% skimmed milk in Tris buffer saline (TBS), pH 7.4 for 120 minutes. The strips were then washed for 10 minutes with Tris buffered saline, pH 7.4 containing Tween 20 (TBST). Strips were incubated with plasma samples from *S. haematobium* infected and resistant individuals diluted 1 in 100 in TBS for a further 120 minutes. The strips were again washed three times (10 minutes/wash) in TBST to remove excess unbound antibodies. The processed membranes were probed with immunoglobulin conjugates - anti human (IgA, IgE, IgG and IgM) each conjugated to horse raddish peroxidase (HRPO). The conjugates were incubated separately, each for 60 minutes. The strips were again washed four times (10 minutes/wash) in TBST. Finally, the strips were incubated in substrate solution containing 0.025mg 3,3' - diaminobenzidine (DAB), 7.5ul of 30% hydrogen peroxide (H₂O) and 300ul cobaltous nitrite for 5 – 10 minutes. Positive results appeared as bluish-black bands on the strips. The reaction was stopped by washing strips with excess distilled water.

4.3 RESULTS

4.3.1 Protein concentration of crude *S. haematobium* worm and egg antigens

The concentration of protein in *S. haematobium* crude antigen extracts was determined using the Bio Rad reagent (Cat. 500-0006, Bio-Rad Laboratories) prepared as described by the manufacturer with microplate reader. *S. haematobium* crude worm antigens had higher protein concentration (1.5mg/ml) compared to that of the crude soluble egg antigens (0.5mg/ml). The protein concentration of antigens was generally higher in the 1st extract compared to the 2nd extracts.

4.3.2 SDS-PAGE analysis of *S. haematobium* worm and egg antigens

Figure 4.2.2, illustrates SDS-PAGE analysis of *S. haematobium* worm and egg antigens and comparison of the polypeptide band profiles. As shown in the stained membrane, the *S. haematobium* crude worm protein extract gave more than 14 polypeptide bands with molecular weight ranging from 115kDa to 16kDa (lane 2). In the case of *S. haematobium* egg antigen lane 3, only four polypeptide bands ranging from (105 – 52) kDa were identified. However, two polypeptide bands (105 and 78) kDa were common to both *S. haematobium* worm and egg antigens, with the remaining bands being different.

For both worm and egg antigen preparations, the 1st extracts gave very prominent band compared to the 2nd extracts. Crude parasite antigens in the

1st extracts were therefore utilized in further analysis by the western immunoblot analysis.

4.3.3 Selection of sera from *S. haematobium* resistant and susceptible groups for western blotting analysis

As communicated in Chapter 3, Section 3.3.6 11 *S. haematobium* putative resistant subjects with high Water Contact Activity Index (WCAI \geq 300) were identified. Sera from 6 of those individuals aged not less than 15 years were selected for the western immunoblot analysis. This consisted of three females aged 16, 40 and 64 years, and three males aged 22, 38 and 55 years. Similarly, 2 infected individuals (a male and a female) were selected from the 15 infected subjects with WCAI \geq 300. Two individuals who had never lived in an endemic area were included in the study as controls.

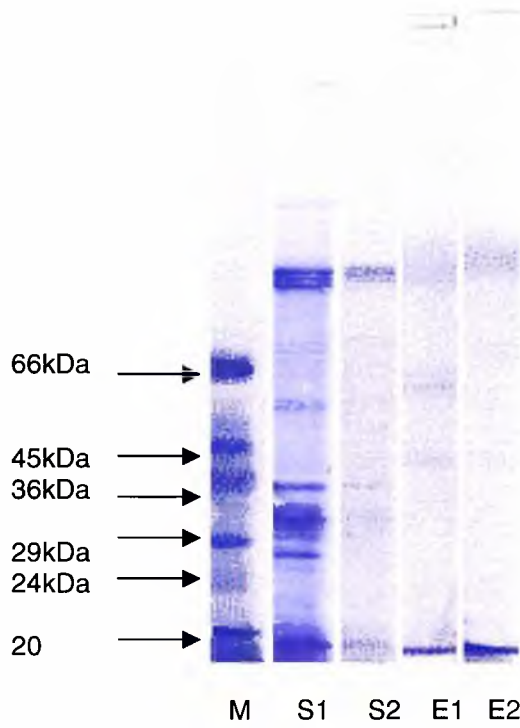


Figure 4.2.2 SDS-PAGE analysis of *S. haematobium* worm and egg antigens. Lane M is molecular weight markers, S1, S2 (adult worm antigens) and E1, E2 (egg antigens).

4.3.4 Analysis of *S. haematobium* resistant and susceptible human sera

Sera from *S. haematobium* resistant and susceptible individuals were analysed in the western immunoblot assay to reveal the reactivity of anti-schistosome IgG, IgA, IgE and IgM against crude adult worm and egg antigens.

Table 4.3.4a summarizes the reactivity of anti-schistosome IgG, IgA, IgE and IgM against adult worm antigens. As shown, two (2) bands (102 and 104) Kda were detected by both *S. haematobium* susceptible and resistant sera analyzed, as well as the normal controls. These two protein bands were revealed by all the different classes of immunoglobulins (anti-schistosome IgG, IgA, IgE and IgM) that were tested. With the exception of anti-schistosome IgG, the remaining immunoglobulin isotypes (IgA, IgE and IgM) reacted with a 32 kDa antigen band in all the sera analyzed. On the other hand, the anti-schistosome IgG detected 2 higher molecular weight antigens (112 and 110) kDa which were not bound by the other immunoglobulin isotypes. Anti-schistosome IgG detected more protein bands in the sera analysed as compared to anti-schistosome IgA (3 bands), IgE (5 bands) and IgM (5 bands). Although very clear distinct bands were obtained from anti-schistosome IgG probes (Figure 4.3.4), reactive bands from the IgA, IgE and IgM were faintly detected.

Table 4.1a Reactivity of anti- *S. heamatobium* antibody in sera against adult worm antigens

Individuals	Immunoglobulin classes			
	IgG (kDa)	IgE (kDa)	IgM (kDa)	IgA (kDa)
<i>S. heamatobium</i> resistant	112, 110, 105, 104, 102, 100, 78,68, 64, 40, 36	104, 102, 32, 29, 26	104, 102, 37, 32, 31	104, 102, 32,
<i>S. heamatobium</i> infected	112, 110, 104, 102, 90, 86, 78, 73, 60, 20.5	104, 102, 32, 29, 26	104, 102, 37, 32, 31	104, 102, 32
Normal	112, 110, 104, 102,	104, 102, 32, 29, 26	104, 102, 37, 32, 31	104, 102, 32

Anti-schistosome IgG detected a unique 100 Kda protein band in five out of six resistant sera, and this was absent when infected and/or normal subjects sera were analysed. One of the resistant subjects interestingly revealed a broad band of 36kDa, and this was missing from the others. However, there were no unique band differences between resistant and susceptible sera utilizing the anti-schistosome IgA, IgE and IgM probes against crude adult worm antigens. As revealed by the anti-schistosome IgG analysis (Figure 4.3.4), most of the resistant subjects sera detected more antigen components compared to the infected and normal subjects. However,

the number of protein bands detected by different immunoglobulin classes in resistant subjects was not significantly different from those infected individuals ($P>0.05$).

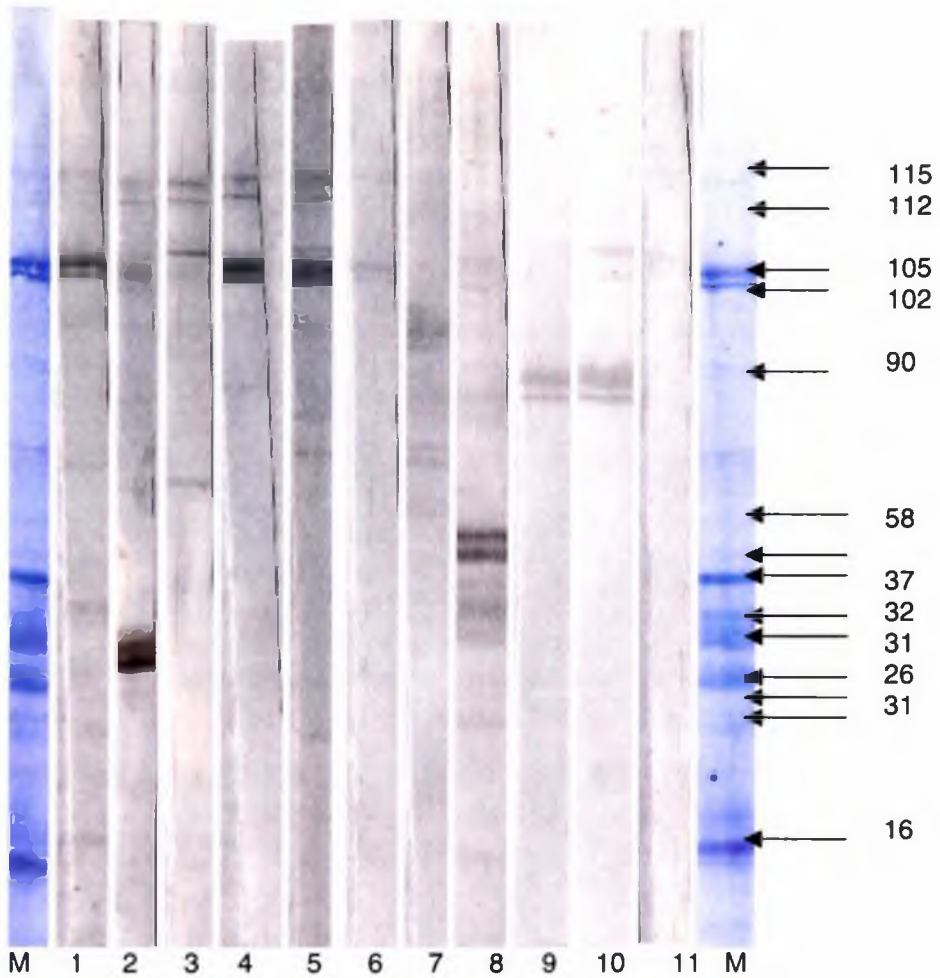


Figure 4.3.4 Reactivity of blotted *S. haematobium* adult worm antigens with anti-schistosome IgG: M, is marker; Lanes 1, 2, 3, 4, 5, and 6 are for sera from resistant individuals; lanes 7, 8, 9 are for sera from infected individuals; lanes 10 and 11 are normal control sera.

As shown in Table 4.1b, anti-schistosome IgE and IgM from sera of resistant and susceptible individuals failed to detect antigens in the egg extracts. On the other hand, anti-schistosome IgA and IgG detected one protein band each that was common to all the sera (*S. haematobium* susceptible, resistant and normal controls). These were the 42kDa band for anti-schistosome IgA and the 58kDa band for IgG. Table 4.3.4b also summarizes data on inconsistent differences in reactivity of anti-schistosome IgG among *S. haematobium* susceptible and resistant groups.

4.1b. Reactivity of anti- *S. haematobium* antibodies in sera against egg antigens, showing sizes (kDa) of bands identified.

Individuals	Immunoglobulin classes			
	IgG (kDa)	IgE (kDa)	IgM (kDa)	IgA (kDa)
<i>S. haematobium</i> resistant	58	-	-	42
<i>S. haematobium</i> infected	58	-	-	42
Normal	58	-	-	42

- means no band

4.4 SUMMARY

S. haematobium crude worm antigen extracts were found to have higher protein content (1.5mg/ml) compared to the crude soluble egg antigens (0.5mg/ml). SDS-PAGE analysis of *S. haematobium* worm and egg antigens and comparison of the polypeptide band profiles showed that the *S. haematobium* crude worm protein extract gave more than 14 polypeptide bands with molecular weight ranging from 115kDa to 16 kDa, whereas *S. haematobium* egg antigen revealed only four polypeptide bands (105 – 52). However two polypeptide bands (105 and 78) kDa were common to both *S. haematobium* worm and egg antigens.

Reactivity of anti-schistosome IgG, IgA, IgE and IgM against crude adult worm antigen extract in western immunoblot assay using sera from *S. haematobium* resistant and susceptible individual revealed two (2) common bands (102 and 104) kDa. Immunoglobulin isotypes (IgA, IgE and IgM) also reacted with a 32 kDa antigen band in all the sera analyzed. On the other hand, the anti-schistosome IgG detected 2 higher molecular weight antigens (115 and 112) Kda, which were not bound by the other immunoglobulin isotypes. Anti-schistosome IgG detected more protein bands in the sera analysed as compared to anti-schistosome, IgM (5 bands), IgE (5 bands) and IgA (3 bands). Although very clear distinct bands were obtained from anti-schistosome IgG probes reactive bands from the IgA, IgE and IgM were faintly detected.

Anti-schistosome IgG detected a unique 100 kDa protein band in five out of six resistant sera, and this was absent when infected and/or normal

subjects sera were analysed. No unique band differences were found between resistant and susceptible sera utilizing the anti-schistosome IgA, IgE and IgM probes against crude adult worm antigens. In anti-schistosome IgG analysis, most of the resistant subjects sera detected more antigen components compared to the infected and normal subjects. Anti-schistosome IgE and IgM from sera of resistant and susceptible individuals failed to detect antigens in the egg extracts. However, anti-schistosome IgA and IgG detected one protein band each that was common to all the sera (*S. haematobium* susceptible, resistant and normal controls). These were the 42kDa band for anti-schistosome IgA and the 58kDa band for IgG.

4.5 DISCUSSION

The aim of the work described in this Chapter was to analyze sera from *S. haematobium* resistant and susceptible individuals from an endemic community using the Western immunoblot assay and to identify antigens that could be useful in protection against the disease. Sera collected from individuals identified as susceptible and resistant to *S. haematobium* (Chapter 4) were employed in the analysis.

It has been reported that the major antigenic stimulation of the host immune system in schistosome infections are from the adult worms and eggs (Kelly, 1987). During a normal schistosomal infection, a complex array of antigens is presented to the host immune system. Investigations carried out by

Butterworth *et al.* (1987), Dessien *et al.* (1988), Correa-Oliveira *et al.* (1989) and Grzych *et al.* (1993), revealed that several schistosomal antigens and their antigenic preparations have putative roles in the induction of protective immune responses. These include soluble secretory/excretory antigens, and structural antigens on the surface of the different developmental stages of the parasites that occur in the host. In the present study, the approach was to compare the antibody responses of *S. haematobium* resistant and susceptible groups [with water contact activity index (WCAI) ≥ 300] to egg and worm antigens and identify unique antigens.

The multiple polypeptide bands revealed by SDS-PAGE analysis of *S. haematobium* worm and egg antigens was similar to earlier findings. Aronstein and Strand (1983), and Norden and Strand (1984) identified about 20 – 30 antigenic polypeptides of adult worms from each of the three major human schistosome species, *S. haematobium*, *S. mansoni* and *S. japonicum*.

Kelly *et al.* (1985), showed that adult worms rather than eggs stimulated the production of most antibody response directed against schistosome infections in the host. The present study observed more reactive polypeptide bands in adult worm extract compared to the egg extract. In the search for a vaccine candidate for schistosomiasis, the adult worms have been extensively investigated and certain proteins have been identified as potential vaccine candidates (Rollinson and Simpson, 1987).

The detection of two bands from the worm antigen by all the immunoglobulin isotypes of susceptible, resistant and normal control sera may suggest that those polypeptide antigens are not protective (Kelly *et al.*, 1985). During early schistosome infections the major antibodies that the

immune response stimulate are blocking antibodies (IgM, IgG2 and IgG4) which serve no protective role. Later in infection, other antibodies like IgG1, IgE and IgA, which have protective roles, are stimulated. However there is always the active interference and competition from the blocking antibodies for binding sites. This phenomenon may probably explain why reactive species of all the immunoglobulin classes were found in the three groups of study subjects. In this study, whole IgG detection system was used. Hence, the detection of 2 antigens may be due to reactivity with any of the IgG subclasses. There is the need for further studies to determine the role of immunoglobulin sub-classes and protective or blocking IgG antibodies. The multiple distinct bands revealed by anti-schistosome IgG for both the adult worm and the egg extracts compared to fewer and faint bands by anti-schistosome IgA, IgE, and IgM suggest that IgG may play a significant role during *S. haematobium* infections.

Earlier studies by Capron *et al.* (1987), suggest that IgE is the main protective antibody produced during schistosome infections. However, this study identified IgG as the major antibody reactive to worm antigenic. In earlier works, Corea-Oliveira *et al.* (1989; 2000) all showed high IgG antibody response in resistant individuals than infected people.

The detection of a unique antigen band (100kDa) using resistant individuals sera appear to suggest a protective role by this antigen and immunoglobulin. However there is the need to do further work to identify the IgG subclasses involved in the specific reactivity. Furthermore, despite the potential of the target antigen as a protective vaccine candidate, the IgG antibody alone may not confer resistant to schistosomiasis. James and

Pearce (1988), and Sher *et al.* (1990) demonstrated that there was no successful transfer of protection by antibodies alone. They demonstrated that sensitization of lymphocytes for proliferation and IFN gamma production are very crucial in protection against schistosomes. Generally, antibodies act in concert with effector and inflammatory cells such as macrophages and eosinophils to destroy schistosome parasites. Immunologically, studies of naturally acquired resistance in putative resistant individuals show the involvement of both Th1 and Th2 responses in the development of protection.

In conclusion, this study provides the basis for exploration of a 100kDa -antigen component from adult worms as a potential vaccine candidate against schistosomiasis. It also provides evidence that anti- schistosome IgG are important antibodies that may possibly contribute to protection in *S. heamatobium* resistant individuals in endemic communities.

REFERENCES

- Ahlquist, D.A., McGill, D.B., Schwartz, S., Taylor, W.F., and Owen, R.A. (1985). Faecal blood levels in health and disease. A study using HemoQuant. *New England Journal of Medicine*; **312**: 1422-1427
- Amankwa, J.A., Bloch, P., Meyer Lassen, J., Olsen, A. and Christensen, N.O. (1994). Urinary and intestinal schistosomiasis in the Tono Irrigation Scheme, Kassena/Nankana District, Upper east region, Ghana. *Tropical Medicine and Parasitology*; **45**: 319-323.
- Amanor, J. D., Bosompem, K. M., Arishima, T., Assoku, R. K. G. and Kojima, S. (1996). Characterization of monoclonal antibodies reactive with *Schistosoma haematobium* soluble egg and infected human urinary antigens, *Hybridoma*, **15**; (3); 219-224.
- Anderson, R.M. (1987). Determinants of infection in human schistosomiasis. Bailliere's Clinical Tropical Medicine and Communicable Diseases. Vol.2. No.2; 279-300.
- Arnon, R. (1990). Life span of parasite in schistosomiasis patients. *Israeli Journal of Medical Science*; **126**: 404-5.
- Aronstein, W.S. and Strand, M. (1983). Identification of species specific and gender specific proteins and glycoproteins of three human schistosomes. *Journal of Parasitology*; **69**: 1006-1017.
- Aryeetey, M.E., Wagatsuma, Y., Yeboah, G., Asante, M., Mensah, G., Nkrumah, F.K. and Kojima, S. (2000). Urinary schistosomiasis in Southern Ghana.

- I. Prevalence and morbidity assessment in three (defined) rural areas drained by the Densu river. *Parasitology International*; 49(2): 155-163
- Aryeetey, M.E, Ahorlu, C., Wagatsuma, Y., Bentil, G. Nkrumah, F.K. and Kojima, S. (1999). Health education and community participation in the control of urinary schistosomiasis in Ghana. *East Africa Medical Journal*; 76:324-329
- Badran, A., El Alfi, O., Pfischner, W.C., Killough, J.H., and Burns T.W. (1955). The value of routine rectal biopsy in the diagnosis of schistosomiasis. *American Journal of Tropical Medicine and Hygiene*; 4: 1068-1071
- Balloul, J.M., Sondermeyer, P., Dreyer, D., Capron, M., Grzych, J.-M., Pierce, R.J., Carvalho, D., Lecocq, J.P. and Capron, A. (1987a). Molecular cloning of a protective antigen of schistosomes. *Nature*, 326; 149-153.
- Basch, P.F. and Samuelson, J. (1990). Cell biology of Schistosomes. I. Ultrastructure and transformation. In: Wyler, D.J. (ed.), *Modern Parasite Biology: Cellular Immunological and Molecular Aspects*. Freeman, New York. pp 91-106.
- Basch, P.F. (1976). Parasitological review; intermediate host specificity in *Schistosoma mansoni*. *Experimental parasitology*, 39; 150-169.
- Bawden, M.P. and Weller, T.H. (1974). *Schistosoma mansoni* circulating antigen: Detection by complement fixation in sera from infected hamsters and mice. *American Journal of Tropical Medicine and Hygiene*, 16; 602-612.
- Bell, D.R. (1963). A new method for counting *Schistosoma mansoni* eggs in faeces. *Bulletin of the World Health Organization*, 29; 525 - 530.

- Berggren, W.L. and Weller, T.H. (1967). Immuno-electrophoretic demonstration of specific circulating antigen in animals infected with *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene*, **23**; 1077-1084.
- Bergquist, N.R. (1995). Controlling Schistosomiasis by vaccination: A realistic Option ?. *Parasitology today*, **11:5(119)**; 191-194.
- Bergquist, N.R. (1987). Schistosomiasis. In *WHO Report*, WHO/TDR/BERG/87.
- Bickle, Q.D., Taylor, M.G., James, E.R., Nelson, G.S., Hussein, M.F., Andrews, B.J., Dobinson, A.R. and de C. Marshall, T.F. (1979). Further observations on immunization of sheep against *Schistosoma mansoni* and *S. bovis* using irradiation-attenuated schistosomula of homologous and heterologous species. *Parasitology*, **78**; 185-193.
- Billing, P.B. (1985). Basic Procedures for the production and selection of monoclonal antibody-secreting hybridomas. In: Application of Genetic Engineering to Research on Tropical Disease Pathogens with special reference to *Plasmodia*: A laboratory manual of selected techniques. (Panyim, S., Wilairat, P. Yuthovong Y. Eds). From an international laboratory workshop sponsored by the UNDP/WORLD BANK/WHO/TDR and Mahidol University, Bangkok, Thailand.
- Bloom, B.R. (1979). Games parasites play: how parasites evade immune surveillance. *Nature*, **279**, 21-26.
- Boothroyd, J.C. and Komuniecki, R. (1995): Molecular Approach to Parasitology, MBL Lectures Volume 12, Wiley-Liss, Inc., USA, Pages 21, 301

- Boros, D.L. and Waren, K.S. (1970). Delayed hypersensitivity-type granuloma formation and dermal reaction induced and elicited by a soluble factor isolated from *Schistosoma mansoni* eggs. *Journal of Experimental Medicine*; **132**: 488-492.
- Bosompem, K.M., Otchere, J., Kojima, S. (1999). Comparison of a rapid monoclonal antibody-based dipstick and a questionnaire approach of screening for urinary schistosomiasis in school children. Presentation; 20th African Health Sciences Congress, Ghana.
- Bosompem K.M., Asigbee J., Otchere J., Abdul H., Kpo K. H., Kojima S. (1998) Accuracy of diagnosis of urinary schistosomiasis: Comparison of parasitological and a monoclonal antibody-based dipstick method *Parasitology International* 47 211-217
- Bosompem, K.M., Ayi, I., Anyan, W.K. Nkrumah, F.K. and Kojima, S. (1997). A monoclonal antibody-based dipstick assay for diagnosis of urinary schistosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*; **91**: 554-556.
- Bosompem, K.M., Arishima, T., Yamashita, T., Ayi, I., Anyan, W.K. and Kojima, S. (1996a). Extraction of *Schistosoma haematobium* antigens from infected human urine and generation of potential diagnostic monoclonal antibodies to urinary antigens. *Acta Tropica*, **62**; (2), 91-103.
- Bosompem, K.M., Assoku, R.K.G. and Nantulya, V.M. (1996b). Differentiation between culture- derived insect stages of *T. brucei*, *T. vivax*, *T.*

- congolense*, and *T. simiae* using a monoclonal antibody- based dot-ELISA. *Parasitology*, **112**: 59-66.
- Bosompem, K.M., Ayi, I., Anyan, W.K. and Kojima, S. (1996c). A new monoclonal antibody-based dipstick assay for specific diagnosis of urinary schistosomiasis. *Journal of Immunological Methods*.
- Bosompem, K.M., Ayi, I., Anyan, W.K. Nkrumah, F.K and Kojima, S. (1996d). Limited field evaluation of a rapid monoclonal antibody-based dipstick assay for urinary schistosomiasis. *Hybridoma*; 15: 443-447
- Bradley, D.J. (1965). The measurement of bilharziasis- prevalence and schistosomal egg output. *Bulletin of the Medicine and Hygiene*; **4**: 23-28.
- Brindley. P.J., Ramirez, B, Tiu, W., Wu, G., Wu, H-W and Yi, X (1995). Networking Schistosomiasis Japonica. *Parasitology today* Vol. **11** No 5 (119); 163 - 165.
- Brown, D.S. (1980). "Freshwater Snails of Africa and their Medical Importance." Taylor and Francis Ltd., London. 406pp.
- Burchard, G.D. and Kern P. (1985). Probable hybridization between *S. intercalatum* and *S. haematobium* in Western Gabon. *Tropical Geographical Medicine*; **37**: 119-123.
- Burnette, W.N. (1981). "Western blotting" electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibodies and radioiodinated protein A. *Anal Biochem.*; **112**(2):195-203.
- Butterworth, A.E., Dunne, D.W., Fulford, A., Thorne, K.J, Gachuhi, K., Ouma, J.H. and Sturrock, R.F. (1992). Human immunity to *Schistosoma*

- mansoni: observation on mechanisms and implication for control. *Immunology Invest.*; **21**: (5) 391-407.
- Butterworth, A.E., Dunne, D.W., and Fulford, A. (1988). Immunity in human *Schistosoma mansoni*: cross reactive IgM and IgG2 antibodies block the expression of immunity. *Biochimie*, **70**; 1053-1063.
- Butterworth, A.E, Bensted-Smith, R., Capron, A., *et al.*, (1987). Immunity in human schistosomiasis: Prevention by blocking antibodies of the expression of immunity in young children. *Parasitol.* **94**: 269-280
- Butterworth, A.E., Capron, M., Cordingley, J.S. Dalton, P.R., Dunne, D.W., Karinki, H.C., Kimani, G., Koech, D., Mugambi, Ouma, J.H., Prentice, M.A., Richardson, B.A., Arap-Siongok, T.K., Sturrock, R.F. and Taylor, D.W. (1985). Immunity after treatment of human schistosomiasis mansoni. II. Identification of resistant individuals and analysis of their immune responses. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **79**; 393-408.
- Butterworth, A.E., Dalton, P.R., Dunne, D.W., Mugambi, M., Ouma, J.H., Richardson, B.A., Arap-Siongok, T.K and Sturrock, R.F. (1984). Immunity after treatment of human schistosomiasis mansoni. I. Study design, pre-treatment observations and the results of treatment. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **78**; 108-123.
- Butterworth, A.E., Taylor, D.W., Veith, M. C., Vada, M.A., Dessaint, J.P., Capron, M. Joseph, M. and Topier, G. (1982). Studies of the mechanisms of immunity in human schistosomiasis. *Immunological Reviews*, **61**; 5-39.

- Butterworth, A. E., Sturrock, R. F., Houba, V., Mahmoud, A. A., Sher, A. and Rees, P. H. (1975). Eosinophils as mediators of antibody-dependent damage to schistosomula. *Nature* **256**; 727-729.
- Byram, J.E., Doenhoeff, M.J., Musallam, R., Brink, L.H. and von Lichtenberg, F. (1979). *Schistosoma mansoni* infections in a T cell deprived mouse, and the ameliorating effect of administering homologous chronic infection serum. II. Pathology. *American Journal of Tropical Medicine and Hygiene*; **28**; 274-285.
- Campbell, A.M. (1984). Antibody production and purification. In: Monoclonal antibody technology; laboratory techniques in biochemistry and molecular biology. (Burdon, R.H. and van Knippenberg, P.H. Eds), Elsevier, Oxford, Vol. **13**; 166-184.
- Cancado, J.R., Sales de Cunha, A., Garcia de Carvalho, D. and Santos Cambráia, J.N., (1965). Evaluation of the treatment of human *Schistosoma mansoni* infection by the quantitative oogram technique. *Bulletin of the World Health Organization*; **33**: 557-566.
- Capron, A., Dessaint J.P., Capron, M., Joseph, M and Topier, G. (1982). Effector mechanisms of immunity to schistosomes and their regulation. *Immunological Reviews*, **61**; 41-66.
- Capron, A., Pearce, R., Balloul, J.M., Grzych, J.M., Dissous, C., Sondermeyer, P. and Lecocq, J.P. (1987). Protective antigens in experimental schistosomiasis. *Acta Tropica*; **44**; 63-69.
- Carter, C.E. and Colley, D.G. (1978). An electrophoretic analysis of egg *Schistosoma mansoni* soluble egg antigen preparation. *Journal of Parasitology*, **64**; 385-400.

- Carter, C.E. and Colley, D.G. (1979) Partial purification and characterization of *Schistosoma mansoni* soluble egg antigens with Con-A sepharose chromatography. *Journal of Immunology*, **122**; 2204-2210.
- Chernin, E. (1970). Behavioral response of miracidia of *Schistosoma mansoni* and other trematodes to substances emitted by snails. *Journal of Parasitology*, **56**; 287-296.
- Chitsulo, L., Engels, D., Montessor, A. and Savioli, L. (2000). The global status of schistosomiasis and its control. *Acta Tropica*; **77**: 41-51.
- Chu, K.Y., Kpo, H.K. and Klumpp, R.K. (1978). Mixing of *Schistosoma haematobium* strains in Ghana. Bulletin of the WHO; **56**: 601-608.
- Clark, M. and Waldmann, M.C.H. (1986). Production of murine monoclonal antibodies. In: Methods in Hematology: Monoclonal antibodies. (Beverley, P.C.L.Ed) Churchill Livingstone, New York, Vol. **13**; 1-20.
- Clegg, J.A. and Smithers, S.R. (1972). The effect of immune rhesus monkey serum on schistosomula of *Schistosoma mansoni* during cultivation in vitro. *Int. J. Parasitol.*; **2**(1):79-98.
- Colley, D.G., and Colley, M.D. (1989). Protective Immunity and Vaccines to Schistosomiasis. *Parasitology Today*; **5**: 350-354.
- Colley, D.G. (1987). Dynamics of the human immune response to schistososomes. In: " Bailliere's Clinical Tropical Medicine and Communicable Diseases. Vol.2. No.2; 315-332.
- Colley, D.G. (1981). Immune responses and immunoregulation in experimental and clinical schistosomiasis. In: Parasitic Diseases (Mansfield, J.M. Ed), vol. **1**. pp 1-83. New York; Marcel Dekker.

- Correa-Oliveira, R., Rodrigues Caldas, I., Assis-Martins Filho, O., Carvalho Queiroz, C., Lambertucci, J.R., Renan Cunha-Meio, J., Soares Silveira, A., Prata, A., Wilson, A. and Gazzinelli, G. (2000). Analysis of the effect of treatment of human *Schistosoma mansoni* infection on the immune response of patients from endemic areas. *Acta Tropica*; **77** (1): 141-6.
- Correa-Oliveira, R., Pearce, E.J. Oliveira, G.C. Golgher, D.B., Katz, N., Bahia, L.G., Carvalho, O.S., Gazzinelli, G. and Sher, A. (1989). The human immune response to defined immunogens of *Schistosoma mansoni*: elevated antibodies levels to paramyosin in stool-negative individuals from two endemic areas in Brazil. *Transaction Royal Society Medicine Hygiene*; **83**(6): 798-804.
- Cousin, C.E., and Dorsey, C.H. (1991). Nervous System of *Schistosoma mansoni* cercariae: organization and fine structure. *Parasitology Research*; **2**: 132-41.
- Cousin, C.E, Stirewalt, M.A. and Dorsey, C.H. (1981). *Schistosoma mansoni*: ultrastructure of early transformation of skin- and shear-pressure derived schistosomules. *Experimental Parasitology*; **51**: 341-365.
- Crabtree J.E and Wilson, R.A. (1980). *Schistosoma mansoni*: a scanning electron microscope study of the developing schistosomulum. *Parasitology*; **81**: 553-564.
- da Cunha, A.S.; (1982). [Therapeutic evaluation of oxamniquine in schistosomiasis mansoni by the oogram method with biopsy of rectal mucosa]. *Rev. Inst. Med. Trop. Sao Paulo*; **24**(2): 88-94.

- Dalton, P.R., and Pole, D., (1978). Water- contact pattern in relation to *Schistosoma haematobium* infection. *Bulletin of the World Health Organization*; **56**: 417-426.
- Davern, K.M, Wright, M.D., Herrmann, V.R., and Mitchell, G.F. (1991). Further characterization of the *Schistosoma japonicum* protein Sj 23, a target antigen of an immunodiagnostic monoclonal antibody. *Molecular Biochem. Parasitol.*; **48**(1): 67-75.
- Davis, G. M. (1980). Snail hosts of Asian *Schistosoma* infecting man: evolution and co-evolution. In: "The Mekong *Schistosoma*". (Bruce, J.I., Sommani, S., Asch, H.L., and Crawford, K.A. eds). *Malacodological Review*; Suppl. **2**; 195-238.
- Davis, B.J., (1964). Disc electrophoresis. II. Method and Application to Human serum proteins. *Annals of New York Academy of Science*; **121**: 404-427.
- De Jonge, N., Fillie, Y.E., Hilberath, G.W., Krijger, F.W., Lengeler, C., De savigny, D. H., van Vliet, N. G., and Deelder, A.M., (1989). Presence of the schistosome circulating anodic antigen (CAA) in urine of patients with *Schistosoma mansoni* or *S. haematobium* infections. *American Journal of Tropical Medicine and Hygiene*, **41**; 563-569.
- Dean, D.A., Mangold, B.L., Georgi, J.R., Jacobson, R.H. (1984). Comparison *Schistosoma mansoni* migration pattern in normal irradiated cercaria immunized mice by means of autoradiographic analysis. Evidence that worm elimination occurs after skin phase in immunized mice. *Am J. Trop. Med Hyg.*; **33**(1):89-96
- Dean, D.A. (1983). *Schistosoma* and related genera: acquired resistance in mice. *Experimental Parasitology*, **55**; 1-104.

- Dean, D.A., Bukwoski, M.A., Cheever, A.W. (1981). Relationship between acquired resistance, portal hypertension and lung granulomas in ten strains of mice infected with *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene*, **30**; 806-814.
- Deelder, A.M., Miller, R.L., de Jonge, M. and Krijger, F.W. (1990). A new technique in diagnosis of an orient disease: assaying schistosome antigen in mummy tissue. *The Lancet*, **i**: 31-81.
- Deelder, A.M., DeJonge, N., Fillie, Y.E., Kornelis, D., Helelia, D., Qian, Z., DeCluwe, P. and Polderman, A.M. (1989). Quantitative determination of circulating antigens in human schistosomiasis *mansoni* using an indirect haemagglutination assay. *American Journal of Tropical Medicine and Hygiene*; **40**: 50-54.
- Deelder, A.M., Kornelis, D., Marck, E.A.L., Van, Eveliegh, P.C. and Egmond, J.G. (1980). *Schistosoma mansoni*: Characterization of two circulating polysaccharide antigens and the immunological response to these antigens in mouse, hamsters and human infections. *Experimental Parasitology*, **50**: 16-22.
- Deelder, A.M., Klappe, H.J.M., Van den Aardweg, G.J.M.J. and Van Meerbeke, E.H.E.M. (1976). *Schistosoma mansoni*: demonstration of two circulating antigens in infected hamsters. *Experimental Parasitology*, **40**; 189-197.
- Derban, L.K.A. (1984). Health impact of the Kpong dam in Ghana. In: "Water Power and Dam Construction. Volta River Authority Report pp 13-15.
- Dessien, A.J., Begley, M., Demeure, C., Caillol, D., Feuri, J., dos Reis, M.G., Andrade, Z.A., Prata, A., Bina, J.C. (1988). Human resistance to

- Schistosoma mansoni* is associated with IgG reactivity to a 37-kDa larval surface antigen. *J. Immunol.* **27**(3): 244-246
- Dissous, C., Balloul, J.M., Pierce, R and Capron, A. (1987). Protective antigens of schistosomes. In; "Bailliere's Clinical Tropical Medicine and Communicable Diseases." Vol.2. No.2; 267-278.
- Dissous, C., Grzych, J.M. and Capron, A. (1986). *Schistosoma mansoni* shares a protective oligosaccharide epitope with freshwater and marine snails. *Nature*, **323**; 443-445.
- Dissous, C., and Capron, A. (1983). *Schistosoma mansoni*: antigenic community between schistosomula surface and adult worm incubation products as a support for concomitant immunity. *FEBS Letters*, **162**; 355-359.
- Dissous, C. and Capron, A. (1982). Isolation of surface antigens from *Schistosoma mansoni* schistosomula. In: "Protides of the Biological Fluids", (Peeters, H. Ed), pp 179-182. *Oxford: Pergamon Press*.
- Dissous, C., Dissous, C. and Capron, A. (1981). Isolation and characterization of surface antigens from *Schistosoma mansoni* schistosomula. *Molecular and Biochemical Parasitology*, **3**; 215-225.
- Dissous, C., Grzych, J.M. and Capron, A. (1982). *Schistosoma mansoni* surface antigens defined by a rat monoclonal IgG_{2a}. *Journal of Immunology*, **129**; 2232-2234.
- Doehring, E., Vester, U., Ehrich, J.H.H. and Feldmeier, H. (1985a). Circadian variation of ova excretion, proteinuria haematuria and leukocyturia in urinary schistosomiasis. *Kidney International*; **27**:667-71.

- Doenhoff, M., Musallam, R., Bain, J. and McGregor, A. (1979). *Schistosoma mansoni* infections in a T-cell deprived mice and the ameliorating effect of administering homologous chronic infection serum. I. Pathogenesis. *American Journal of Tropical Medicine and Hygiene*, **28**; 260-273.
- Doumenge, J.P., Mott, K.E., Cheng, C., Villenave, D., Chappuis, O., Perrin, M.F., Reaud-Thomas, G. (1987). Atlas of the global distribution of schistosomiasis. Talence, CEDGET-CNRS; Geneva, OMS/WHO.
- Dunne, D.W. and Doenhoff, M.J. (1983). *Schistosoma mansoni* egg antigens and hepatocyte damage in defective T-cell deprived mice. *Contributions to Microbiology and Immunology*, **7**; 22-29.
- Dunne, D.W., Lucas, S., Bickle, Q., Pearson, S., Madgwick, L., Bain, J. and Doenhoff, M.-J. (1981). Identification and partial purification of an antigen (omega-) from *Schistosoma mansoni* eggs which is putatively hepatotoxic in T-cell deprived mice. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **75**; 54-71.
- Eklun-Natey, D.T., Wuest, J., Swiderski, Z., Striebel, H.P. and Huggel, H. (1985). Comparative scanning electron microscope (SEM) study of miracidia of four human schistosome species. *International Journal of Parasitology*, **15**: 33-42.
- Erasmus, D.A. (1987). The Adult Schistosome: Structure and Reproductive Biology. In: *The Biology of Schistosomes: From Genes to Laterines*. (Rollinson D. and Simpson, A.J.G. eds.) Academic Press Ltd. pp 51-82.
- Faust, E.C. and Hoffman, W.A. (1934). Studies on schistosomiasis mansoni in Puerto Rico. III. Biological studies I. The extramammalian phases of the

- life-cycle. *The Puerto Rica Journal of Public Health Tropical Medicine*; **10**: 1-47.
- Feldmeier, H., Nogueira-Queiroz, J.A., Peixoto-Queiroz, M A., Doehring, E., Dessaint, J.P., de Alencar, J.E., Daffalla, A.A. and Capron, A. (1986). Detection and quantification of circulating antigen in schistosomiasis by monoclonal antibody. II. The quantification of circulating antigens in human schistosomiasis mansoni and haematobium: relationship to intensity of infection and disease status. *Clinical and Experimental Immunology*, **65**; 232-243.
- Feldmeier, H., Poggensee, G., and Kruntz, I (1993). A synoptic inventory of needs for research on women and tropical parasitic diseases. II Gender-related biases in the diagnosis and morbidity assessment of schistosomiasis in women. *Acta Tropica*; **55**: (3) 139-169.
- Ford, M.J., Bickle, Q.D, Taylor, M.G. and Andrew, B.J. (1984). Passive transfer of resistance and the site of immune-dependent elimination of the challenge infection in rats vaccinated with highly irradiated cercariae of *Schistosoma mansoni*. *Parasitology*; **89** (Pt 3): 461-482.
- Frandsen, F. (1979). Studies of the relationships between *Schistosoma* and their intermediate hosts.I. The genus *Bulinus* and *Schistosoma haematobium* from Egypt. *Journal of Helminthology*, **53**; 15-29.
- Furu, P. (1987). Ghana: Atlas of the global distribution of schistosomiasis. CETGET-CNRS/OMS-WHO; **15**; 115-122.
- Garcia, L.S. and Shimuzu, A. (1981). Comparison of clinical results for the use of ethyl acetate and diethyl ether in the formalin-ether

- sedimentation technique performed on polyvinyl alcohol preserved specimens. *Journal of Clinical Microbiology*, **13**; 709 - 713.
- Gazzinelli, G., Lambertucci, J.R, Katz, N., Rocha, R.S., Lima, M.S. and Colley, D.G. (1985). Immune responses during human schistosomiasis mansoni. XI. Immunologic status of patients with acute infections and after treatment. *Journal of Immunology*, **135**; 2121-2127.
- Goding, J. W. (1986). Monoclonal antibodies: Principles and practice. In; Production and Application of Monoclonal Antibodies in cell Biology, Biochemistry and Immunology. 2nd edition. Academic Press, London; pp 1-33.
- Gold, R., Rosen, S. and Weller, T.H. (1969). A specific circulating antigen in hamsters infected with *Schistosoma mansoni*: detection of antigen in serum and urine and correlation between antigenic concentration and worm burden. *American Journal of Tropical Medicine and Hygiene*, **18**; 545-550.
- Goldring, O.L, Kusel, J.R. and Smithers, S.R. (1977). *Schistosoma mansoni*: origin in vitro of host-like surface antigens. *Experimental Parasitology*; **43** (1): 82-93.
- Goldsmith, R.S. (1985). Infectious disease: Helminth. In: Current Medical Diagnosis and Treatment. (Krupp, M.J., Werdegar, D., Eds.). Los Altos: Lauge, pp918.
- Gounni, A.S., Lankhioued, B., Ochiai, K., Takaka, Y., Delaporte, E., Capron, A. Kinet, J.P. and Capron, M. (1994). High affinity IgE receptor on eosinophils is involved in defined against parasite. *Nature*; **367** (6459): 183-186.

- Gryseels, B. (1989). The relevance of schistosomiasis for public health. *Tropical Medicine and Parasitology*, **40**: 134-142
- Grzych, J.M., Grezel, D., Xu, B.C., *et al.*, (1993). IgA antibodies to the protective antigens in human Schistosomiasis mansoni. *Journal of Immunology*, **150**: 527-535.
- Grzych, J.-M., Capron, M., Lambert, P.H., Dissous, C., Torres, S., and Capron, A. (1985). An anti-idiotypic vaccine against experimental schistosomiasis. *Journal of Immunology*, **133**: 998-1004.
- Grzych, J.-M., Capron, M., Dissous, C., and Capron A. (1984). Blocking activity of rat monoclonal antibodies in experimental schistosomiasis. *Journal of Immunology*, **129**: 2739-2743.
- Grzych, J.M., Capron, M., Bazin, H. and Capron, A. (1982). In vitro and in vivo effector function of rat IgG_{2a} monoclonal anti-*S. mansoni* antibodies. *Journal of Immunology*, **129**; 2739-2743.
- Hackett, F., Simpson, A.J.G., Knight, M., Ali, P., Payares, G., Smithers, S.R. (1986). *Schistosoma mansoni*: antigen preparations which induce antibodies to schistosomula surface antigens *Experimental Parasitology*, **40**; 294-303.
- Hagan, P., Wilkins, H.A., Blumenthal, U.J., Hayes, R.J. and Greenwood, B.M. (1985b). Eosinophilia and resistance to *Schistosoma haematobium* in man. *Parasite Immunology* **7**, 617-624
- Hagan, P., Blumenthal, U.J., Dunn, D., Simpson, A.J.G. and Wilkins, H.A. (1991). Human IgE, IgG4 and resistance to reinfection with *Schistosoma haematobium*. *Nature* **349**; 243-245.

- Hamburger, J., Lustigman, S., Arap siongok, T.K., Ouma, J.H. and Mahmoud, A.A.F. (1982). Characterization of a purified glycoprotein from *S. mansoni* eggs: specificity, stability and the involvement of carbohydrate and peptide moieties in its serological activity. *Journal of Immunology*, **128**; 1864-1869.
- Hamburger, J., Pelley, R.P. and Warren K.S. (1976). *Schistosoma mansoni* soluble egg antigens: determination of the stage and species specificity of their serological reactivity by radioimmunoassay. *Journal of Immunology*, **177**; 1561-1570.
- Hang, L.E., Warren, K.S. and Boros, D.L. (1974). *Schistosoma mansoni*: antigenic secretions and the etiology of egg granuloma in mice. *Experimental Parasitology*, **35**; 288-294.
- Harn, D.A., Mistuyama, M., Huguenel, E.D. and David, J.R. (1985a). *Schistosoma mansoni*: detection by monoclonal antibody of a 22,000 dalton surface membrane antigen which may be blocked by host molecules on parasites. *Journal of Immunology*, **135**; 2115-2120.
- Harn, D.A., Mistuyama, M., Huguenel, E.D. Oigino, L. and David, J.R. (1985b). Identification by monoclonal antibody of a major (28kDa) surface membrane antigen of *Schistoosma mansoni*. *Molecular and Biochemical Parasitology*; **16**: 345-354.
- Harn, D.A., Mistuyama, M., and David, J.R. (1984). *Schistosoma mansoni*: anti-egg monoclonal antibodies protect against cercarial challenge in vivo. *Journal of Experimental Medicine*; **159**: 1371-1387
- Harris, A.R., Russell, R.J., and Charters, A.D. (1984). A review of Schistosomiasis in immigrants in Western Australia, demonstrating the

- unusual longevity of *Schistosoma mansoni*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*; **78**: 385-388
- Harrison, R.A., Bickle, Q., Doenhoeff, M.J. (1982). Factors affecting the acquisition of resistance against *Schistosoma mansoni* which mediate resistance during early patent infections may lack immunological specificity. *Parasitology*; **84**; 93-110.
- Hiatt, R.A., Sotomayor, Z.R., Sanchez, G., Zombrana, M. and Knight, W.B. (1979). Factors in the pathogenesis of acute schistosomiasis mansoni. *Journal of Infectious Diseases*, **139**; 659-666.
- Hoffman, K.F. and Strands, M. (1996). Molecular Identification of a *Schistosoma mansoni* Tegumental Protein with Similarity to Cytoplasmic Dynein Light Chains. *The Journal of Biological Chemistry*, **271** (42): 26117-26123
- Hoffman, D.B., Lehman, J.S., Stott, V.C., Warren, K.S. and Webbe, G. (1979). Control of Schistosomiasis: report of a workshop. *American Journal of Tropical Medicine and Hygiene*; **29**; 586.
- Inatomi, S. (1962). Submicroscopic structures of the egg shell of helminths. *Okayama Medical Journal*; **74**; 31-81.
- Iskander, R., Das, P.K. and Aalberse, R.C. (1981). Ig4 antibodies in Egyptian patients with schistosomiasis. *International Arch. Allergy Immunology*, **66**(2):200-207.
- James, S.L., Cheever, A.W. (1985). Comparison of immune responses between high and low responder strains of mice in the concomitant immunity and vaccine models of resistance of *Schistosoma mansoni*. *Parasitology*; **91**; 301-315.

- James, S.L., Pearce, E.J. (1988). The influence of adjuvant on protective immunity by a non living vaccine against schistosomiasis. *J. Immunol.*; 140(8): 2753-2759
- Jobin, W.R. (1979). Cost of snail control. *American Journal of Tropical Medicine and Hygiene*; **28**: 142-154.
- Jordan, P and Webbe, R.F., (1989) Control in Human Schistosomiasis (eds P Jordan, G Webbe & RF Sturrock) CAB International, Wallingford, UK
- Jourdane, J. and Theron, A. (1987). Larval Development: Eggs to Cercariae. In: The Biology of Schistosomes; from genes to laterines (Rollinson, D. and Simpson, A.J.G Eds) Academic Press, Harcourt Bruce, pp. 83-106.
- Kagan, I.G. (1968). A critical review of immunological bilharziasis. *Bulletin of New York Academy of Medicine*; **25**: 611
- Kamiya, H., Smithers, S.R., McLaren, D.J. (1987). Schistosoma mansoni: autoradiographic tracking studies of isotopically labeled challenge parasite in naïve and vaccinated CBA/Ca mice. *Parasite Immunology*; **4**:515-529.
- Kato, K. (1960). A correct application of the thick-smear technique with cellophane paper cover. A Pamphlet, pp 1-9 (in Japanese).
- Kato, T. and Miura, M. (1954). On the comparison of some stool examination methods. *Japanese Journal of Parasitology*, **3**; 35-42.
- Katz, N., Chaves, A. and Pelligrino, J. (1972). A simple device for quantitative stool thick-smear technique in schistosomiasis mansoni. *Revista do Instituto de Medicina Tropical de Sao Paulo*, **14**; 397-400.
- Kelly, C. (1987). Molecular studies of schistosome immunity. "In The Biology of Schistosomes; from genes to laterines (Rollinson, D. and Simpson,

- A.J.G. Eds.) Academic Press , Harcourt Bruce Javanovich, Publishers, London.
- Kelly, C., Hagan, P., Knight, M., Hodgson, J., Sampson, A.J., Hackett, F., Wilkins, H.A., Smithers, S.R. (1987). Surface and Species specific antigens, *Shistosoma haematobium*. *Division of Parasitology, National Institute for Medical Research, Mill Hill, London. (Pt 2):* 253-266.
- Kelly, C., Payares, G., Simpson, A.J., Smithers, S.R. (1985). *Schistosoma mansoni*: complexity of antigenic surface. *Exp. Parasitol.*; 60(3): 304-308
- Khalife, J., Capron, M., Capron, A., Grzych, J., Butterworth, A.E., Dunne, D.W. and Ouma, J.H. (1986). Immunity in human schistosomiasis mansoni. Regulation of protective immune mechanisms by IgM blocking antibodies. *Journal of Experimental Medicine*, **164**; 1626-1640.
- Khalil, S.B. and Mansour, N.B. (1990). In vivo and in-vitro studies on the development of *S. haematobium* and *S. mansoni* worm under various experimental conditions. *Abstract presented at the 39th annual meeting of the American Society of Tropical Medicine and Hygiene, New Orleans.*
- Kloos, H., Higashi, G., Schniski, V.D., Mansour, N.S., Murrell, K.D. and Miller, F.D. (1990). Water contact and *Schistosoma haematobium* infection: a case study from an upper Egyptian village. *International Journal of Epidemiology*, **19**(3): 749-758.
- Knopf, D.M, Cioli, D., Mangold, B.L. and Dean, D.A. (1986). Migration of *Schistosoma mansoni* in normal and passively immunized laboratory rats. *American Journal of Tropical Medicine and Hygiene*; **35** (6): 1173-1184.

- Komiya, Y. and Kobayashi, A. (1966). Evaluation of Kato's thick smear technique with cellophane cover for helminth eggs in faeces. *Japanese Journal of Medical Science and Biology*, **19**; 59-64.
- Kremsner, P.G., Enyong, P., Krijger, F.W., De Jonge, N., Zotter, G.M., Thalhammer, F., Muhlschlegel, F., Bienzle, U., Feldmeier, H., and Deelder, A.M., (1994). Circulating anodic and cathodic antigen in serum and urine from *Schistosoma haematobium* infected Cameroonian children receiving praziquantel: A longitudinal study. *Clinical Infectious Diseases*, **18**; 408-413.
- Kruatrachue, M., Upatham, E.S., Kunatham, L. (1984). Effect of cercarial concentration and length of exposure on the infection of mice by *Schistosoma japonicum* and *Schistosoma mekongi* cercariae. *Southeast Asian J. Trop. Med. Public Health*; **15**(2): 254-260
- Kuntz, R.E. (1955). Biology of the schistosome complexes. *American Journal of Tropical Medicine*; **32**; 784-824.
- Kusel, J.R. (1970). Studies on the structure and hatching of the eggs of *Schistosoma mansoni*. *Parasitology*; **60**; 79-88.
- Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, **227**; 680-685.
- Lanar, D.E., Pearce, E.J. James, S.L and Sher, A. (1986). Identification of paramyosin as schistosome antigen recognized by interdermally vaccinated mice. *Science*, **234**; 593-596.
- Le Roux, P.L. (1954). A new mammalian schistosome (*S. leiperi* sp. nov.) from herbivora in Northern Rhodesia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*; **49**: 293-294.

- Liese, B. (1986). The organization of schistosomiasis control programmes. *Parasitology Today*, **2**: 339-345.
- Lopes, J.D. and Alves, M.J.A. (1984). Production of monoclonal antibodies by Somatic Cell Hybridization. In: Genes and Antigens of Parasites. Proceedings of a course sponsored by UNDP/WORLD BANK/WHO (Moore, C.M. Ed) pp 419-433. Fundacao Oswaldo Cruz-Rio de Janeiro, Brazil.
- Lunde, M.N., Ottesen, E.A., and Cheever, A.W. (1979). Serological differences between acute and chronic schistosomiasis mansoni detected by enzyme-linked immunosorbent assay (ELISA). *American Journal of Tropical Medicine and Hygiene*, **28**; 87-91.
- Mangold, B.L. and Dean, D.A. (1986). Passive transfer with serum and IgG antibodies of irradiated cercaria-induced resistance against *Schistosoma mansoni* in mice. *Journal of Immunology*, **136**; 2644-2648.
- Mangold, B.L. and Knopf, P.M. (1981). Host protective immune responses to *Schistosoma mansoni* in the rat. Kinetics of hyperimmune serum-dependent sensitivity and elimination of schistosomes in a passive transfer system. *Parasitology*; **83**: 559-574.
- Manson P.E.C. (1989). Schistosomiasis. In: Trematode Infection. Manson's Tropical Diseases (Manson, P.E.C. and Bell, E. eds.) Bailliere Tindall, London pp 448-485.
- Manson-Bahr P. E. C. and Bell O. R. (1991): Manson's Tropical Diseases, Ninth Edition, Bath Press Avon, Great Britain. Pages (449-558).

- Mao, S.P. and Shao, B.R. (1982). Schistosomiasis control in the Peoples Republic of China. *American Journal of Tropical Medicine and Hygiene*, **31**; 92-99.
- Martin, L.K. and Beaver, P.C. (1968). Evaluation of Kato thick smear technique for quantitative diagnosis of helminth infections. *American Journal of Tropical Medicine and Hygiene*, **17**; 382-391.
- McCullough, F.S. and Ali, Y.M. (1965). The distribution and prevalence of *Schistosoma haematobium* and *S. mansoni* in Ghana. *Ghana Medical Journal*; **4**: 83-84.
- McCullough, F.S. (1957) The distribution of human schistosomiasis and the potential snail host in Ghana. *West African Medical Journal*, **VI**; 87-97.
- McLaren D.J. and Smithers S.R. (1987). The immune response to Schistosomes in Experimental Hosts. "In The biology of schistosomes; from genes to laterines (Rollinson D. and Simpson A.D.J. Eds.) Academic Press, Harcourt Bruce Jovanovich, Publishers, London.
- McLaren, M., Draper, C.C., Roberts J.M., Minter-Goedbloed, E., Lighthart, G.S., Teesdale, C.H., Amin, M.A., Omer, A.H.S., Bartlet, A. and Voller, A. (1987). Studies on the enzyme-linked immunosorbent assay (ELISA) test for *Schistosoma mansoni* infections. *Annals of Tropical Medicine and Parasitology*, **72**: 243-249.
- McLaren D.J: and Smithers, S.R. (1985). *S. mansoni*. Challenge attrition during the lung phase of migration in vaccinated and serum-protected rats. *Experimental Parasitology*, **60**: 1-9.
- McLaren, D.J., Pearce, E.J. and Smithers, S.R. (1985). Site potential for challenge attrition in mice, rats and guinea pigs vaccinated with

- irradiated cercariae of *Schistosoma mansoni*. *Parasite Immunology*, **7**; 29-44.
- McLaren, D.J. (1985). Parasite defence mechanism. In “ *Inflammation: Basic Mechanism, Tissue Injuring Principles and Clinical Models*’ (Venge, P. and Lindbom, A., eds), pp. 219-254. Almquist and Wiksell, Sweden.
- McLaren, D.J. (1982). Role of granulocytes in immune defense against parasites. *Zentralblatt fur Bacteriologie und Parasitenkunde*; **12**: 57-73.
- McLaren D.J and Incani, R.N. (1982). *Schistosoma mansoni*: acquired resistance of developing schistosomula to immune attack in vitro. *Experimental Parasitology*, **53** (2): 215-298.
- McLaren, D.J. (1980). *Schistosoma mansoni*: the parasite surface in relation to host immunity. In “ *Tropical Medicine Research Studies*’, Vol. 1 (Brown, K.N., ed.), pp. 1-229. John Wiley, Chichester.
- McLaren D.J and Ramalho-Pinto, F.J. (1979). Eosinophil-mediated killing of schistosomula of *Schistosoma mansoni* in vitro: Synergistic effect of antibody and complement. *Journal of immunology*, **4**: 1431-1438.
- McLaren, M.L., Long, E.G., Goodgame, R.W. and Lilleywhite, J.E. (1979). Application of the enzyme-linked immunosorbent assay (ELISA) for the serodiagnosis of *Schistosoma mansoni* infections in St. Lucia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **73**; 636-642.
- McLaren, D.J. and Hockley, D.J. (1977). Blood flukes have a double outer membrane. *Nature (London)*; **265**: 147-149.

- McLaren, D.J. Clegg J.A. and Smithers, S.R. (1975). Acquisition of host antigens by young *S. mansoni* in mice: correlation with failure to bind antibody in vitro. *Parasitology*, **70** (1): 67-75.
- McMahon-Pratt, D., Bennet, E. and David, J.R. (1982). Monoclonal antibodies that distinguish subspecies of *Leishmania braziliensis*. *Journal of Immunology*, **129**; 926-927.
- Michelson, E.A. and Dubois, L. (1978). Susceptibility of Bahian populations of *Biomphalaria glabrata* to an allopatric strain of *Schistosoma mansoni*. *American Journal of tropical Medicine and Hygiene*; **27**: 782-787.
- Mitchell, G.F. (1984). Monoclonal antibodies as parasite-typing reagents. In: New approaches to the identification of parasites and their vectors. (Newton, B.N. and Michal, F. eds). Tropical Diseases Research Series 5. Schwabe and Co., A.G., Basel; 95-105.
- Moloney, N.A. and Webbe, G. (1987). The duration of species-specific immunity against *Schistosoma japonicum* by exposure of rats to ultra-violet attenuated cercariae. *Parasitology*, **94**; 149-154.
- Moloney, N.A. and Webbe, G. (1983). The host-parasite relationship of *S. japonicum* in CBA mice. *Parasitology*; **87**: 327-42.
- Mott, K.E. (1987). Epidemiological considerations for the development of a schistosome vaccine. *Acta Tropica*, **12**; 13-20.
- Mott, K.E. (1982). A reusable polyamide filter for diagnosis of *Schistosoma haematobium* infection by urine filtration. *Bulletin de la Société de Pathologie Exotique et de ses Filiales*; **76**: 101-104.
- Nantulya, V.M., Musoke, A.J., Rurangirwa, F.R. and Moloo, S.K. (1984). Resistance of cattle to tsetse-transmitted challenge with *T. brucei* and

- T. congolense after spontaneous recovery from syringe passage infections. *Infection and Immunity*, **43** (2): 735-738.
- Nantulya, V.M., Musoke, A.J., Rurangirwa, F.R., Saigar, N., and Minja, S.H. (1987). Monoclonal antibodies that distinguish *Trypanosoma congolense*, *T. vivax*, and *T. brucei*. *Parasite Immunology*, **9**: 421-431.
- Nash, T.E., Garcia-Coyco, C., Ruis-Tiben, E., Nazaro-Lopez, H.A., Vazquez, G. and Torres-Borges, A. (1983). Differentiation of acute and chronic schistosomiasis by antibody responses to specific schistosome antigens. *American Journal of Tropical Medicine and Hygiene*, **32**; 776-784.
- Nash, T.E. (1974). Localization of the circulating antigen within the gut of *Schistosoma mansoni*. *American Journal of Tropical Medicine and Hygiene*; **23**: 1085-1087.
- Nojima, H., Santos, A.T., Blas, B.L. and Kamiya, H. (1980). The emergence of *S. japonicum* cercariae from *Oncomelania quadrasi*. *Journal of Parasitology*, **66**: 1010-13
- Norden, A.P. and Strand, M. (1984). *Schistosoma mansoni*, *S. haematobium* and *S. japonicum*: identification of genus-, species- and gender-specific antigenic worm glycoproteins. *Experimental Parasitology*, **57**: 110-123.
- Nyame, A.K., Kuwar, L.S., Cummings, R.D., (2004). Antigenic glycans in parasitic infection: implication for vaccines and diagnostics. *Arch. Biochem. Biophys.*; **426**(2): 182-200
- Nyame, A.K., Lewis, F.A., Doughty, B.L., Correa-Oliveira, R., Cummings, R.D., (2003). Immunity to schistosomiasis: glycans are potential antigenic targets for immune intervention.; *Exp. Parasitol.* **104** (1-2): 1-13

- Odei, M.A.(1975). Distribution and dynamics of population of snails transmitting schistosomiasis in Ghana. *Brasilia Medica*; **11**; 15-26.
- Okoh, V.O. (1994). Case study of the Volta Lake. The Health Aspect. Report by Lakeside Health Unit, Volta River Authority. pp 1-8.
- Olveda, R.M. and Domingo, E.O. (1987). Schistosomiasis japonicum. Bailliere's Clinical Tropical Medicine and Communicable Diseases. **2:2**; 397-418.
- Omer-Ali, P., Magee, A.I., Kelly C. and Simpson A.J.G. (1986). A major role for carbohydrate epitopes preferentially recognized by chronically infected mice in the determination of *Schistosoma mansoni* schistosomulum surface antigenicity. *Journal of Immunology*, **137**; 3601-3607.
- Onori, E., McCullough, F.S. and Rosei, L. (1963). Schistosomiasis in the Volta region of Ghana. *Annals of Tropical Medicine and Parasitology*, **57**:59-70
- Omstein, L. (1964). Disc Electrophoresis. I. Background and Theory. *Annals of New York Academy of Science*; **121**: 321-349.
- Ouchterlony, O. (1976). Immunodiffusion and immunoelectrophoresis. In: Handbook of experimental immunology (Wier, D. M. Ed). Blackwell Scientific Publications, Oxford, pp 655 - 706.
- Owhashi, M. and Ishii, A. (1982). Purification and characterization of a high molecular weight eosinophil chemotactic factor from *Schistosoma japonicum*. *Journal of Immunology* **129**; 2226-2231.
- Pan, C.T. (1965). Studies on the host-parasite relationship between *Schistosoma mansoni* and the snail *Australorbis glabrata*. *American Journal of Tropical Medicine and Hygiene*, **14**; 931-976.
- Pao, T.C. (1959). Description of a new schistosome in Szechuan Province. *Chinese Medical Journal, Peking*, **78**; 278.

- Paperna, I. (1969). Studies on the transmission of schistosomiasis in Ghana: V. Transmission of *Schistosoma haematobium* in the forest and savannah of south east Ghana. *Ghana Medical Journal*; **8**: 35-46.
- Pearce, E.J., James, S.H., Hieny, S., Lanar, D.E. and Sher, A. (1988). Induction of protective immunity against *Schistosoma mansoni* by vaccination with schistosome paramyosin (Sm 97), a non surface antigen. *Proceedings of the National Academy of Sciences U.S.A.*; **85**: 5768-5782
- Pelley, R.P., Pelley, R.J., Hamburger, J., Peters, K. and Warren, K.S. (1976). *Schistosoma mansoni* soluble egg antigens and the employment of radioimmunoassay for their further characterization. *Journal of Immunity*, **177**; 1553-1560.
- Peresan, G. and Cioli, D. (1980). Resistance to cercarial challenge upon transfer of *Schistosoma mansoni* into mice. *American Journal of Tropical Medicine and Hygiene*, **29**; 1258-1262.
- Pesigan, T.P., Hairston, N.G., Jauregui, J.J., Garcia, E.G., Santos, A.T., Santos, B.C. and Besa, A.A. (1958). Studies on *S. japonicum* infection in the Phillipines. 2. The molluscan host. *Bulletin of the World Health Organization*; **18**: 481-578.
- Peters, P.A. (1976) Rapid accurate quantification of schistosome eggs via nuclepore filters. *Journal of Parasitology*, **62**; 154-155.
- Peters, P.A., Warren, K.S. and Mahmoud, A.A.F. (1976). Field studies of a rapid, accurate means of quantifying *Schistosoma haematobium* eggs in urine samples. *Bulletin of the World Health Organization*, **54**; 159 - 162.

- Peters, P.A.S. and Kazura, J.W. (1987). Update on diagnostic methods for schistosomiasis. *Bailliere's Clinical Tropical Medicine and Communicable Diseases*; **2**(2): 419-434.
- Pitchford, R.J. (1977). A check list of definitive hosts exhibiting evidence of the genus *Schistosoma* Weinland, 1858 acquired naturally in Africa and the Middle East. *Journal of Helminthology*; **51**: 229-252.
- Pitchford, R.J. and Viser, P.S. (1981). *Schistosoma* from *Hippotamus amphibius* in Krugger National Park. *Ondersteproet Journal of Veterinary Research*; **49**: 33-235.
- Polman, K., De Vlas, S.J., Van Lieshout, L., Deelder, A.M., Gryseels, B., (2001). Evaluation of density-dependent fecundity in human *Schistosoma mansoni* infections by relating egg counts to circulating antigens through Deming regression. *Parasitology*; **122**(Pt 2): 161-167.
- Proietti, F.A. and Antunes, C.M.F. (1989). Sensitivity, specificity and positive predictive value of selected clinical signs and symptoms associated with schistosomiasis mansoni. *International Journal of Epidemiology*; **18**: 680-683.
- Pugh, R.N.H., Bell, D.R. and Gilles, H.M. (1980). The potential medical importance of bilharzias in Northern Nigeria: a suggested rapid, cheap and effective solution for control of *Schistosoma haematobium* infection. *Annals of Tropical Medicine and Parasitology*; **74**: 597-613.
- Ramasay, R. (1979). Surface proteins on schistosomula and cercariae of *Schistosoma mansoni*. *International Journal of Parasitology*; **9**:491-493.

- Ratard, R.C. and Greer, G.J. (1991). A new focus of *S. haematobium* *S.intercalatum* hybrid in Cameroun. *American Journal of Tropical Medicine and Hygiene*; **45**:332-8
- Reiner, J., Carter, R., Rosenberg, Y. and Miller, L.H. (1980). Anti-gamete monoclonal antibodies synergistically block transmission of Malaria by preventing fertilization in the mosquito. *Proceedings of the National Academy of Sciences (USA)*, **77**; 6797.
- Rogers, M.V. (1986). *Schistosoma mansoni*: Studies of Antigen Recognition and Immune Expression in Chronically Infected and Vaccinated Guinea Pigs. *Ph.D. Thesis, Brunel University, Uxbridge, Middlesex, U.K.*
- Rollinson, D. and Simpson, A.J.G. (1987). The Biology of Schistosomes; from genes to latrines. 1-50. Academic Press. London.
- Rollinson, D. and Southgate, V.R. (1987). The genus *Schistosoma*; A Taxonomic Appraisal. In: "The Biology of Schistosomes; from genes to latrines. (Rollinson, D. and Simpson, A.J.G. Eds.) 1-50. Academic Press. London."
- Ruiz Peres, A.J., Ramos Garcia, A., Pelaez Castro, K. and Garcia Humaran, G. (1990). Estudio clinico, laparoscopico e histologico en 60 pacientes con esquistosomiasis mansoni. *Revista Cubana Medicina Tropical*; **42**:321-239.
- Savioli, S., Hatz, C., Dixon, H., Kisumku, U.M. and Mott, K.E. (1990). Control of morbidity due to *Schistosoma haematobium* on Pemba Island: egg excretion and haematuria as indicators of infection. *An. J Trop. Med. Hyg.* **43**: 289-295

- Savioli, L. and Mott, K.E. (1989). Urinary schistosomiasis on Pemba Island: Low-cost diagnosis for control in a primary Health Care Setting. *Parasitology Today*, **5**; 333-337
- Schutte, C.H.J. (1974a). Studies on the South African strain of *Schistosoma mansoni* I. Morphology of miracidium. *South African Journal of Science*, **70**; 299-302.
- Schwetz, J. (1953b). Sur le probleme bilharzien de Sakinia (Katanga, Congo Beige). *Annales de la Societe Belge de Medecine Tropicale*; **33**: 463-482).
- Sellin, B., Simonkovich, E., Ovazza, L., Sellin, E., Desfontaine, M. and Rey, J.L. (1982). Valeur de l'examen macroscopique des bandelettes reactives pour la detection de l'hematurie et de la proteinurie dans le diagnostic de masse de la schistosomiase urinaire avant et apres traitement. *Medicine Tropicale*; **42**: 521-526.
- Sher, A., Coffman, R.L., Hieny, S., Cheever, A.W. (1990). Ablation of eosinophil and IgE responses with anti-IL-5 or anti-IL-4 antibodies fail to affect immunity against *Schistosoma mansoni* in the mouse. *J. Immunol.*; **145**(11): 3911-3916.
- Sher, A., Hieny, S., James, S.L. and Asofsky, R. (1982). Mechanisms of protective immunity against *Schistosoma mansoni* infection in mice vaccinated with irradiated cercariae. II. Analysis of immunity in hosts deficient in T lymphocytes, B. lymphocytes and complement. *Journal of Immunology*, **128**: 1880-1884.

- Sher, A., Hall, B.F. and Vadas, M.A. (1978). Acquisition of murine major histocompatibility complex gene products by schistosomula of *Schistosoma mansoni*. *Journal Experimental Medicine*; **148**(1): 46-57.
- Sher, A., Mackenzie, P. and Smithers, S. R. (1974). Decreased recovery of invading parasites from the lungs as a parameter of acquired immunity to schistosomiasis in the laboratory mouse. *J. Infect. Dis.* **130**; 626-633
- Shiff, C. and Kriel, R.L. (1970) A water soluble product of *Bulinus (Physopsis) globosus* attractive to *S. haematobium* miracidia. *Journal of Parasitology*; **56**: 281-6.
- Sikora, K. and Smedley, H.M. (1984). *Monoclonal antibodies* . Blackwell Scientific Oxford. p. 132.
- Simpson ,A.J.G. and Cioli, D. (1987). Progress towards a molecularly defined vaccine for schistosomiasis. *Parasitology Today*, **3**; 26-28.
- Simpson, A.J.G., Chandler, M., Kelly, C., Rumjanek, F., Martin, S. and Smithers, S.R. (1987). Characterization of the structure and expression of the gene encoding a major female specific polypeptide of *Schistosoma mansoni*. *Molecular Biochemical Parasitology*, **22**; 169-176.
- Simpson, A.J.G., Hacket, F., Kelly, C., Knight, M., Payares, G., Ali, P., Lilleywhite, J., Fleck, S.L. and Smithers, S.R. (1986). The recognition of *Schistosoma mansoni* surface antigens by antibodies from patients infected with *S. mansoni* and *S. haematobium*. *Transactions of the Royal society of Tropical Medicine and Hygiene*, **80**; 261-270.
- Simpson, A.J.G., Knight, M., Hagan, P., Hodgson, J., Wilkins, H.A. and Smithers, S.R. (1985). The schistosomulum surface antigens of *S. haematobium*. *Parasitology*; **90**: 499-508.

- Simpson, A.J.G. and Smithers, S.R. (1985). Schistosome: surface, egg and circulating antigens. In: "Current Topics in Microbiology and Immunology". Vol. 120 (Parkhouse, R.M.E. Ed) pp 205-239. Springer-Verlag, Berlin and Heidelberg.
- Simpson, A.J.G., James, S.L. and Sher, A. (1983). Identification of surface antigens of schistosomula of *Schistosoma mansoni* recognized by antibodies from mice immunized by chronic infection and by exposure to highly irradiated cercariae. *Infection and Immunity*, **41**; 591-597.
- Sleigh, A., Hoff, R., Mott, K.E., Barreto, M., Maisk de Paiva, T., Sonza Pedrosa, S. and Sherlock, I (1982). Comparison of filtration staining (Bell) and thick smear (Kato) for the detection and quantification of *Schistosoma mansoni* eggs in faeces. *Transaction of the Royal Society of Tropical Medicine and Hygiene*; **81**: 468-475.
- Smith, H.V. and Kusel, J.R. (1979). The acquisition of antigens in the intercellular substance of mouse skin by schistosomula of *Schistosoma mansoni*. *Clinical Experiment of Immunology*; **36**(3): 430-435.
- Smith, M.A., Clegg, J.A., Snary, D. and Trejdosiewicz, A.J. (1982). Passive immunization of mice against *Schistosoma mansoni* with IgM monoclonal antibody. *Parasitology*, **84**; 83-89.
- Smithers, S.R. (1986). Parasitology: Improving prospects for a schistosome vaccine. *Nature*; **323**(6085): 205-206.
- Smithers, S.R. and Doenhoff, M.J. (1982). Schistosomiasis. In: Immunology of Parasitic infections. 2nd Ed. (Cohen, S. and Warren, K.S. eds) pp. 328-584. Blackwell Scientific Publications, Oxford, London.

- Smithers, S.R. and Gammage, K. (1980) Recovery of *S. mansoni* from the skin, lungs and hepatic portal system of naïve mice and previously exposed to *S. mansoni*: evidence for two phases of parasite attrition in immune mice. *Parasitology*, **80**: 289-300.
- Smithers, S.R., Miller, K.L. (1980). Protective immunity in murine schistosomiasis mansoni; evidence for two distinct mechanisms. *American Journal of Tropical Medicine and Hygiene*; **29**: 832-841.
- Smithers, S.R., McLaren, D.J. and Ramalho-Pinto, F.J. (1977). Immunity to schistosomes: The targets. *American Journal of Tropical Medicine and Hygiene*; **26**: 11-19.
- Smithers, S.R., Terry, R.J. (1969). Immunity in schistosomiasis. *Annals of the New York Academy of Sciences*; **160**: 826-840
- Smithers, S.R., Terry, R.J. (1967). Resistance to experimental infection with *Schistosoma mansoni* in rhesus monkeys induced by the transfer of adult worms. *Transactions of the Royal Society of Tropical Medicine and Hygiene*; **61**: 517-533.
- Smyth, J.D. and Halton, D.W. (1983). *The Physiology of Trematodes*. Cambridge; Cambridge University Press. 446pp.
- Southgate, V.R., Van Wyk, J.B. and Wright, C.A. (1976). Schistosomiasis at Loum, Cameroun: *S. haematobium*, *S. intercalatum* and their natural hybrid. *Zeitschrift fur Parasitenkunde*; **49**: 145-59
- Stirewalt, M.A. (1974). *Schistosoma mansoni*: cercaria to schistosomula. *Advances in Parasitology*, **12**: 115-182

- Sturrock, R.F. (1987). Biology and ecology of human schistosomes. *Bailliere's Clinical Tropical Medicine and Communicable Diseases*; **2** No.2: 249-266.
- Taylor, D.W. and Butterworth, A.E. (1982). Monoclonal antibodies against surface antigens of schistosomes of *Schistosoma mansoni*. *Parasitology*, **84**: 65-82.
- Taylor, D.W., Haguya, E.G. and Vannier, W.E. (1981). Surface antigens of *S. mansoni*. *Molecular and Biochemical Parasitology*, **3**; 157-168.
- Taylor, M.G. (1970). Hybridisation experiments on five species of African Schistosomes. *Journal of Helminthology*, **44**: 253-314.
- Taylor, P., Chandiwana, S.K. and Matanhire, D. (1990). Evaluation of reagent strip test for haematuria in the control of *Schistosoma haematobium* infection in school children. *Acta Tropica*, **47**; 91-100.
- Teesdale, C.M. and Amin, M.A. (1976). Comparison of the Bell technique, a modified Kato thick smear, technique and a digestion method for the field diagnosis of schistosomiasis mansoni. *Journal of Helminthology*, **50**; 17-20.
- Threadgold, L.T. (1984). Parasitic plathyhelminths. In *"Biology of the Intergument"*. (Bereiter-Hahn, J., Matoltsky, A.G. and Richards, K.S. eds.); **1** (11): 132-191.
- Towbin, H., Staehelin, T. and Gordon, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: Procedure and some applications. *Proceedings of the National Academy of Sciences, USA*; **79(9)**: 4350-4354.

- van der Kleij, D., van Remoortel, A., Schuitemaker, J.H., Kapsenberg, M.L., Deelder, A.M., Tielens, A.G., Hokke, C.H., Yazdanbakhsh, M., (2002). Triggering of innate immune response by schistosome egg glycolipids and their carbohydrate epitope GalNAc beta 1-4 (Fuc alpha 1-2 Fuc alpha 1-3). *J. Infect. Dis.*; **185**(4): 531-539
- van Wijk, H.B., (1969). *Schistosoma intercalatum* infection in school-children of Loum, Cameroun. *Tropical and Geographical Medicine*; **21**: 375-382
- Viana, I.R.C., Correa-Oliveira, R., Carvalho, O.D.S., Massara, C.L., Colosimo, E., Colley, D.G. and Gazzinelli, G. (1995). Comparison of antibody isotype responses to *Schistosoma mansoni* antigens by infected and putative resistant individuals living in an endemic area. *Parasite Immunological*; **17**: 297-304.
- Viana, I.R.C. Sher, A., Carvalho, O.S., Massara, C.L., Eloi Santos, S.M., Pearce, E.J., Colley, D.G., Gazzinelli, G. and Correa-Oliveira, R. Correa-Oliveira, R., (1994). Interferon gamma production by peripheral blood mononuclear cells from residents of an area endemic for *Schistosoma mansoni*. *Transactions of the Royal Society of Tropical Medicine and Hygiene*; **88**: 393-408.
- Vogel, M., Bruckner, D. and Bruce, J.I. (1978). *Schistosoma mekongi* sp. n. from man and animals compared with four geographic strains of *S. japonicum*. *Journal of Parasitology*; **64**: 577-584.
- von Lichtenberg, F.V. (1987). Consequences of infections with schistosomes. In: "The Biology of Schistosomes: from genes to latrines. (Rollinson, D and Simpson, A.J.G., eds) Academic Press, Harcourt Bruce. pp 185-224.

- von Lichtenberg, F.V. Bawden, M.P. and Shealy, S.H. (1974). Origin of circulating antigen from the schistosome gut. An immunofluorescent study. *American Journal of Tropical Medicine and Hygiene*; **23**; 1088-1091.
- Waine, G.J., Becker, M., Yang, W., Kalina, B. and McManus, D.P. (1993). Cloning, molecular characterization and functional activity of *Schistosoma japonicum* glyceraldehydes-3-phosphate dehydrogenase, a putative vaccine candidate against *Schistosoma japonicum*. *Infect immunology*; **61** (11): 4716-4723.
- Walker, A.R.P., Walker, B.T., and Richardson, B.D. (1970). Studies on Schistosomiasis in a South African Bantu Schoolchild population. *American Journal of Tropical Medicine and Hygiene*; **19**: 792-814.
- Warren, K.S. (1987). Determinants of disease in human schistosomiasis. *Bailliere's Clinical Tropical Medicine and Communicable Diseases*, 2:2; 301-314.
- Warren, K.S., Boros, D.L., Hang, L. and Mahmoud A.A.,F. (1975). The *Schistosoma japonicum* egg granuloma. *American Journal of pathology*; **60**: 279-294.
- Warren K.S. (1973). Regulation of the prevalence and intensity of schistosomiasis in man. Immunology or ecology. *Journal of Infectious Diseases*; **127**: 595-609.
- Warren, K.S. (1972). The immunopathogenesis of schistosomiasis: a multidisciplinary approach. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **66**; 417.

- Webbe, G., James, C., Nelson, G.S., Smithers, S.R. and Terry, R.J. (1976). *Schistosoma haematobium* in the baboon (*Papio anubis*) after cercariae exposure and adult worm transplantation. *Annals of tropical Medicine and Parasitology*, **70**; 411-424.
- Weber, K. and Osborn, M. (1969). The reliability of molecular weight determination by Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis. *Journal of Biological Chemistry*, **244**; 4406-4412.
- Wen, S.T. and Chu, K.Y. (1984). Preliminary schistosomiasis survey in the lower Volta River below Akosombo Dam, Ghana. *Annals of Tropical Medicine and Parasitology*, **78**: 129-133.
- Wilkins, A., and Gilles, H. (1987). Schistosomiasis haematobia. *Bailliere's Clinical Tropical Medicine and Communicable Diseases*. **2:2** pp 333-348. London.
- Wilkins, H.A., Blumenthal, V.J., Hagan, P., Hayes, R.J and Tulloch, S. (1987). Resistance to reinfection after treatment of urinary schistosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*; **81**: 29-35.
- Wilkins, H.A., Goll, P., Marshall, T.F. and Moore, P. (1979). The significance of proteinuria and haematuria in *Schistosoma haematobia* infection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **73**; 74-80.
- Wilkins, H.A. and Scott, A. (1978). Variation and stability in *Schistosoma haematobium* egg counts: A four year-year study of Gambia children. *Transactions of the Royal Society of Tropical Medicine and Hygiene*; **16** (1): 73-79.

- Wilson, R.A. (1987). Cerceriae to Liver worms: Development and Migration in the Mammalian Host. In: "The Biology of Schistosomes: from genes to latrines. (Rollinson, D and Simpson, A.J.G., eds) Academic Press, Harcourt Bruce. pp 115-146.
- Wilson, R.A., Coulson, P.S. and Dixon, B. (1986). Migrating bof Schistosomula of *Schistosoma mansoni* in mice vaccinated with radiation-attenuated cercariae, and normal mice: an attempted to identify the timming and site of parasite death. *Parasitology*; **92**: 101-116.
- Wilson, R.A., Coulson, P.S., Metting, S.M. (1983). A significant part of the "concomitant immunity" of *Schistosoma mansoni* is a consequence of a leaky hepatic portal system not immune killing. *Parasite Immunology*; **5**; 595-601.
- Wilson, R.A. and Barnes, P.E. (1977). The formation of the membranoclyx on the tegument of *Schistosoma mansoni*. *Parasitology*; **74**: 61-64.
- Woolhouse, M.E.J. and Hagan, P.(1999). Seeking the ghost of worms past. *Nature Medicine*; **5**: 1225-1227
- Woolhouse, M.E., Etard, J.F., Dietz, K., Ndhlovn, P.D. and Chandiwana, S.K. (1998). Heterogeneities in schistosome transmission dynamics and control. *Parasitology*; **117**: (Pt.5) 475-482.
- Woolhouse, M.E., Taylor, P., Matanhire, D. and Chandiwana, S.K. (1991). Acquired immunity and epidemiology of *Schistosoma haematobium*. *Nature*; **351**: (6329) 757-759.
- World Health Organization (1993). The control of schistosomiasis. *Technical Report Series*, 830, Geneva, 86 pp

- World Health Organization (1992). Report of a meeting on application of immunodiagnosis. 15-16 January, N.Y., USA.
- World Health Organization (1985). The control of Schistosomiasis. *World Health Organization Technical Report Series* **728**, 113.
- World Health Organization (1983a). Diagnostic techniques in schistosomiasis control. WHO/SCHISTO/83.69
- World Health Organization (1983b). Cellophane thick smear examination technique (Kato) for diagnosis of intestinal schistosomiasis and gastrointestinal helminth infections. PDP/83.3
- World Health Organization (1983c). Urine filtration for *S. haematobium* infection. PDP/83.4
- Wright, C.A. and Ross, G.C. (1980). Hybrids between *Schistosoma haematobium* and *Schistosoma mattheei* and their identification by isoelectric focusing of enzymes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*; **74** (3): 326-332.
- Wright, C.A., Southgate, V.R., Van Wyk, J.A. and Moore, P.J. (1974). Hybrids between *Schistosoma haematobium* and *Schistosoma intercalatum* in Cameroun. *Transactions of the Royal Society of Tropical Medicine and Hygiene*; **68**: 413-14.
- Wright, C.A., and Knowles, R.J. (1972). Studies on *Schistosoma haematobium* in the laboratory. III. Strains from Iran, Mauritius and Ghana. *Transactions of the Royal Society of Tropical Medicine and Hygiene*; **66**: 108-118.

- Wurapa, F., Barakamfitye, D., and Mott, K.E. (1989). Current status of the epidemiology and control of Schistosomiasis in Africa. *Tropical Medical Parasitology*, **40**; 49-152.
- Xu, C.B., Verwaerde, C. Grzych, J.M., Fantaine, J. and Capron, A. (1991) A monoclonal antibody blocking the *Shistosoma mansoni* 28Kda glutathione S-transferase activity reduces female worm fecundity and egg viability. and high anti-larval IgE levels. *European Journal of Immunology*, **21**: 1801-1807
- Yi, X., Omer-Ali, P., Kelly, C., Simpson, A.J.G. and Smithers, S.R. (1986). IgM antibodies recognizing carbohydrate epitopes shared between schistosomula and miracidia of *Schistosoma mansoni* that block in vitro killing. *Journal of Immunology*, **137**; 3949-3954.
- Yoshida, N.R.S., Nussenzweig, R.S., Potocnjak, P., Nussenzweig, V. and Aikawa, M. (1980). Hybridoma produces protective antibodies directed against the sporozoite stage of malaria parasite. *Science*, **207**; 71.
- Zwingenberger, K. de Jonge, N. Oellinger, H. and Feldmeier, H. (1989). New approaches to measurement of morbidity in intestinal schistosomiasis. *Tropical Medicine and Parasitology*, **40**: (163-168)

Appendices

Appendix A.

Chemicals/reagents for preparation of resolution and stacking gels

Acrylamide,
 N,N'-methylene bis-acrylamide
 Tris-aminomethane)-HCL pH 8.8,
 Sodium dodecyl sulphate (SDS)
 Ammonium persulphate (APS)
 N,N,N',N'-tetramethylethylenediamine (TEMED)
 Distilled water
 Methanol

Appendix B.

Reagents/chemicals for preparation of (10%) resolution and (3%) stacking gels

Solution A. 10% resolution gel (one gel)

29.2% (w/v) acrylamide,	
0.8% (w/v) N,N'-methylene bis-acrylamide	12ml
1.5M (Tris-aminomethane)-HCL pH 8.8,	
0.4% (w/v) sodium dodecyl sulphate (SDS)	9ml
10% (w/v) Ammonium persulphate (APS)	20mg
N,N,N',N'-tetramethylethylenediamine (TEMED)	6ul
Distilled water	15ml

Solution B, 3% stacking gel (one gel)

29.2% (w/v) acrylamide,

0.8% (w/v) n,n'-methylene bis-acrylamide 1.6ml

1.5M (Tris- aminomethane)-HCL pH 6.8,

0.4% (w/v) sodium dodecyl sulphate (SDS) 3ml

10% (w/v) Ammonium persulphate (APS) 4mg

N,N,N',N'-Tetramethylethylenediamine (TEMED) 12ul

Distilled water 7.2ml

Appendix C.**Materials for Western Immunoblot**Antigen samples (*S. haematobium* worm and egg antigen extracts)

Protein molecular weight standards

Electroblotting/transfer buffers

Anode buffer 1 (0.3M Tris, 10% methanol, pH 10.4)

Anode buffer 2 (0.025M Tris, 10% methanol, pH 10.4)

Cathode buffer (0.025M Tris, 0.04M Glycine, 10% methanol, pH 9.4)

Plasma

Tris buffer saline (TBS) pH 7.4)

Anti-human IgG HRPO

Diaminobenzedine

Cobaltous Nitrite

Whatmann 3MM filter paper

Immobilon-P transfer membrane

Semi dry blotter

Trough

Plastic bowl