

**EPIDEMIOLOGICAL STUDY OF UROGENITAL
SCHISTOSOMIASIS IN APPARENTLY HEALTHY AND HIV-
INFECTED FEMALES IN JOS, PLATEAU STATE, NIGERIA**

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CERTIFICATION

This is to certify that the research work for this thesis and the subsequent preparation of this thesis by JANE CHIZOMA NJOKU (PGNS/UJ/0098/04) were carried out under my supervision.

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DECLARATION

I hereby declare that this work is the product of my own research efforts, undertaken under the supervision of Prof. D.A. Dakul and has not been presented elsewhere for the award of a degree or certificate. All sources have been duly distinguished and appropriately acknowledged.

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DEDICATION

Dedicated to
my dear children, especially little Anita Njoku,
for their love and care.

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Abstract

This study is aimed at determining the status, prevalence and significance of female urogenital schistosomiasis (FUGS) in schistosomiasis endemic population at risk of HIV transmission in Jos, Plateau State. Epidemiological circumstances that predisposed individuals to urogenital schistosomiasis and HIV in Jos Plateau State, was investigated. FUGS was determined from parasitological and immunological techniques. Circulating cathodic (worm) antigen (CCA) was used as an additional immunodiagnostic tool to measure the worm burden of the study population. Commercial ELISA was used to determine HIV status while CCA and cytokine plasma levels were used to assess pathology and determine indices of FUGS. Immunological studies to provide evidence of FUGS morbidity, to establish the presence of immunopathology and other markers of FUGS were carried out using important regulatory T - cells and cytokines involved in cellular and humoral immune response. The physical nature and chemistry profile of study subjects were determined for urinary tract pathology, using urinalysis test strips. Data analyses, using expressive percentage, parametric and non-parametric tests were used to assess the significance of varied observations. Two Way analysis of variance was used for multiple comparisons while regression for linear relationships, were applied appropriately. Observations were confirmed significant at $p = 0.05$ in SPSS version 15.1 and all statistics; while Optical Density values were transformed by ELISA LogIt 2005 software. One thousand, two hundred and forty-five (1245) females were screened for *S. haematobium*. Urogenital schistosomiasis was confirmed in 265 (26.3%), in 11-20 year olds (28.3%) accounting for the highest infections ($p < 0.05$). HIV infection was 27(6.8%) and (9.4%) in 21-30 year olds. Students accounted for most infection (39.7%). Only 5.3% urinary schistosomiasis/HIV co-infections were recorded. Economic activities including, irrigation agriculture and domestic chores accounted for most water contacts (64.8%). About 70.9 % (381) presented with indices of FUGS pathology, including: blood, nitrate,

protein, leucocyte in >45% of positive persons. Worm burden (from CCA level) was as high as 127 (67.8%; $p > 0.05$) among study groups. About 79.3% schistosomiasis and HIV infected groups expressed high level of IFN- γ (5-fold, $p < 0.05$). Tumor necrosis factor-alpha (TNF- α) revealed elevation in 58.0% of the schistosomiasis, HIV and HIV/schistosomiasis co-infected subjects ($p < 0.05$). Schistosomiasis infected group had a 7-fold plasma TNF compared to only a 2-fold elevation obtained in co-infected subjects. Interleukin (IL) - 4 presented more irregular interaction with a 42.3 fold elevation observed in HIV infected group, compared to 23.1 and 15.3-fold elevations recorded for single and co-infections. In all, changes in cytokines were marked with a general inconsistency across all groups. This demonstrates FUGS, HIV associated immunopathology and inflammatory reaction to *Schistosoma* infection. Rises in the levels of inflammatory cytokines (IFN- γ , TNF- α , IL-4) in schistosomiasis and HIV infection append to schistosomiasis morbidity via up-regulation of immunopathology of schistosomiasis and HIV replication, thus contributing to cytokine disorder. Results confirmed high FUGS and inflammatory cytokines; whose presence may constitute a significant risk factor in HIV acquisition and pathogenesis in Jos and perhaps north central Nigeria. It is hoped that this knowledge on urogenital schistosomiasis and HIV will rekindle public health interest and help to highlight the need for curbing the risk of these diseases. It is also hoped that it will emphasize the urgent need for the provision of safe water, sanitary facilities in endemic Nigerian communities.

CHAPTER ONE INTRODUCTION

1.1 FEMALE UROGENITAL SCHISTOSOMIASIS (FUGS) AND HUMAN IMMUNODEFICIENCY VIRUS (HIV)

Schistosomiasis, a systemic helminthes infection, is one of the most important socio-economic and poverty-related health problems that affect human development in developing countries. It is also the second most prevalent tropical disease after malaria (Vennervald and Dunne, 2004; Harms and Feldmeier, 2002). It affects approximately over 200 million people in Africa, Asia, South America and the Caribbean. Though schistosomiasis leads to death; it is generally a disease of morbidity. Its morbidity depends on the infecting *Schistosoma* species, the intensity of infection, the topographic site affected by sequestered eggs and the immune responsiveness of the host. In Africa, especially Nigeria; *Schistosoma mansoni*, *S. intercalatum* and *S. haematobium* are the most predominant species (Agi and Okafor, 2005). The effects of the disease are varied. Among the pathogenic effects of the disease include chronic diarrhoea, hepatosplenomegaly, liver fibrosis, ulceration of the genital organs and haemorrhage (Oliveira and Andrade, 2001). Schistosomiasis of the urethral tract; which leads to the destruction of the mucosal cells of some of the reproductive organs - by the piercing action of the oval spines -has become significant; particularly in women.

Thus, as a disease, FUGS is a peculiar pathology of *S. haematobium* disease morbidity, marked by the presence of *S. haematobium* eggs and or worms in the upper or lower urogenital tract, the release of certain antigens (circulating cathodic antigens-CCA; circulating anodic antigens-CAA); as well as the increase in tissue egg load. FUGS are also associated with obvious chronic symptomatic lesions, inflammations and activation of immune cells (Feldmeier *et al.*, 1998; 2001, Poggensee *et al.*, 1999; 2000a). Genital manifestations include lesions of the vulva, vagina and cervix as well as

thinning, erosion and ulceration of the epithelium. Although it has been reported that sexually transmitted diseases (STDs), increases the probability of HIV transmission, presumably through lesions in the genital mucosal, FUGS has been implicated as an important risk factor in the transmission of HIV/AIDS (Feldmeier *et al.*, 1998, 2001; Poggensee *et al.*, 2001).

Globally, Acquired Immunodeficiency Syndrome (AIDS) has so far claimed over 20 million lives since 1984. As at the end of 2007, approximately 33.2 million people are estimated to be living with HIV, the causative agent of AIDS; with about 2.5 million newly infected individuals in 2007 (UNAIDS, 2007). HIV infection causes cytolytic effects on T-lymphocytes, which results to immune suppression of infected individuals. HIV and parasitic infections interact and affect each other mutually. Whereas HIV infections may alter the natural history of parasitic diseases, impede rapid diagnosis or reduce the efficacy of anti-parasitic treatment, while parasitoses facilitate the infection with HIV as well as the progression from asymptomatic infection to AIDS. Some known parasitic interactions with HIV include: schistosomiasis, malaria, leishmaniasis, onchocerciasis, lymphatic filariasis (Idoko *et al.*, 2001; Harms and Feldmeier, 2002).

Though, a prospective post-mortem study of FUGS-related schistosomiasis (involving vulva, vagina, cervix, ovaries), have been reported in Nigeria (Edington *et al.*, 1975a); recent data on the prevalence and significance of FUGS in HIV/AIDS is still limited.

1.2 BACKGROUND AND RATIONALE

Schistosomiasis, an important public health challenge is second only to malaria in tropical countries and is estimated to affect 207 million people in 74 developing countries of Africa, the Caribbean and Asia. In Africa alone, Nigeria is the most schistosomiasis-endemic country (Carter Center, 2007; McIntosh *et al.*, 2006;

Steinmann *et al.*, 2006). Children shoulder the majority of schistosomiasis' consequences, such as poor growth and impaired cognitive function, especially for communities already burdened by poverty and ravaged by scourges such as malaria and HIV/AIDS. Apart from children, the disease affects farmers, women and those who depend on daily water contact patterns for domestic and occupational activities. Of the 207 million infected, 120 million (58%) are symptomatic with 20 million having severe manifestations and another 750 million at risk of infection. Sub-Saharan Africa accounts for 85% of all people suffering from schistosomiasis and is home to the causative agents, including *S. mansoni*; *S. haematobium* and *S. intercalatum* (Chitsulo *et al.*, 2000). The pathogenic effects of the species vary, intestinal schistosomiasis caused by *S. mansoni* and *S. intercalatum* gives rise to hepatobiliary and gastrointestinal symptoms such as bloody diarrhoea, abdominal pain and hepatosplenomegaly. On the other hand, urinary schistosomiasis caused by *S. haematobium* is associated with urogenital symptoms such as haematuria, dysuria, bladder carcinoma and kidney failure (Danso-Appiah *et al.*, 2004). In addition, the involvement of gynecological organs such as the vulva, cervix, ovaries, fallopian tubes and uterus (a peculiar pathology of *S. haematobium*) has earned itself in females, 'Female urogenital schistosomiasis' (FUGS).

This condition of schistosomiasis morbidity is characterized by the presence of *Schistosoma* eggs in the upper or lower organs of the reproductive tract (Poggensee and Feldmeier, 2001; Poggensee *et al.*, 1998). FUGS has been neglected despite reports of vaginal schistosomiasis which has been reported by Madden (1899) when he observed a tumorous growth with numerous egg granulomas in the vagina of a young Egyptian woman (Feldmeier *et al.*, 2001). FUGS commonly occur in *S. haematobium* endemic areas, with a prevalence rate ranging from 30-75% (Poggensee *et al.*, 1999, 2001; Feldmeier *et al.*, 1998). In Africa alone, more than 13 million women are estimated to

suffer from FUGS (Carey *et al.*, 2001). Unlike other sexually transmitted ulcerative diseases that leave the various genital organs intact, FUGS is associated with multiple organ damage, myriad of complications and lesions that co-exist with an altered epithelium (Mabey, 2000). Studies have shown that due to these alterations, disruptions and ulcerative lesions in the epithelium of FUGS infected individuals, HIV is easily transmitted (Poggensee *et al.*, 2000). Furthermore, inflammation around egg-associated lesions recruits activated immune cells expressing CD4 cells and the chemokines receptor CCR5 into the epithelium providing HIV virus the ample opportunity to bind (Rumbley and Phillips, 2000).

In sub-Saharan Africa, several epidemiological studies on HIV and *S. haematobium* infection shows a substantial overlap in many areas where urinary schistosomiasis is endemic and women have a high HIV prevalence (UNAIDS, 2007). These studies observed that *S. haematobium* infection; which is a rural phenomenon, occurs more often among women with limited access to clean water while HIV prevalence peaked in the younger rural women than in the urban women (Barongo *et al.*, 1992). This put together could have resulted to the unexplained gender quotient of HIV-1 infection which disfavors rural women, with a prevalence that is eight times higher in young women than in the young men in these rural areas since both diseases meet in migrating populations, travelers, commuting spouses and roadside villages (UNAIDS, 2008; Laga *et al.*, 2001). For example, in countries like Central African Republic, Kenya, Malawi and Uganda where HIV prevalence was at its peak and *S. haematobium* infection was 70%, HIV prevalence was observed to be 1.2 – 1.7 times higher in women than in men (Barongo *et al.*, 1992; Feldmeier *et al.*, 1994). These findings were attributed to social, behavioural and cultural norms, even though risk factors for HIV

acquisition are different for both rural and urban populations (Barongo *et al.*, 1992; Nunn *et al.*, 1994; Quigley *et al.*, 1997).

In Nigeria, studies on urinary schistosomiasis/HIV co-infection are scanty, with recent reports from Benin, Edo State and Otukpo, Benue State showing a 0.3% and 20% prevalence respectively (Olusegun *et al.*, 2011; Okwori and Alao, 2009). The true prevalence of FUGS infection is rather difficult to estimate since only reports on urinary schistosomiasis abound which were based on parasitological observations in urine alone (Opara *et al.*, 2007; Anosike *et al.*, 2006; Agi and Okafor, 2005; Ukwandu and Nmorsi, 2004; Dakul *et al.*, 1997; Akufongwe *et al.*, 1996). Thus, only the very early direct studies of Edington *et al.*, (1975a) on FUGS detection were relied upon. However, in Jos, Plateau State; urinary schistosomiasis is still endemic with recent reports (Gutman *et al.*, 2010, 2009, 2008, Njepuome *et al.*, 2009; Carter Centre, 2007). Conversely, studies on FUGS have been greatly impaired by the invasive nature of sample collection and analysis prior to the current era of serology and knowledge of specific immunological markers. From previous studies on the seroprevalence of FUGS Njoku *et al.*, (2004) confirmed the existence of known markers of FUGS prevalence in a small population (of apparently healthy and HIV-positive individuals) from schistosomiasis endemic communities of Jos.

1.3 SIGNIFICANCE OF THE STUDY

FUGS is widespread and on the increase in schistosomiasis endemic areas causing significant morbidity and mortality (Mosunjac *et al.*, 2003). Recent data has shown that the disease is especially important in poor, rural areas, where attempts to alleviate poverty, has promoted small-to-large scale water-related development projects that may increase transmission (Danso-Appiah *et al.*, 2004). Diagnosis of FUGS has mainly been by the demonstration of eggs in tissues such as Quantitative Compressed

Biopsy Technique (QCBT), the histopathological examination and the cytological examination (Pap smear) (Poggensee *et al.*, 2001). These biopsy-based methods are invasive and not feasible in primary healthcare settings in developing countries like Nigeria except cervical smears which are routinely used during gynecological examinations (Savioli *et al.*, 1990). Ultrasonography, on the other hand, although sensitive and specific, is very expensive and needs a trained staff to operate it and this limits its large-scale applicability in many endemic areas (Hatz *et al.*, 1998). However, it has a low sensitivity to mild lesions and early inflammatory changes which places it at a disadvantage for use in the diagnosis of FUGS in schistosomiasis endemic areas (Vennervald *et al.*, 2000). The fact that the effects of the disease are varied (Feldmeier *et al.*, 1998), suggests that FUGS could be considered not only as a risk factor for contracting different STDs, but also plays a significant role in modifying the natural history and immunological response of the hosts to the infections, particularly HIV (Danso-Appiah *et al.*, 2004; Mosunjac *et al.*, 2003).

In the same vein, HIV is endemic and a major cause of death in the world generally (UNAIDS, 2007), and more common in Africa, the same continent where schistosomiasis is most endemic. Though the availability and use of antiretroviral medicines have reduced HIV disease and spread of the virus by half, the disease is still without a cure and preventive vaccine does not appear feasible in the very near future (UNAIDS, 2007). In addition, reports have shown that HIV is mainly transmitted through the heterosexual route in sub-Saharan Africa (UNAIDS, 2007). This then means that FUGS is a major risk factor to HIV and STIs for more females. Thus, these relationships make case for the full understanding of opportunistic infections especially FUGS in HIV infection situations, bearing in mind the consequences of their co-infection occurrence.

This study therefore, aspired to investigate the epidemiology of FUGS in Jos, Plateau State, with the aim of revealing the link of female populations and communities to urinary schistosomiasis/FUGS endemicity. At this stage, we have tried to establish the preliminary parasitological status of individuals, FUGS status and relevant epidemiological factors in the transmission of HIV in the population (Njoku *et al.*, 2004). Perhaps it may be justified to conclude that people living in the tropics not only face a health threat with the expanding HIV epidemic, they also have to fear that HIV infection will alter the natural history of parasitic infections which they are exposed to, in an unfavorable way. This is possible since the parasites, harboured in the host, impairs the immune response towards HIV, thus, making rapid progression from HIV to AIDS more likely (Harms and Feldmeier, 2002). In addition, recent pathophysiological and etiological evidences, suggest that FUGS is taking a life threatening course in HIV. This study, thus, adopted more sensitive and less invasive techniques in the diagnosis of FGS using simple molecular diagnostics.

1.4 HYPOTHESES

1. **Hypothesis I:** Epidemiological factors do not expose individuals to *S. haematobium* infection and does not determine the transmission of urinary schistosomiasis in the study communities.
2. **Hypothesis II:** Schistosomiasis exposed subjects are not at risk of symptoms and indicators of female urogenital schistosomiasis.
3. **Hypothesis III:** Females who are infected with *S. haematobium* with presence of FUGS indicators do not have immunological markers that may increase the subjects to higher risk of HIV transmission
4. **Hypothesis IV:** Schistosomiasis/HIV co-infected subjects will not exhibit distinctive immunological changes based on immunological responses of CD4 T cell and cytokines (IL-4, TNF- γ and IFN- α)
6. **Hypothesis V:** *S. haematobium* infected subjects will not demonstrate higher HIV risk exposure compared to normal population of individuals without *S. haematobium* infection

- 7. Hypothesis VI:** Praziquantel treatment will not exert a detectable influence on immunological response to schistosomiasis and will not show clear evidence of the relationship and contributions of FUGS morbidity in HIV/schistosomiasis co-infection

1.5 AIM AND OBJECTIVES

The main aim of the study is to determine the status and characteristics of FUGS in HIV infected and apparently healthy females in Jos.

Specific Objectives include:

1. To determine the prevalence of FUGS and HIV in apparently healthy and immunosuppressed individuals.
2. To study FUGS as a risk factor in HIV transmission by identifying and characterizing the potential agents.
3. To assess the association between FUGS and HIV seropositivity using specific immunological markers.
4. To study immune mechanisms and their potentials capable for use in serodiagnosis and disease control.

CHAPTER TWO LITERATURE REVIEW

2.1 HISTORICAL BACKGROUND

The parasite of the genus *Schistosoma* is the causative agent of the age long schistosomiasis. As early as 1850, Egyptian pharaohs wrote of urinary disturbances, notably haematuria, which appeared in classically young boys and was once deemed to be a sign of puberty. It was not until 1851, when a young German pathologist, Theodore Bilharz discovered the causative parasite; *Schistosoma* (El-Khoby *et al.*, 1998). He described the worm “*Distoma haematobium*” (subsequently *S. haematobium*) and published his finding together with his teacher, Professor Siegbold in Breslau. The connection to urinary disease (haematuria) with presence of eggs in urine was made a year later (WHO, 2010). A few other studies reported Manson to have observed laterally spined eggs in the feces of a patient in 1903, who had no haematuria and this further suggested that more than one species of the worm was involved. It took half a century for the controversy regarding terminal and laterally spined eggs to be resolved because double infections with *S. haematobium* and *S. mansoni* were so common in the Nile Delta, a situation persisting into this day. Eventually Learner, an English scientist gave definite proof that two species were in existence with different snail intermediate hosts (Sturrock *et al.*, 1990; Frandsen and Christensen 1984; Cridland, 1955). More reports of *S. haematobium* eggs detected in the kidneys of Egyptian Mummies and the detection of circulating schistosome antigens were also described (Deelder *et al.*, 1989; Reyman *et al.*, 1977; Ruffer, 1910).

Furthermore, Archeological studies in China have shown that schistosomiasis japonica has been present for more than 2200 years (Mao and Shao, 1982). The first detailed description of Katayama disease (acute schistosomiasis) in rice farmers was

made by a Japanese physician Dario Fuji in 1847. He also noted that cattle and horses could be affected. The connection of the disease, to the occurrence of schistosome eggs were made more than 60 years later, when *Schistosoma* eggs were found in a patient with Katayama disease (Mao and Shao, 1982). More recently, the genomes of *Schistosoma mansoni* and *S. japonicum* have been decoded revealing new ways for targeted treatments (Zhou *et al.*, 2009; Berriman *et al.*, 2009). These studies also reported that the genome of *S. mansoni* contain 11,809 genes which produces enzymes for breaking down proteins which helps the parasite to bore through tissue.

2.2 MORPHOLOGY OF *Schistosoma* SPECIES

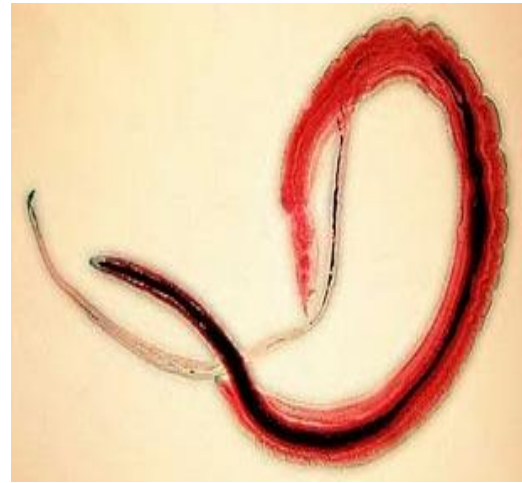
The Human *Schistosoma* and most of the other mammalian species belong to the phylum Platyhelminthes, family Schistosomatidae and are a group of digenetic, dioecious trematodes requiring definitive and intermediate hosts to complete their life cycles. They show affinity with strigeids and certain other flukes in having miracidia with two pairs of flame cells, daughter sporocysts instead of rediae, and cercariae with forked-tails. Schistosomes, in particular *Schistosoma, spp.* are known for their peculiarity in having separate males and females that are morphologically different. The male worm, usually about 8 to 16mm. long, has a unique cylindrical appearance that is actually flat, with the sides of the body which is posterior to the ventral sucker, rolled ventrally to form a groove or “gynecophoric canal” in which the longer and more cylindrical female projecting free at each end, but enclosed in the middle, (lies safe in the arms of her spouse) (Garcia and Bruckner, 1993).

Most schistosomes seem to remain permanently wedded and monogamous, with the uncoupled females remaining spinsters. In *S. mansoni*, however, the union is of a more companionate nature. Oddly enough, the female worms do not become sexually

mature until they become associated with the males, although the males are capable of developing quite independently of the females (Ambroise-Thomas and Andrews, 1976). Both male and female worms are provided with oral and ventral suckers of which the ventral sucker in the males are large and powerful. Their digestive tract has no pharynx, and the esophagus forks, as usual, just anterior to the ventral sucker, but the forks reunite in the middle portion of the body to be continued as a single tube. The male worm has several testes just behind the ventral sucker, and it is here that the genital pore opens (Plate 1). The female on the other hand, has an elongated ovary situated in the fork, from where the intestinal cerca rejoin. Most of the posterior half of the worm is occupied by the yolk glands. Anterior to the ovary is a straight uterus; which contains a small number of eggs, 1 to 50 or more in the different species. Unlike most flukes, the schistosomes do not develop great numbers of eggs all at once but instead develop them gradually and have only a few in the oviduct at any one time (Garcia and Bruckner, 1993).



a.



b.

Plate 1: *Schistosoma* adult worm parasite. (Source: CDC, 2012)

1a: *S. mansoni* adult worm

1b: *S. haematobium* adult worm

2.3 TAXONOMY

Schistosoma, a digenetic trematode, belongs to the Phylum Platyhelminthes, Superfamily - Schistosomatoida and Genus – *Schistosoma*. Ten species of schistosomes can infect man but a vast majority of infections are caused by *S. haematobium*, *S. mansoni*, *S. japonicum*, and *S. mekongi* (Frandsen and Christensen, 1984), of which *S. haematobium*, *S. mansoni*, and *S. japonicum* has a wide distribution. Moreover, within each of the three principal species of man, there are physiologic strain differences, perhaps due to geographic isolation and mollusk host adaptations. The taxonomic classification of schistosomes is presented (Table 1 and 2).

Table 1: The Taxonomic Classification of *Schistosoma* Species.

Classification	Name	Biological Characteristics
Sub-Kingdom	Metazoa	Are triploblastic, possess a skin; possess a mouth of sorts, body systems are mainly alimentary and reproductive; possess primitive nervous and excretory systems.
Phylum	Platyhelminthes	Are flattened, segmented or unsegmented; gut may or may not be present; no body cavity, viscera in gelatinous matrix
Class	Trematoda	Unsegmented, leaf-like or cylindrical; generally are hermaphroditic; reproduction (digenetic) are either oviparous or multiply within larval forms; infection mainly by larval stages entering intestinal tract, sometimes through the skin.
Subclass	Digenea	Almost all species are endoparasitic; an organ of attachment consisting of one or more suckers, of which one is circumoral. Excretory pores open posterior; development complicated, with an alternation of three or more generations and an alternation of hosts, of which that harboring the intermediate stages is a mollusk; larva hatched from egg has ciliated epithelium.
Super order, Anepitheliocystida		Definitive stage monoecious or dioeciously, in intestine or blood stream of vertebrates; anterior sucker almost always present; one or more ventral acetabula usually present; cercariae with a bi-forked tail; two pairs of flame cells in the miracidium.
Order: Strigeatoidea		
Suborder: Strigeata		
Family	Schistosomatidae	The females live in gynecophoric canal of the males; Cercaria is adapted for penetrating skin of hosts by cytolysis. The worms are parasitic in circulatory system.
Genus	<i>Schistosoma</i>	Separate males and females that are morphologically different. Males have a unique cylindrical gynecophoric canal which houses the longer and more cylindrical females.

Source: Ukoli, 1984

Table 2: Species of *Schistosoma* and their Definitive Hosts

Species	Definitive host(s)	Reference
<i>S. haematobium</i>	Man, monkeys,	Manson-Bahr and Bell, 1987
Subspecies		
<i>S. bovis</i> , <i>S. curassoni</i> , <i>S. guineensis</i>	Cattle, goats and sheep.	Kane <i>et al.</i> , 2003
<i>S. intercalatum</i>	Man	Pages <i>et al.</i> , 2001
<i>S. kisumuensis</i>	Man	Manson-Bahr and Bell, 1987
<i>S. leiperi</i> , <i>S. matthei</i>	Ruminants	Kaukas <i>et al.</i> , 1994
<i>S. margrebowiei</i>	Man	Webster <i>et al.</i> , 2006
<i>S. mansoni</i>	Man, baboons, monkeys, rodents and raccoons.	Manson-Bahr and Bell, 1987
Subspecies		
<i>S. hippotami</i> , <i>S. edwardiense</i>	Hippopotamus	Manson-Bahr and Bell, 1987
<i>S. rodhaini</i>	Rodents and dogs	Manson-Bahr and Bell, 1987
<i>S. japonicum</i> ,	Man , cats, dogs, water buffalo, goats, horses	Manson-Bahr and Bell, 1987
Subspecies		
<i>S. malayensis</i>	Rat (<i>Rattus muelleri</i>)	Attwood <i>et al.</i> 2005
<i>S. mekongi</i>	Man and dogs	Manson-Bahr and Bell, 1987
<i>S. incognitum</i>	Man and pigs	Webster and Littlewoods, 2012
<i>S. indicum</i>	Horses, ruminants	Manson-Bahr and Bell, 1987
<i>S. spindale</i>	Ruminants, cattle	Liu <i>et al.</i> , 2010
<i>S. nasale</i>	Ruminants	Manson-Bahr and Bell, 1987
<i>New spp.: S. bomfordi</i> , <i>S. datta</i>	Ruminants	Aldhoun and Littlewood, 2012
<i>S. turkestanicum</i> , <i>S. harinasutai</i>	Ruminants	Aldhoun and Littlewood, 2012

These three principal species are representatives of groups of other schistosomes which some experts (Tsang *et al.*, 1984) consider as species while others refer them as subspecies and species complexes (Cridland, 1955). Related to *S. haematobium* are three other African schistosomes and one in India, which differ in the shape of their eggs and in their definitive hosts, though all use *Bulinus* as intermediate hosts. These are the widely distributed *S. bovis* of cattle, sheep, and goats; *S. matthei* of sheep in South Africa; *S. intercalatum* of man in Belgian Congo; and *S. indicum* of horses and other animals in India. Those related to *S. mansoni* as subspecies include *S. mansoni var. rodentorum* of rodents, and *S. rodhaini* of rodents and dogs, which have eggs with a sub-terminal spine. Other subspecies that have been related to *S. japonicum* include *S. margrebowiei* which is found in Congo and South Africa, and *S. incognitum* which is a pig schistosome, also found in the feces of a human being in India (Frandsen and Christensen, 1984). However, of all people suffering from schistosomiasis, 85% live in sub-Saharan Africa where *S. haematobium*, *S. mansoni* and *S. intercalatum* are prevalent (Chitsulo *et al.*, 2000). In Nigeria, the major species found are *S. mansoni*, *S. haematobium*, and *S. intercalatum*.

2.4 LIFE CYCLE

The Life Cycle of *Schistosoma* species is a complex one. It involves a phase of sexual reproduction by adult schistosomes in the definitive human host and an asexual phase in the intermediate host, usually a freshwater snail. Although the basic life cycles of schistosomes are similar, there are many species differences that allow one to easily differentiate among them. These differences include variations in morphology, development and differentiation, snail intermediate host species and pathology (Thors, 2006; Elias *et al.*, 2005).

A succession of stages is involved; the egg, miracidium, first stage sporocyst, second stage sporocyst, cercariae, schistosomulae and adult. All species of schistosomes are contracted in the same way; by direct contact with infested surface water containing free living forms of the parasite known as cercariae.

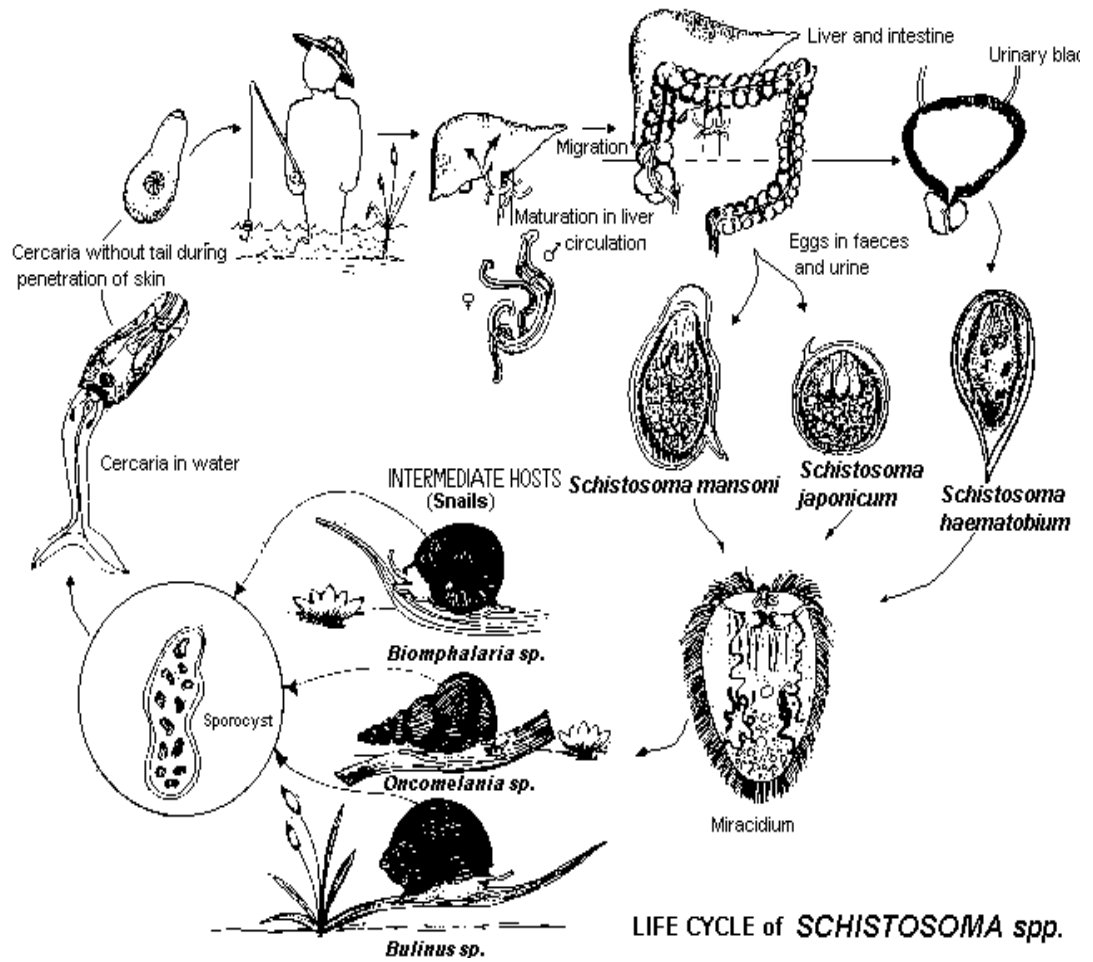
These schistosome cercariae are non-feeding organisms that possess a short life span of between 36 and 48hrs. The cercariae consist of a tail, used for motility in the water, and a head region which is used for attachment to host skin and glands containing proteolytic enzyme to facilitate penetration of the skin. When a human host is found, the cercariae are able to penetrate the skin while the human is in contact with the infested water during occupational and/or recreational activities. After penetration, the Cercaria sheds its tail and several other major changes accompany transformation into a new form called the schistosomulum. Inside the walls of a nearby vein, schistosomulum is carried in the host blood flow, reaching the liver where they grow and reach sexual maturity. The mature male and female worms pair and then, depending on species, migrate to the vessels of the bowel or bladder where egg production occurs. Many eggs pass through the tissues to the intestinal or bladder wall where they are excreted in the feces or urine. *Schistosoma* species can be subdivided into three groups characterized by the size, shape and appearance of the eggs produced by the female worm:

1. *S. haematobium* and *S. intercalatum* produce ovoid eggs with a size of 6 x 140 – 170mm and a terminal spine.
2. *S. mansoni* eggs have a similar shape and size but with a lateral spine.
3. *S. japonicum* and *S. mekongi* produce smaller rounded eggs with a size 50-90mm and a rudimentary spine (WHO, 1994).

However, the schistosome life cycle (Fig.1), is completed when the eggs hatch, releasing free-swimming miracidia, which in turn re-infect freshwater snails. Interestingly, there are only very few snail species that are compatible and species-

specific. In Cote d'Ivoire, for instance, *Biomphalaria pfeifferi* was reported as the only intermediate snail host for *S. mansoni* (Rollinson *et al.*, 2001), while both *Bulinus globosus* and *B. truncatus* act as intermediate hosts for the transmission of *S. haematobium* (Brown *et al.*, 1994, 1981; Southgate and Knowles, 1977; Sturrock, 1965). The miracidia penetration into the intermediate snail host is predominantly via the foot of the snail. After penetration, they develop into mother sporocyst where an asexual multiplication phase of reproduction takes place and thousands of cercariae are released back into the water. However, due to biological, environmental and physical determinants it takes between 4 and 6 weeks, from the penetration of a miracidium to the production of mature cercariae (Thors, 2006).

Some of the schistosome eggs (especially *S. haematobium*), rather than being excreted, often lodge in the tissues of the host. It is the presence of these retained eggs that causes the pathology of schistosomiasis. In intestinal schistosomiasis, eggs lodged in the mucosa or sub mucosa of the gut causes granulomatous reactions, which often extend into the gut lumen as pseudopapillomas resulting in colonic obstruction and blood loss. Booth *et al.*, (2004) observed that eggs lodged in the liver result in portal fibrosis leading to portal hypertension, splenomegaly and ascites while esophageal and gastric varices (exsanguinations from bleeding esophageal varices), are the major cause of death.



Adapted from National Communicable Disease Center (NCDC) USPHS, Atlanta, Georgia, USA

Fig.1: Life Cycle of *Schistosoma* Species

2.5 DISTRIBUTION

The global distribution of schistosomiasis, one of the most important tropical diseases, has changed significantly in the past 50 years with control successes achieved in Asia, the Americas, North Africa and Middle East. About 750 million people are reported to be at risk and of those infected; 120 million are symptomatic with 20 million having severe manifestations (Frandsen and Christensen, 1984). Recently, the concept of disability-adjusted life years (DALY) was developed in order to assess and refine estimates of the global burden of the disease (King *et al.*, 2005). For sub-Saharan Africa, a morbidity burden due to schistosomiasis was estimated to be 3.5 million DALYs. In comparison with other communicable diseases, schistosomiasis ranked ten, after respiratory infections (31.6 million DALYs), malaria (31.5 million), diarrhea diseases (30.4 million), HIV infections (18.4 million), measles (16.1 million), tuberculosis (13.7 million), sexually transmitted diseases excluding HIV (7.5 million), tetanus (5.8 million) and pertussis (4.8 million) DALYs (King *et al.*, 2005, Ejezie and Ade-Serrano, 1981).

Schistosoma japonicum and *S. mekongi*, which causes intestinal schistosomiasis, are prevalent in 7 African countries and the pacific region. *Schistosoma intercalatum* on the other hand is found in 10 African countries while *S. haematobium* which causes urinary schistosomiasis affects 54 countries in Africa and Eastern Mediterranean. In Nigeria, however, up to 102 million people are said to be at risk of schistosomiasis with nearly 26 million infected (Chitsulo *et al.*, 2000). There is general agreement that the global prevalence of schistosomiasis will most likely increase due to the increasing numbers of irrigation systems for agriculture and cattle breeding. It is also believed that construction of dams and man-made lakes for hydroelectric power production, civil strife and war which contribute to additional human migration, and the introduction of

the disease from endemic communities to non-endemic communities will aid the transmission (Mott *et al.*, 1995).

2.6 EPIDEMIOLOGY

Humans are the most important reservoir hosts, although naturally infected monkeys, baboons and chimpanzees have been reported in endemic areas. For instance in Tanzania, a tribe of wild baboons have been reported as sustaining the life cycle of schistosomiasis without human intervention (King *et al.*, 2005, 2006, 2008; Kariuki and Farah, 2005). For a comprehensive understanding of the epidemiology of human schistosomiasis three major factors of the disease transmission are of central importance and this drives the epidemiology of human schistosomiasis as follows:

2.6.1 Environmental Transmission

People are infected through contact with infested water during domestic (washing of utensils/laundry), recreational (swimming/bathing) or economic (irrigation farming/fishing) activities. Poverty, insufficient knowledge of the risks of schistosomiasis, lack of public health facilities plus the unsanitary conditions in which millions of people lead their daily lives, are all factors contributing to the risk of contracting *Schistosoma* infection. Due to lack of information and insufficient attention to good hygiene practices, infected individuals contaminate their water supply with their feces and urine (Harms and Feldmeier, 2002). Sewage from homes in endemic foci, which are emptied into streams, with feces/urine found in or around water bodies, all add to the propagation of the infection. Thus, the eggs of schistosomes in the excreta or urine of an infected person hatch on contact with water and release the cercariae which invariably must find a suitable host to survive and the cycle continues. Irrigated agriculture which creates permanent shallow freshwater channels has also been a major

factor promoting the spread of schistosomiasis. According to WHO report in 1993, the reason for the increase of schistosomiasis, was the construction of large scale water projects, dams and irrigation canals, which when put together create ideal environment for snails hosts. These smaller water channels that run through rural lands, allow for a closer water contact and a high rate of infection, since people who live near these channels use the water for their daily household chores and farming. Overall, the number of large-scale water projects in developing nations is on the rise and it has been noted that schistosomiasis is on the increase in these areas as well. Some of these water projects include dams, canals and man-made lakes which in all create ideal snail habitat. For instance, Lake Volta, the world's largest artificial lake in Ghana has been reported with approximately 90% of all the neighboring villages' children infected with schistosomiasis (Youssef *et al.*, 1970). These children when infected contaminate their environment with urine and stool samples creating a continual cycle that leads to poor school performance and growth. Other dams such as the Diama dam in Senegal, the Akosombo dam in Ghana, Kainji Dam in Nigeria and Kariba dam in Zimbabwe as well as other smaller reservoirs have all been reported as major transmission foci, responsible for the spread of schistosomiasis in Africa (Flok *et al.*, 2005; Scott *et al.*, 2003; Kloos *et al.*, 1988; Youssef *et al.*, 1970).

2.6.2 Intermediate Hosts and Transmission of Schistosomiasis

The transmission of schistosomiasis also depends on the intermediate host. The construction of numerous large-scale water projects, irrigation canals and other smaller water reservoirs create ideal vegetation/environment for snails to thrive. There are only a few snails that can act as host, restricting the disease to tropical and subtropical areas. In most, schistosomiasis endemic areas the disease is, characterized by seasonal

transmission patterns; though this is also linked to the source of transmission. In numerous studies, it has been shown that the distribution and density of the intermediate snail host is an important determinant, accounting to a large extent for the observed variability in rates of schistosomiasis infection (WHO, 2011; Woolhouse *et al.*, 1991). There is firm evidence that in lotic environments, water current velocity is the key determinant influencing the distribution of snails, while the lentic environments, water temperature plays the key role for snail abundance. Both the water current velocity and temperature vary over time and show seasonal pattern. Different species of the intermediate hosts exist depending on the *Schistosoma species* they harbour. The species include; *Bulinus* species; which harbour *S. haematobium* (*B. globosus*; *B. truncatus*; *B. africanus*, *B. nasutus*; *B. forskalii*, *B. senegalensis*) (Scott *et al.*, 2003; Wright *et al.*, 1979; Sturrock, 1965) and *Biomphalaria*; which harbour *S. mansoni* (e. g. *B. pfeifferi*; *B. alexanderi*) (Esterre *et al.*, 1994) (Tables 3 and 4; Plate 2 and 3).

Table 3: Snail Intermediate Hosts of *Schistosoma* species

Human Schistosomes		
Scientific Name	First Intermediate Host	Endemic Area
<i>Schistosoma guineensis</i>	<i>Bulius forskalii</i>	West Africa
<i>Schistosoma intercalatum</i>	<i>Bulinus</i> spp.	Africa
<i>Schistosoma haematobium</i>	<i>Bulius</i> spp.	Africa, Middle East
<i>Schistosoma japonicum</i>	<i>Oncomelania</i> spp.	China, East Asia, Philippines
<i>Schistosoma malayensis</i>	Not known	South East Asia
<i>Schistosoma mansoni</i>	<i>Biomphalaria</i> spp.	Africa, South East Asia, Caribbean, Middle East
<i>Schistosoma mekongi</i>	<i>Neotricula apertaz</i>	South East Asia

Source: Manson-Bahr and Bell, (1987)



*Bulinus
forskalii*



Bulinus wright



Bulinus truncatus



*Bulinus
globosus*

Plate 2: Snail intermediate hosts of *Bulinus species*

Table 4: Different Snail Intermediate Hosts Species of *Bulinus* group

<i>Bulinus africanus</i> group - 10 species	<i>Bulinus forskalii</i> group - 11 species
<i>Bulinus africanus</i> , <i>Bulinus globosus</i> , <i>Bulinus nasutus</i> (Kane <i>et al.</i> , 2008; Brown, 1994)	<i>Bulinus canescens</i> (Brown, 1994; 1996)
<i>Bulinus ugandae</i> , <i>Bulinus hightoni</i> , <i>Bulinus jousseaumei</i> , <i>Bulinus obtusus</i> (Brown, 1994)	<i>Bulinus browni</i> , <i>Bulinus crystallinus</i> , <i>Bulinus bavayi</i> , <i>Bulinus scalaris</i> , <i>Bulinus senegalensis</i> - the type species of the genus (Brown, 1994)
<i>Bulinus obtusispira</i>, <i>Bulinus umbilicatus</i>, <i>Bulinus abyssinicus</i> (Brown, 1994)	<i>Bulinus camerunensis</i> , <i>Bulinus cernicus</i> , <i>Bulinus forskalii</i> , <i>Bulinus barthi</i> , <i>Bulinus beccarii</i> (Kane <i>et al.</i> , 2008; Brown, 1994)
<i>Bulinus reticulatus</i> group – two species	<i>Bulinus truncatus/tropicus</i> complex - 14-15 species
<i>Bulinus reticulatus</i> , (Brown, 1994; Appleton <i>et al.</i> , 2009; Kane <i>et al.</i> , 2008)	<i>Bulinus angolensis</i> , <i>Bulinus depressus</i> , <i>Bulinus hexaploidus</i> , <i>Bulinus liratus</i> , <i>Bulinus octoploidus</i> , <i>Bulinus trigonus</i> , <i>Bulinus permembranaceus</i> , <i>Bulinus succinoides</i> , <i>Bulinus transversalis</i> , <i>Bulinus yemenensis</i> , (Brown, 1994)
<i>Bulinus wrighti</i> (Kane <i>et al.</i> , 2008; Brown, 1994)	<i>Bulinus mutandensis</i> (Kyambadde, 2004)
	<i>Bulinus natalensis</i> (Brown, 1994; Curtis <i>et al.</i> , 2009; Kane <i>et al.</i> , 2008) <i>Bulinus nyassanus</i> , <i>Bulinus tropicus</i> , <i>Bulinus truncatus</i> (Kane <i>et al.</i> , 2008; Brown, 1994)

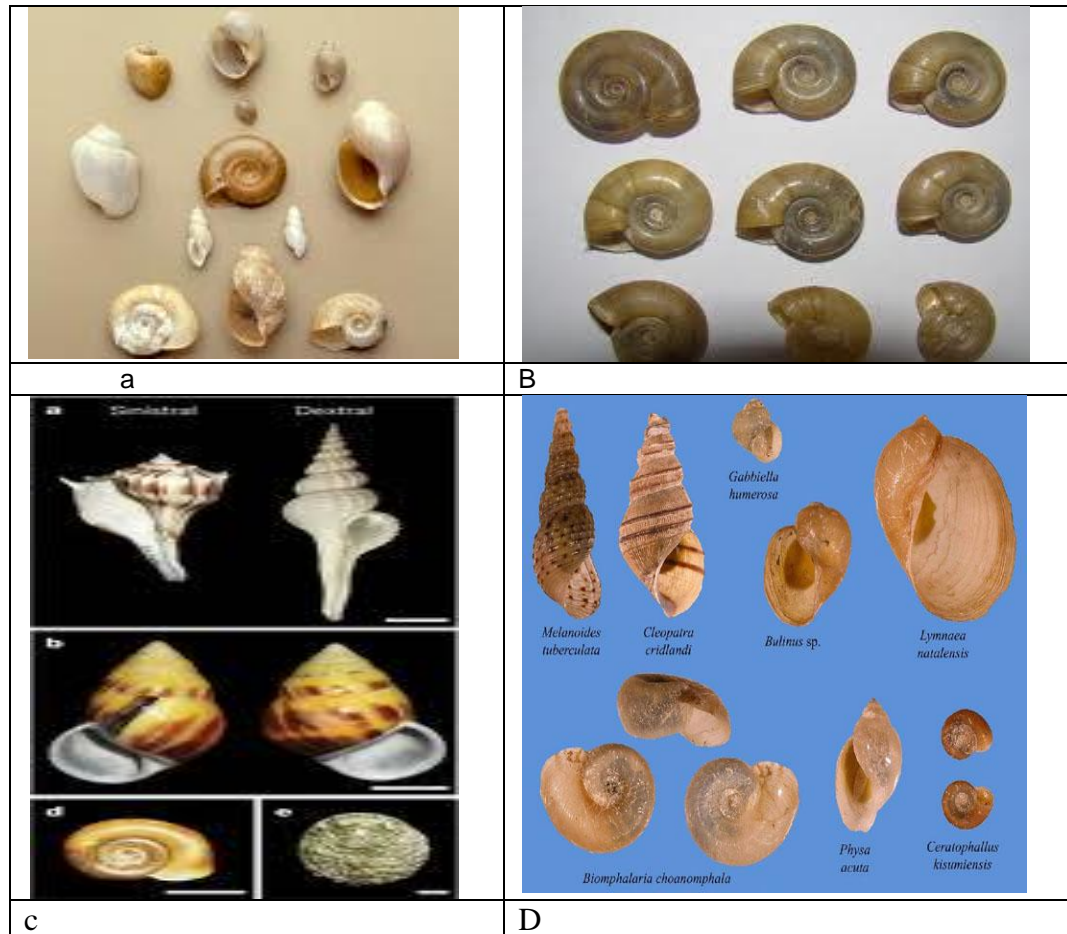


Plate 3: Different snail intermediate hosts

- a. Different snail species
- b. *Biomphalaria* species
- c. Ancient snail intermediate hosts
- d. Other snail intermediate hosts such as *Lymnaea natalensis*, *Physa acuta*, *Cleopatra oridlandi*, *Melanoides tuberculata*, *Gabbrella humerosa*

Bulinus truncatus have been implicated as the major snail host involved in urinary schistosomiasis in Senegal (Scott *et al.*, 2003), while in Zambia and USA, *B. globosus* and *B. abyssinicus* were implicated as the snail host for *S. haematobium* respectively (Kane *et al.*, 2008; Brown, 1994). *Oncomelania hupensis robertsoni* has been identified as a subspecies of the *S. japonicum* snail host and is found near Xi Chang, China. Interestingly, the snail host for *S. japonicum* is so small compared to the vectors of other species of *Schistosoma* (Spear *et al.*, 2004). In Kano state, Nigeria for instance, *Bulinus senegalensis*, *B. forskalii*, *B. globosus*, *B. rohlfsi* are the major intermediate hosts associated with *S. haematobium* transmission while *Biomphalaria pfeifferi* was the only intermediate host for *S. mansoni* (Anosike *et al.*, (1992). In another study, in Bauchi State of Nigeria, Anosike *et al.*, (1992) reported *Bulinus truncatus*, *B. globosus*, *B. forskalii*, *Biomphalaria pfeifferi*, *Lymnea natalensis*, *Lanistes ovum*, *Lanistes libycus* and *Melanoides tuberculata* collected in the area, as intermediate hosts of *Schistosoma* species.

2.6.3 Human Infection Pattern

Schistosomiasis affects adult workers in rural areas employed either in agriculture or the fresh water fishing sector (WHO, 1999). Children, farmers and women who depend on daily water contact for domestic and occupational activities are the most affected (Danso-Appiah *et al.*, 2004). In most endemic foci, children have been reported as more frequently and more heavily exposed and infected than the adults. The disease is especially important in poor, rural areas where attempts to alleviate poverty also promote small-to-large scale water related development projects that often increase transmission. In many endemic areas, a high proportion of children between the ages of 10 and 14 are infected. Urinary schistosomiasis has been estimated to affect 66 million

children throughout 54 countries (Anosike *et al.*, 2006; UNAIDS, 2007). As with other tropical diseases, population movements and refugees in unstable regions contribute to the transmission of schistosomiasis. Rapid urbanization, migration and tourist activities have been implicated as risk factors for schistosomiasis transmission. For instance, refugee movements, population displacement in the Horn of Africa, introduced intestinal schistosomiasis to Somalia, where adequate knowledge and healthcare was not available (WHO, 1993). In addition, WHO (1999), noted the impact of the disease on economic conditions and the general health situation of majority of infected rural workers in affected areas, such as north-east Brazil, Egypt and Sudan. Furthermore, they added that areas confirmed to be free from schistosomiasis at a particular point in time could rapidly become important disease foci, which invariably may challenge previously unexposed populations. Often times environmental alterations, such as caused by water resource development projects (damming and irrigation); have also been implicated as causes of the onset of schistosomiasis endemicity (Mott, 1982).

2.7 CLINICAL MANIFESTATIONS OF SCHISTOSOMIASIS

Schistosomiasis morbidity as stated by Harms and Feldmeier, (2002) and Vennervald and Dunne (2004); depend on the schistosome species involved, the intensity of infection, the topographic site affected by sequestered eggs and the immune responsiveness of the host. The clinical manifestations reflect developmental stages of the parasite and host responses to toxic or antigenic substances derived from the parasite and eggs. During the early stage of intestinal schistosomiasis (caused by *S. mansoni* and *S. japonicum*), a patient may present with signs such as cercarial dermatitis, which is as a result of cercarial penetration of the host skin. This is followed by more dramatic symptoms often known as Katayama disease. It presents itself as malaise, weight loss, gastrointestinal symptoms, eosinophilia and fever (Danso-Appiah *et al.*, 2004). As the

female worms lay eggs that develop into miracidia, antigenic substances are excreted from the miracidia and this elicits acute inflammation in the surrounding tissues resulting in the rupture of the vascular wall, escape of the eggs into the mucosa and submucosa of the intestinal lumen. Due to these inflammations, recurrent daily fever, abdominal pain, enlarged tender liver and spleen and dysentery or diarrhea results (Booth *et al.*, 2006). Blood chemistry has revealed a transient elevation of glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) and alkaline phosphatase, 5 - 6 weeks after infestation. Increase in eosinophilia production and IgE levels in serum have also been reported (Booth *et al.*, 2006).

The chronic form of intestinal schistosomiasis on the other hand is characterized by hepatosplenomegaly, although development of polyps or mucosal proliferation of the intestine is observed in most cases. Egg granulomas are replaced by fibrotic tissues which are prominent in the periportal areas that lead to the development of periportal fibrosis (Oliveira and Andrade, 2001; Rumbley and Phillips, 2001). Enlargement of the liver and spleen have also been experienced by these patients. However, the extents of liver involvement in intestinal schistosomiasis have been reported to depend mainly on the intensity of infestation and duration. The liver gradually decreases in size, but increases in hardness as fibrosis extends into parenchyma resulting eventually in liver cirrhosis in severe cases and the enlarged spleen expanding to fill most of the abdomen, fibrotic and progressive lesions have been observed to occlude the portal system, leading to portal hypertension that may precipitate haematemesis from ruptured oesophageal varices at advanced schistosomiasis (Danso-Appiah *et al.*, 2004; Poggensee *et al.*, 2001).

Urinary schistosomiasis caused by *S. haematobium* affects the urogenital system. The stage of oviposition manifest into genito-urinary symptoms, such as: cystitis,

haematuria, leukocyturia, dysuria (painful urination), tender abdomen and supra-pubic tenderness. Other clinical manifestations include mild symptoms to chronic iron deficiency and anemia, to scarring and deformity of the ureters and bladder, calcification of the bladder wall, bladder stones, bladder carcinoma, and hydronephrosis and ultimately to renal failure have been observed (Danso-Appiah *et al.*, 2004; Schwartz, 1981). The involvement of gynecological organs has also been reported and as a result, its peculiar pathology has become so significant particularly in women, and contributed to the classification of female urogenital schistosomiasis.

2.8 FEMALE UROGENITAL SCHISTOSOMIASIS

Female urogenital schistosomiasis (FUGS), a morbidity of *S. haematobium* infection is described as the presence of *Schistosoma* eggs in the upper and/or lower parts of the reproductive tract. According to Madden 1899 (in: Midzi *et al.*, 2003), it was first observed about 100 years ago in an Egyptian woman. FUGS is widespread especially in *S. haematobium* endemic regions causing significant morbidity and mortality (Mosunjac *et al.*, 2003). A prevalence ranging from 30-75% has been recorded (Poggensee *et al.*, 2001). In Africa alone, about 9-13 million women, suffer from FUGS (Carey *et al.*, 2001). However, recent studies indicate that the figure may be quite too low (Van Der Warf, 2003). In Jos, Plateau State, north-central part of the country, Njoku *et al.*, (2004) reported 4.3% prevalence among the women. Other reports include a post mortem study of the reproductive tract (Edington *et al.*, 1975b) in Ibadan. Here, he reported the distribution of *S. haematobium* eggs in all the pelvic organs with the cervix as the most common site (30-100%), followed by the vagina (12-100%), ovaries (5-57%), fallopian tubes (3-36%), vulva (7-17%), and uterus (0-11%).

2.9 PATHOGENESIS AND PATHOLOGY

The pathology experienced with schistosome infestation is caused mainly by the deposition of its eggs in various tissues and organs where granulomas or pseudo tubercles are formed around them. In primary infestations, the granulomas formed are composed of aggregations of mononuclear phagocytes, neutrophils, lymphocytes, plasma cells and fibroblasts. Giant cells are also frequently observed in these granulomas. Granulomas may vary in size and cellular components, for example, a dominant cellular infiltration of eosinophils and lymphocytes observed in smaller egg granulomas (Booth *et al.*, 1998, 2006). Granuloma formation around schistosome eggs has been considered to be the result of a delayed-type hypersensitivity reactions mediated through a T-cell mediated immune response to soluble egg antigens (Warren *et al.*, 1978). From recent studies, T helper cells have been demonstrated to be of two types with a CD4 phenotype, termed Th1 and Th2 cells, which can only be distinguished from each other by their cytokine production (Mossman, 1989). The cytokines derived from Th1 cells are the IL-2, Interferon or Tumor Necrosis Factor (TNF) which are responsible for the activation of macrophages and cell mediated immunity, whereas IL-4 or IL-5, the cytokine produced by Th2 cells, stimulates IgE or eosinophilia production, respectively (Burdin *et al.*, 1999; Mwatha *et al.*, 1998).

2.9.1 Definitive Host Response to *Schistosoma* Infection

Granuloma formation is a peculiar host response to mainly carbohydrate antigens. Schistosome granulomas found in infected individuals are of different composition reflecting their different ages (Thors, 2006; Elias *et al.*, 2005). The granulomatous lesions surrounding the tissue-trapped eggs consist of macrophages, eosinophils, CD4⁺ T cells, fibronectin, fibrin, plasmin and collagen fibers. The

granulomas resolve as the eggs die and leave fibrotic nodules (Pearce, 2005; Secor, 2005). However, already infected individuals are less susceptible to new infections (concomitant immunity). One hypothesis, that newly transformed schistosomule, may be more sensitive to immune attack than the adult worms that provoked the partly protective immunity could be true. This is because the hepatic shunt theory which states that eggs clog the intra-hepatic circulatory system which forces the schistosomulae to by-pass the liver via a collateral circulation, results therefore to hepatic damage and fibrosis. Consequently, the maturation of schistosomulae into adult worms, which takes place in the hepatic environment, is inhibited (McHugh *et al.*, 1987).

2.9.2 Clinical Manifestation of Female Urogenital Schistosomiasis

Clinical features of female urogenital schistosomiasis induced signs and symptoms that are rather non-specific and may be compounded by those of other pelvic diseases (Poggensee *et al.*, 2001). The close relation between vessels in the genital organs and the urinary bladder enables the parasite to easily change locations to virtually any organ in the female pelvic area (Helling-Giese *et al.*, 1996). These symptoms concur with the anatomical location of worm pairs and their eggs, and the pathological findings vary depending on the affected organs. Genital schistosomiasis of the ovaries is associated with bleeding disorder, lower abdominal pains with varying intensity and duration, hypogonadism, delayed puberty and infertility (Swai *et al.*, 2006; Feldmeier *et al.*, 2001; Crump *et al.*, 2000). In the fallopian tube, the clinical features include primary or secondary infertility. A high tissue egg burden located at this site is associated with generalized inflammation and fibrosis, which mechanically leads to an impaired tubal motility or tubal occlusion (Reimert *et al.*, 2008; Andrade *et al.*, 2004, 1991). The eventual consequence would be infertility or ectopic pregnancy through altered peristalsis (Feldmeier *et al.*, 1998). In addition, granulomatous reactions observed near

the hilus, resulted to hilar obstruction and par ovarian adhesions leading to an ovulation (Helling-Giese *et al.*, 1996). This was further confirmed, when in a histological sectioning of a fallopian tube, eggs of *S. haematobium* were discovered in all layers of the tube wall accompanied with generalized salpingitis and fibrosis (Crump *et al.*, 2000). However, other associated symptoms include; nodular lesions of the mesosalpinx, adhesions (Leutscher *et al.*, 1997) which has also been linked with haematosalpinx and hydrosalpinx. *Schistosoma haematobium* eggs or adult worms have been detected in endometrial tissue and diagnostic or therapeutic curettage material (Teasdale and Chitsulo, 1985; Sheehan *et al.*, 1984; Harris and Markl, 1999).

In addition, infestation of the placenta resulted to still birth, miscarriage, premature onset of labor and low birth weight (Feldmeier *et al.*, 1998). Clinically, schistosomiasis of cervix shows diverse features, all of which result from granulomatous inflammation. Cauliflower-like growth, nodular hypertrophy, ulcerative and polyploidy lesions have been documented. Such lesions have been reported to resemble a malignancy. They also present with sandy-patches, which are pathologic and may exist on a macroscopically normal cervix but are found often in the vicinity of ulcer and erosions (Kjetland *et al.*, 2005; Poggensee *et al.*, 2001).

2.10 EPIDEMIOLOGY OF FEMALE UROGENITAL SCHISTOSOMIASIS

The epidemiology of FUGS is not uniform to all schistosomiasis endemic communities and countries or comparable between countries. The factors that predispose women to develop genital lesions are not completely known. So far, the frequency of genital involvement can only be inferred from histopathological, post-mortem and a few population based studies where it may be assumed that the intensity of infection played a pivotal role (Poggensee *et al.*, 2001). In post-mortem studies, observed frequencies

were 7-100% for lesions in the lower reproductive tract, and 2-83% for lesion of the upper reproductive tract (Feldmeier *et al.*, 1995). Systemic autopsies revealed that FUGS can affect any genital organ with the cervix as the most common site, followed by the vagina and the fallopian tubes (Poggensee *et al.*, 2001).

Occurrence of FUGS varies widely due to different methodologies used. Four population- based studies were performed to determine the point prevalence of FUGS of the lower reproductive tract (Leutscher *et al.*, 1998; Kjetland *et al.*, 1996). Generally, in all these studies, diagnosis of FUGS was achieved by the detection of eggs in the biopsies of genital tissue. Although, the study design was different, the studies nevertheless, showed that FUGS was a common manifestation in *S. haematobium* infection, with a prevalence range of 30-75% (Poggensee *et al.*, 1999). For instance, in a recent extensive Tanzanian study, more than half of the female populations of child bearing age living in the study area were screened for FUGS of the lower reproductive tract. About 40% and more, of the women had urinary schistosomiasis (Swai *et al.*, 2006). Lower percentages were recorded for women (Feldmeier *et al.*, 1998). Methodologies employed for these studies underestimated the true occurrence of FUGS in such areas. This was demonstrated in a study, where women who accepted gynecological examination were reported to show higher frequencies of FUGS (with symptoms like discharge, lower abdominal pain, inter-menstrual bleeding and infertility) than women who presented only urine specimens. The study could be said to be selection bias, and the prevalence of FUGS underestimated, because it was most unlikely that the women who presented only their urine specimen were not totally free from genital manifestations of schistosomiasis (Poggensee *et al.*, 2000; WHO, 1999). Other histopathological data have also revealed that underestimation of true occurrence of FUGS is possible when a technique with low sensitivity is used. A demonstration by

Renaud *et al.*, (1989), who reported a 22% frequency of placental schistosome eggs and a single case of 0.3% eggs were detected in the histological section of the placenta, revealed that frequency estimation based on histological studies tends to be systematically biased (Poggensee *et al.*, 2001).

False diagnosis, another cause of underestimation of the occurrence of FUGS has been mentioned. This, some studies observed, were related to the unspecific nature of FUGS signs and symptoms, which make false diagnosis highly probable (Stoller and Carrol, 2003; Joesoef *et al.*, 1991; Swart and van der Merwe, 1987). The clinical features of FUGS, some studies further revealed, resemble cervical cancer, resulting therefore to numerous case reports of unnecessary surgery carried out in women with clinical schistosomiasis mistaken for malignancy (Flok *et al.*, 2005, Kjetland *et al.*, 2005). However, few population based studies have provided reliable data on the point prevalence for FUGS of the cervix/vagina which ranged between 50-80% (Kjetland *et al.*, 1996). Some of these women without sexually transmitted diseases but with FUGS accounted for about 38%, while 24% of them expressed clinically detected inflammatory lesions. Furthermore, about 42% had histopathologically assessed moderate to severe cervical inflammation and 28% of cytologically determined moderate to severe inflammation, which they concluded were all attributed to FUGS (Ouma *et al.*, 2000).

2.11 DIAGNOSIS

Diagnosis is of pivotal importance for all aspects of human schistosomiasis (Feldmeier *et al.*, 1998). This is because it directly affects treatment, assessment of morbidity and identification of risk groups/communities (Ejezie and Ade-Serrano, 1981). Monitoring and evaluation of programmes are all based on the results of diagnostic tests. Many different techniques and approaches have been identified which

can be used both at the individual and community levels (WHO, 1999). The selection and application depends not only on the type of information sought but also the resources available. Two major diagnostic techniques have been described as methods mostly used in the diagnosis of genital schistosomiasis of the lower reproductive tracts. They are the direct morphological methods (urine filtration method) and indirect techniques which includes symptoms, clinical examination, serology and immunological methods (Utzinger *et al.*, 2001; Van Etten *et al.*, 1997). Furthermore, diagnosis using imaging techniques to detect pathology due to genital schistosomiasis has also been described (Kabatereine *et al.*, 1998, Edington *et al.*, 1975a).

2.11.1 Direct Methods

Urine filtration technique

The detection of excreted eggs in both feces and urine is the most widely used diagnostic method for schistosome infections. The urine filtration as described by WHO, (1983) has been used to demonstrate the presence of *Schistosoma* eggs in urine. This method is one of the most commonly used techniques in schistosomiasis epidemiological surveys (WHO, 1999). The specificity is very high, but the sensitivity is low due to large day-to-day variations in excretion of eggs (Utzinger *et al.*, 2001; Yu *et al.*, 1998). Unfortunately, examination of urine for *S. haematobium* eggs does not substitute the gynecological examination in the case of genital schistosomiasis because FUGS according to Helling-Giese *et al.*, (1996) and Mosunjac *et al.*, (2003) has frequently been reported in women with scanty or even no egg excretion in their urine. This was confirmed, when in a community based study in Tanzania, urinary and genital schistosomiasis coexisted in 62% of the women while 23% had *S. haematobium* eggs

detected in their cervix without detectable egg excretion in their urine (Poggensee *et al.*, 1998).

2.11.2 Indirect Methods

Symptoms

Many reports have considered symptoms such as dyspareunia, intermittent-menstrual bleeding and bloody vaginal discharge as reliable indicator of vulvae, vaginal or cervical lesions (Naniwadekar, 2008, Poggensee *et al.*, 2001; Szela *et al.*, 1993,). Poggensee *et al.*, (2000a) in a community-based study in Tanzania further explained that perhaps, except post-coital bleeding; self-reported symptoms were neither sensitive nor specific for FUGS diagnosis.

Clinical examination

Macroscopically, studies have observed that lesions in the lower reproductive tract often mimic almost any type of infections or malignant condition found in the vagina or cervix, therefore making it impossible to be pathogenic (Feldmeier *et al.*, 2001; Poggensee *et al.*, 2000b). At a population level, sandy-patches, leukoplakia and erosions are alterations that are significantly associated with the presence of schistosome eggs in the epithelial tissues (Crump *et al.*, 2000; Poggensee *et al.*, 2001). Thus, the use of colposcopy invariably enhances the diagnostic value of a routine gynecological examination as lesions would be examined more clearly as unseen disrupted areas of the epithelium become more visible (Hamilton *et al.*, 1998).

Demonstration of eggs in tissues

This method includes Quantitative Compressed Biopsy Technique (QCBT), the histopathological examination and the cytological examination (i.e. Pap smear)

(Poggensee *et al.*, 2001; Kjetland *et al.*, 1996). The QCBT involves the use of forceps in collecting biopsy from suspicious lesions or from the anterior lip of the cervix and then the collection obtained is compressed between two glass slides. With this method, several biopsies of patients with presumptive diagnosis of FUGS are easily and rapidly screened for the presence of schistosome eggs. Though invasive, QCBT is sensitive enough to detect a single egg in cervical tissues with specificity close to 100% (Poggensee *et al.*, 2001). For the histopathological examination of cervical biopsies, the method of collection is similar to that of the QCBT, except that it has a very low sensitivity as compared to QCBT. Furthermore, the sensitivity of histopathological examination depends on the number of eggs found in the tissues. The higher the egg burden in the cervix, therefore, the more likely, genital schistosomiasis will be diagnosed histopathologically. Biopsy-based methods may not be feasible in primary healthcare settings in developing countries except for cervical smears which are methods that are routinely used in gynecology (Poggensee *et al.*, 2001).

Cervical smear or Pap smear is a cytological examination of biopsy collected from cervical tissues with the corner of a glass slide (Feldmeier *et al.*, 2001). The cervical smears are fixed in 95% alcohol and stained by a routine papanicolaou method. This technique has a disappointingly low sensitivity and specificity (Savioli *et al.*, 1990). Thus if genital schistosomiasis of the cervix is considered in a differential diagnosis of cervical genital lesions, the QCBT is the diagnostic method of choice. However, it could be complemented with histopathology and cytology, if other clinical conditions such as pre-malignant or malignant alterations of the cervix are excluded (Poggensee *et al.*, 2001; Leutscher *et al.*, 1998).

Indirect diseases markers

Till now, diagnosis of schistosomiasis has been dependent on the detection of schistosome eggs in either urine (or stool) and tissue. Because of low sporadic egg production, the risk of not diagnosing infected individuals is tremendous thus transmission of the disease continues. However, in the last 10 years, remarkable progress has been achieved with serology and understanding of immunological disease markers (Diallo *et al.*, 2004; Kjetland *et al.*, 1996; Poggensee *et al.*, 1996; Kahama *et al.*, 1998). The existence of detectable amounts of circulating antigens, eosinophils in schistosomiasis-infected individuals prompted research into their potential for immunodiagnosis. Subsequently, a variety of assay methods were developed and these include; antibody detection methods, Cop test, Gut Associated Antigens (GAA), Somatic Antigens (SA), CAA and CCA, Eosinophil Cationic Proteins (ECP), Keyhole Limpet Hemocyanin (KLH) antigens and other diagnostic methods (Thors, 2003, 2006; Elias *et al.*, 2005; Poggensee *et al.*, 2001; van Lieshout *et al.*, 2000). The determination of these specific antibodies may be used diagnostically only in special situations such as, in people originating in non-endemic areas. It can also be used for estimates of prevalence in not previously treated populations. However, the antigens utilized in most serological assays have generally been crude preparations. Therefore, they continue to suffer from criticisms of specificity (Midzi, *et al.*, 2003; Joseph, *et al.*, 2004; Secor, 2005).

Soluble egg antigen

The detection of SEA for *S. haematobium* in urine using Enzyme Linked Immunosorbent Assay (ELISA) is a non-invasive urine analysis method. It is used to assess live *Schistosoma* eggs in urine of FUGS positive subjects (Kahama *et al.*, 1998). Six monoclonal antibodies produced and characterized against *S. haematobium* antigens

only, had three that detected SEA in the infected subjects (Nibbeling *et al.*, 1998; De Jonge *et al.*, 1990). In addition, Kahama *et al.*, (1998) reported SEA to be very stable in urine together with the fact that diurnal or day to day variation were less, compared to that seen in egg counts. The SEA ELISA also provides a good quantitative measurement of tissue egg load, compared to that obtained from egg counts (Vennervald and Dunne, 2004; Vennervald *et al.*, 2000). This was confirmed in a field study in Kaldeni district of Kenya where Vennervald *et al.*, (2000) observed a parallel slower reduction in urinary tract pathology and levels of SEA in infected patients whereas egg counts declined much faster in infected patients treated with Praziquantel.

Circulating adult worm antigens

These are assays that have been developed to target freely circulating parasite antigens in serum, urine, saliva and breast milk to adequately differentiate between past and current infections. Circulating antigens are produced due to regular regurgitation of ingested contents of parasite's gut into host's body circulation. They are predominantly polysaccharides. Two major proteoglycans used are the Circulating Anodic Antigen (CAA) and Circulating Cathodic Antigens (CCA) (Deelder *et al.*, 1989). These two proteoglycans are partially characterized in their chemical structure. The CAA is currently the most thoroughly investigated antigen, negatively charged with the carbohydrate chains consisting of multiple disaccharide units (which contain N-acetyl galactosamine and glucuronic acid) (van Leishout, 1993). The detection of these two antigens, CCA and CAA in a sensitive and highly specific manner involves the use of a monoclonal antibody (MAb) based sandwich ELISA. The presence of these two antigens in schistosomiasis patients correlates well with actual infection (De Jonge *et al.*, 1990). The advantage with an antigen detection assay is to prove an ongoing infection

and it also gives the possibility to follow treatment effects. Improvement on the sandwich ELISA have been achieved since its first description by further, optimizing the assay and making available highly standardized mass *in vitro* culture of the MAbs (Polman *et al.*, 1998; van Leishout, 2000). Secondly, the antigen levels are expressed as concentrations instead of titers, thereby allowing for a more statistical analysis of data. Furthermore, a specificity of virtually 100% has been reported, using CAA ELISA in serum (Polman *et al.*, 1998). Many false results were occasionally observed when CCA ELISA was applied in urine. Thus, in order to secure an optimal specificity using the CCA ELISA, a much higher cut off level than the actual detection limit for the assay has to be utilized (van Leishout, 2000). The sensitivity for both CAA and CCA assays ranged from 65% to 100% (Polman *et al.*, 1998), although it is not sensitive enough for diagnosing individuals with light infections (Doenhoff *et al.*, 2004; Leishout *et al.*, 2000). Nonetheless, CCA assay in urine has been reported as the best diagnostic tool followed by CAA in serum, since it involves a non-invasive method of sample collection. CAA level in serum have further been considered as the best indicator of worm burden, even though it depends on the level of endemicity in target populations (Nibbeling *et al.*, 1998; Kremsner *et al.*, 1994; Deelder *et al.*, 1989). Thus the detection of CAA in serum and CCA in urine was said to exhibit the same sensitivity, as careful parasitological examination would. It could therefore either be used in communities with high intensity of infection or as a complementary diagnostic tool in low endemic areas (van Leishout, 2000; van Dam, 1993).

Eosinophil cationic proteins (ECP)

These are highly basic and potent cytotoxic single chain zinc-containing proteins that make up the eosinophilic granulocytes. ECP appears to be involved in defense against parasites and in the tissue damage of subjects with allergies and inflammatory

diseases (Reimert *et al.*, 2006, 1991; Poggensee *et al.*, 1996). Many studies have shown that eosinophils are found in high concentrations in *Schistosoma* egg granulomas (Deelder *et al.*, 1989), and can be detected both in urine and serum of infected hosts using an ECP ELISA (Carey *et al.*, 2001; Reimert *et al.*, 2000). ECP provides a quantitative measure of urinary/tissue egg load, supplementing the information obtained from egg counts and ultrasonography (Vennervald *et al.*, 2000). Poggensee *et al.*, (2001) further stressed that the measurement of ECP was a surrogate for counting eosinophils in urine, therefore exhibiting greater stability than counting eggs both during and between days. This was buttressed in a study, where the levels of ECP were determined both in urine supernatant and extracted cellular urine deposits (Reimert *et al.*, 1993). The results revealed that the levels of ECP in both the supernatant and extracted cellular urine deposits were significantly higher in the affected subjects compared to the control group (Midzi *et al.*, 2003). In another study, concentrations of ECP were also significantly higher in vaginal lavage of FUGS patients as compared to their uninfected counterparts (Poggensee *et al.*, 1998). Vennervald *et al.*, (2000), in yet another study, pointed out ECP as a promising direct marker of urinary bladder morbidity which reflects local inflammatory response of the bladder wall in response to schistosome eggs with optimal conditions for sensitivity and specificity, such as 5ng/ml of urine as best cut-off value for infection and 25ng/ml for bladder morbidity (Poggensee *et al.*, 2001; Reimert *et al.*, 2000). ECP from genital fluid has also been demonstrated to be an indicator of pathology due to FUGS joined with other factors such as allergy and inflammatory microbial infection (Midzi *et al.*, 2003). However, one of the major advantages of ECP diagnostic method is its simplicity and reduced cost of required tools compared to ultrasonography, its ability to detect early undiagnosed FUGS individual. Secondly, it can serve as a useful large-scale epidemiological tool for

the assessment of FUGS impact in control programmes (Midzi *et al.*, 2003; Poggensee *et al.*, 2001; Feldmeier *et al.*, 2001; Ouma *et al.*, 2000).

Ultrasonography

This method is said to be the ‘Gold Standard’ for the detection of urinary tract morbidity. It is safe and non-invasive, feasible, relatively rapid for assessing pathology resulting from schistosome infections (Kabatereine *et al.*, 1998). According to WHO (1999), the method provides a direct image of pathological changes in the urinary tract. It is especially useful in investigating the resolution and reappearance of pathology following treatment (Richter *et al.*, 2003). Nonetheless, some studies have revealed that mild lesions and early inflammatory changes which were missed completely during diagnosis using SEA, CCA and ECP were easily detected using ultrasound (Doenhoff *et al.*, 2004; Vennervald *et al.*, 2004).

However, the use of ultrasonography requires ultrasound equipment, a generator and a trained staff. The fact that the materials needed for the ultrasonography is quite expensive to obtain and also time consuming invariably places it at a disadvantage (WHO, 1999). Recently, however, PCR methods for detecting of *Schistosoma* DNA for both *S. mansoni* and *S. japonicum* have been developed (Ganley-Leal *et al.*, 2005; Hamburger *et al.*, 1991).

2.12 CYTOKINES AND CELL MEDIATED IMMUNITY

It is established that some cytokine patterns helper T cells tend to produce, we understand less about how the patterns themselves are decided. Various evidences suggest that the type of APC presenting the antigen to the T cell has a major influence on its profile. Other evidence suggests that the concentration of antigen presented to the T cell during primary activation influences its choice. The presence of some cytokines

(such as the ones mentioned above) will also influence the response that will eventually be generated, but our understanding is nowhere near complete (Fig. 2, Table 5).

2.13 CD4⁺ T-LYMPHOCYTE CELLS

T helper cells (T_h cells) are a sub-group of lymphocytes, a type of white blood cell, that play an important role in the immune system, particularly in the adaptive immune system. They help the activity of other immune cells by releasing T cell cytokines. They are essential in B cell *antibody* class switching, in the activation and growth of cytotoxic T cells, and in maximizing bactericidal activity of phagocytes such as macrophages (Fig. 2, Table 5).

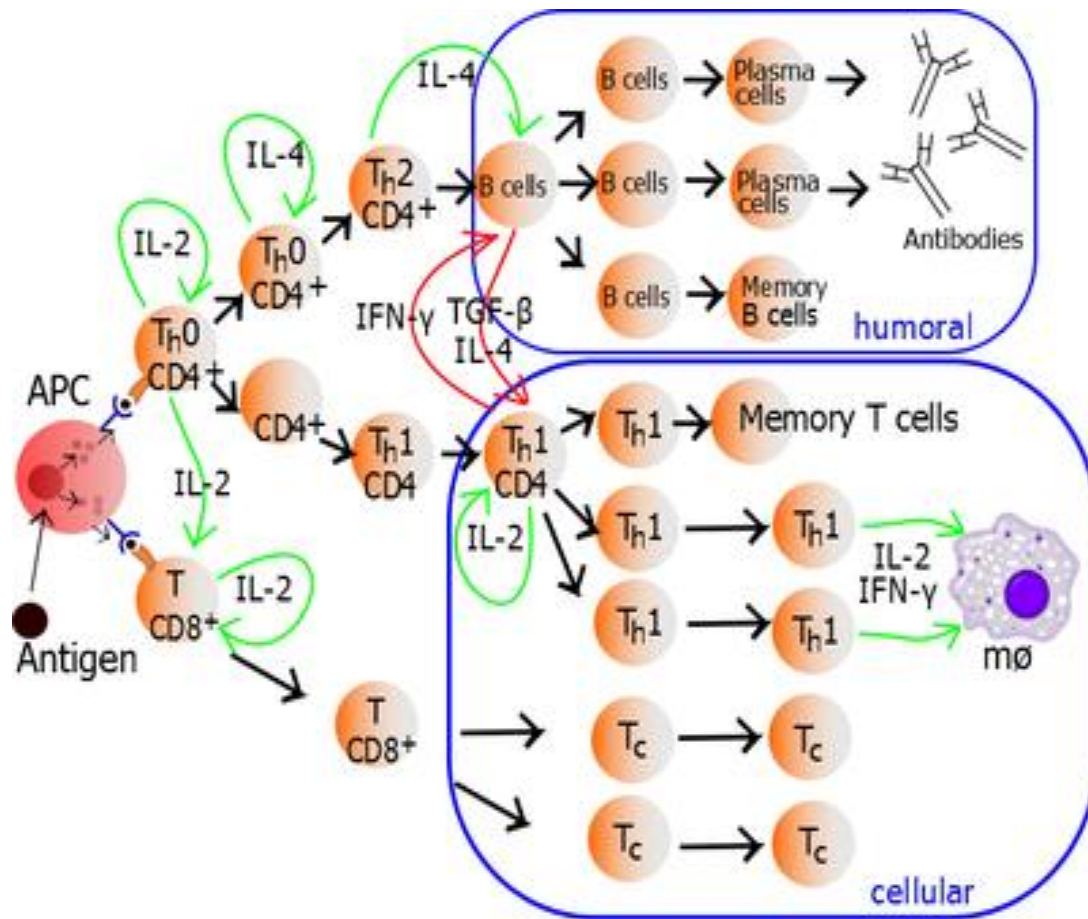


Fig. 2: T cell- helper type 1 (Th-1) and T cell type 2 (Th-2) Immune Response Model: An antigen ingested and processed by an APC (Rang, 2003).

Table 5: Type 1 and Type 2 Immune Response (Rang, 2003))

	Type 1/ T_h1	Type 2/ T_h2
Main partner cell type	<u>Macrophage</u>	<u>B-cell</u>
Cytokines produced	Interferon- γ and TGF-beta. (Interleukin-2 was classically associated with T _h 1 cells, but this association may be misleading; IL-2 is produced by all helper T cells early in their activation.) interleukin-10 production has been shown to be induced in activated Th1 cell ^[2]	interleukin-4, interleukin-5, interleukin-6, interleukin-10, interleukin-13
Immune stimulation promoted	Cellular immune system. Maximizes the killing efficacy of the macrophages and the proliferation of cytotoxic CD8 ⁺ T cells. Also promotes the production of opsonizing antibodies	Humoral immune system. Stimulates B-cells into proliferation, to induce B-cell antibody class switching, and to increase neutralizing antibody production.
Other functions	The Type 1 cytokine IFN- γ increases the production of interleukin-12 by dendritic cells and macrophages, and via positive feedback, IL-12 stimulates the production of IFN- γ in helper T cells, thereby promoting the T _h 1 profile. IFN-gamma also inhibits the production of cytokines such as interleukin-4, an important cytokine associated with the Type 2 response, and thus it also acts to preserve its own response.	The Type 2 response promotes its own profile using two different cytokines. Interleukin-4 acts on helper T cells to promote the production of T _h 2 cytokines (including itself; it is auto-regulatory), while interleukin-10 (IL-10) inhibits a variety of cytokines including interleukin-2 and IFN- γ in helper T cells and IL-12 in dendritic cells and macrophages. The combined action of these two cytokines suggests that once the T cell has decided to produce these cytokines, that decision is preserved (and also encourages other T cells to do the same).

Mature T_h cells express the surface protein CD4 and are referred to as CD4⁺ T cells. CD4⁺ T cells are generally treated as having a pre-defined role as helper T cells within the immune system. For example, when an antigen presenting cell expresses an antigen on MHC class II, a CD4⁺ cell will aid those cells through a combination of cell to cell interactions (e.g. CD40 and CD40L) and through cytokines. Nevertheless, there are rare exceptions; for example, sub-groups of regulatory T cells, natural killer T cells, and cytotoxic T cells express CD4 (although cytotoxic examples have been observed in extremely low numbers in specific disease states, they are usually considered non-existent). All of the latter CD4⁺ T cell groups are not considered T helper cells (Rang, 2003).

The importance of helper T cells can be seen from HIV, a virus that infects CD4⁺ cells. Towards the end of an HIV infection the number of functional CD4⁺ T cells falls, which leads to the symptomatic stage of infection known as the acquired immunodeficiency syndrome (AIDS). There are also some rare disorders that result in the absence or dysfunction of CD4⁺ T cells. These disorders produce similar symptoms, and many of these are fatal. The activities of the CD4 cells also result in the maturation, activation and proliferation of other cells of the immune system. Thus, the CD4 cells play very significant roles in maintaining an efficient response to infection. Therefore the level of circulating CD4 lymphocytes is critical in the immune system of any individual at any point in time. The estimation of CD4 lymphocyte counts has been reported as a critical parameter in the establishment or monitoring of the immune status and function of the individual (Uppal *et al.*, 2003; Holmann, 1971; Njoku *et al.*, 2003). However, several studies have documented that the levels of CD4 cells and other immune-hematological cells in healthy human subjects vary amongst individuals and

within different geographical locations of the world (Wintrobe, 1981; Lee *et al.*, 1996). These differences have been attributed to many factors, which; includes genetic make-up, dietary patterns, sex and age (Chin *et al.*, 1993). Racial differences in the levels of these immune-hematological cells in normal humans have also been reported. The normal ranges of CD4 cell counts have been reported to vary widely amongst populations of Africa and Asia (Oladepo *et al.*, 2009; Njoku *et al.*, 2003; Idoko *et al.*, 2001). More so, earlier studies observed that the ranges of normal CD4 cells in the Africans and Asians were lower than the ranges recorded for North American and European populations (Audu *et al.*, in press; Bussmann *et al.*, 2004). Within African populations, the normal CD4 ranges in healthy Ethiopians (Bentwich *et al.*, 1996) were markedly lower than those in Ugandans (Elliot *et al.*, 1995).

In Nigeria, however, the normal reference CD4 count range for a healthy adult was determined to be between 365 and 1571 cells/ μ l (Oladepo *et al.*, 2009). In the present era of the HIV/AIDS pandemic, the CD4 lymphocyte counts are recognized as the most important measurement of overall HIV induced immune impairment. The CD4 lymphocyte counts are also an established predictor of disease free survival and serves as an important guide in decision to begin prophylactic interventions (Ray *et al.*, 2006). However, as the access to HIV treatment increased in Nigeria, the local reference values for CD4 cell counts became necessary to guide initiate of antiretroviral for people living with HIV (Oladepo *et al.*, 2009, Njoku *et al.*, 2003, 2001).

2.14 IMMUNOEPIDEMIOLOGY OF FEMALE UROGENITAL SCHISTOSOMIASIS

Surviving a chronic parasitic disease requires the generation of a controlled immune response that recognizes the invading pathogen and limits a potentially destructive host response. Several studies using parasites as model systems have shown

that deviating from the host natural immune response during infection can lead to severe consequences including exacerbated tissue pathology and even death (Li *et al.*, 1999; Hunter *et al.*, 1997; Gazzinelli *et al.*, 1996). In schistosomiasis, urinary schistosomiasis has been reported to be responsible for a substantial amount of schistosome associated pathology (WHO, 1993). The role of acquired immunity in reducing *Schistosoma* infection intensity in human populations has been subject to intense analysis (Butterworth *et al.*, 1992; Correa-Oliveira *et al.*, 1998; Dessein *et al.*, 1988; Dunne *et al.*, 1992; King *et al.*, 1989; Woolhouse *et al.*, 1991).

Schistosome immunoepidemiological studies have shown that the development of antigen specific immune responses is related to cumulative exposure to parasite antigens (Anderson, 1987 and Woolhouse and Hagan, 1999) and also the rate of development of different components of these responses give distinct profiles across the host age range (Mutapi *et al.*, 1997). In addition, an understanding of the rate of development of parasite specific immune responses derived from age profiles is useful in interpreting susceptibility and resistance to infection as well as the development of pathology. This is particularly important for the cellular responses, which determine the majority of effector functions and immune mediated schistosome pathology (Stadecker and Colley, 1992; Pearce and MacDonald, 2002; Hoffmann *et al.*, 2002). Over a period of time, detailed studies on the development of schistosome-specific cellular responses in mouse models have been conducted while in humans, it is yet to be conducted (Pearce and MacDonald, 2002). For example, murine studies have shown that chronic schistosomiasis disease is characterized by the establishment of a Th2-associated immune response against egg trapped in organs like the liver and intestines. Hallmarks of this Th2-associated immune response include up-regulation of the collagen-inducing cytokines IL-4 and IL-13 (Fertin *et al.*, 1991; Sempowski *et al.*, 1996; Chiamonte *et al.*, 1999), down regulation of the collagen suppressing cytokine, IFN- γ (Czaja *et al.*, 1993), sequestration of parasite eggs by eosinophil-enriched granulomas and the

development of tissue fibrosis (Cheever and Yap, 1997). According to Pearce and MacDonald (2002), the findings from these murine studies suggest that the development of effector Th1/Th2 and immunomodulatory responses reflects the parasite's developmental stage so that Th1 responses predominate in the early acute phase followed by the emergence of Th2 responses (which is stimulated by egg antigens) and decrease in Th1 responses (which is down modulated through an IL-10 dependent mechanisms) (Hesse *et al.*, 2004). For example, data from mice indicates that the granuloma is a cellular immune response to antigens secreted by viable eggs that recruits CD4⁺, $\alpha\beta$ ⁺- and major histocompatibility complex II dependent T cells.

The immune response is often controlled by counter-regulating cytokines, chemokines and cell adhesion molecules which by expression of their associated receptors successively initiate, maintain and immunomodulate the granuloma (Cheever and Yap, 1997). Given that differences occur in the immunology and immune-pathology of murine and human schistosomiasis (Cheever *et al.*, 2000; Abath *et al.*, 2006), human studies are essential for a clearer definition of human schistosome immunoepidemiology.

2.15 TREATMENT

As female urogenital schistosomiasis is not systematically diagnosed, it is therefore not surprising that knowledge on treatment is scanty (Poggensee *et al.*, 2001).

2.15.1 Chemotherapy

A conservative approach to various forms of FUGS using anti-*Schistosoma* drugs has been reported by a number of studies, showing either regression or disappearance of lesions after treatment (Richter *et al.*, 2003; Poggensee *et al.*, 2001). These anti-schistosomal drugs include Praziquantel, Artemether and Metrifonate. Praziquantel is the drug of choice for treatment (Cioli and Pica-Mathoccia, 2003; N'Goran *et al.*, 2003a, 2003b) and morbidity control of schistosomiasis (Colley and Evan Sector, 2004). Many

studies have reported that the drug has been in use for more than two decades and acts on all human species of *Schistosoma* with virtually no side effects (WHO, 1999). Praziquantel belongs to the pyrazinoquinolones drug category and is said to be stage specific; that is, the cercariae, young schistosomule and adult worms are more susceptible than the juvenile schistosomes (Xiao *et al.*, 1987; Sabah *et al.*, 1986). In animal studies, the drug induces rapid contraction of schistosomiasis by a specific effect on the permeability of the cell membrane (Poggensee *et al.*, 2001), that is to say, the effects of Praziquantel are associated with Ca_2^+ influx into the worm and this leads to a strong muscular and tegumental damage (Becker *et al.*, 1981). As a result of this tegumental injury, schistosome antigens are exposed at the surface (Harnett and Kusel, 1986), and in the mouse model, it has been shown that host immune response/antibodies are necessary to accomplish the effect of Praziquantel (Feldmeier *et al.*, 1998; Brindley *et al.*, 1989; Doenhoff *et al.*, 1987). The cure rate of Praziquantel was observed to be better in mice with heavy, as compared to mild infection (Nessim and Demerdash, 2000). The exact mechanism of action of Praziquantel is not really known, but a recent report claimed that schistosome calcium channels might be the molecular targets of Praziquantel (Kohn *et al.*, 2001, 2003). The rationale to use Praziquantel, however, is based on the fact that the elimination of the adult worms, enhances the resolution of egg induced granulomas and tissue fibrosis (N'Goran *et al.*, 2003, Rumbley and Phillips 2000; Latif *et al.*, 1998; Andrade, 1991; Ballardini *et al.*, 1985). Thus, using a standard regimen of Praziquantel (40mg/kg body weight), in a case study in Malawi; Richter, (2003), observed that symptoms disappeared in patients within 9 weeks, yet in another study, Poggensee *et al.*, (2000) noted that prevalence of FUGS decreased from 100% - 39% over a period of 12months. Simultaneously, the frequency of epithelial lesions such as oedema and disrupted epithelium diminished significantly, from 70% - 25% ($P < 0.05$)

(WHO, 1998). Other studies reported that mass treatment with Praziquantel reduced infection by up to 80% (Kusel and Hagan, 1999). A cure rate after a single dose of Praziquantel has been described as rather low, thus, a repeated administration of the drug, say, three consecutive days (40mg/kg/day) seems to be warranted in moderately to heavily infected women, in order to clear the infection (Alonso *et al.*, 2006; Lawn *et al.*, 2003).

Derivatives of *Artemisia* (artemether, artesunate, dihydroartemisinin) better known as anti-malarial drugs also have anti-schistosomal properties. Artemisinin kills schistosomulae that is: 1-3 weeks old but not the adult worms, of all three schistosome species that affect man (Hatz, 2000). Several clinical trials with artemether have shown its efficacy in reducing the incidence and intensity of infection (N’Goran *et al.*, 2003; Xiao *et al.*, 2000a, 200b, 2000c). A single treatment with artemether, especially when parasite is particularly susceptible between 3 and 4 weeks after infection, gave cure rates of up to 82%. Multiple therapies further raised the cure rate to almost 100% (Ouma *et al.*, 2000). The artemether treatment has been observed to affect the different parasites for slightly different lengths of time. For example, *S. japonicum* responded to the drug in 21 days; *S. mansoni* in 42 days and *S. haematobium* which has a longer time of developing into the adult stage, has an even longer period of sensitivity. Thus, the facts that both Praziquantel and artemether affect schistosomes at different developmental stages, a combined treatment with the two drugs have been reported to work better in combination (Poggensee *et al.*, 2001). Here, Praziquantel kills both the adult and young worms (although realistically, only during the first day in the host) when artemether has no effect at all on the parasite. Conversely, artemether affects the juvenile stages (except immediately after infection) by blocking its developmental growth into adulthood (Xiao *et al.*, 2002, 2003). However, the limitations to using artemether, is that it must be

administered repeatedly every 15 days throughout the transmission cycle in order to be protective. Secondly, apart from cost, it cannot be administered to individuals residing in malaria endemic regions so as to avoid the emergence of artemether-resistant malaria parasites (WHO, 1999; Xiao *et al.*, 2000a; 2000b).

Metrifonate is another alternative to Praziquantel for *S. haematobium* infections. It is an organophosphate derivative with anti-helminthic and anti-cholinesterase activity (Poggensee *et al.*, 2001). The drug is well absorbed from the gastrointestinal tract with peak levels occurring one hour after administration. The standard regimen is 10mg/kg/dose PO 2 weeks for 3 weeks for 3 doses; single doses of 10mg/kg at interval of 3, 6 and 12 months (WHO, 1998). Depression of blood cholinesterase has been reported as the most serious side effect observed, hence it is not administered to persons that have recently been exposed to pesticides or those receiving drugs that exacerbate cholinesterase inhibition (i.e. neuromuscular blocking agents). Thus, a delay of 48 hours after administration of metrifonate is necessary, if patients have already been exposed to the above agents (Poggensee *et al.*, 2001; Kabatereine *et al.*, 1998). The only disadvantage is that it has become very difficult to obtain in some African countries (Cioli and Pica-Mattocchia, 2003; Cioli, 1995).

The fourth drug is Oxamniquine, an antihelminthic that is used exclusively in the treatment of intestinal schistosomiasis in both Africa and South America. It is only effective on cercariae, young schistosomule and adult worms of *S. mansoni* (Cioli, 1995). The regimen is 40-60mg/kg *per os* (PO) over 2-3 days to minimize gastrointestinal discomfort, though increased dosage may be required in endemic areas. However, side effects like fever, dizziness and hallucinations have been reported (Poggensee *et al.*, 2001; Chitsulo *et al.*, 2000).

2.15.2 Surgery

Many lesions of the ovaries and cervix mistaken to be malignancy have been treated by aggressive surgery, such as ovariectomy and hysterectomy (Poggensee *et al.*, 2001). While such an approach may be justified when during laparoscopy, an enlarged ovary or a tubal mass is seen, irreversible surgery is by no means a warranty, for lesions seen in the lower reproductive tract (Richter *et al.*, 2003; Poggensee *et al.*, 2000a). For instance, if the differential diagnosis of a lesion in the cervix were cervical cancer, then the application of the quantitative compressed biopsy technique (QCBT) would invariably confirm FUGS within minutes before a surgery can be carried out (Hatz *et al.*, 1990, 1992).

2.15.3 Vaccination

There is strong need for schistosomiasis vaccination so as to complement the drug therapy. This is because drug treatment in combination with a vaccine would be beneficial and contribute to savings for the healthcare system as a whole, even if the protection afforded by the vaccine were not completely absolute (Vercruyse and Gabriel, 2005). In line with this *Schistosoma haematobium* vaccine project based on the worm enzyme glutathione-S-transferase (GST) has successfully passed Phase I & II testing. The Sh28-GST which is a prostaglandin D2 synthase produced by cercariae is now ready for the phase III vaccination trial (Bergquist *et al.*, 2002). Meanwhile, the Schistosomiasis Vaccine Development Programme (SCVP), based in Egypt and supported by USAID is focusing on two *S. mansoni* antigens; paramyosin, an invertebrate muscular protein, and a synthetic peptide construct containing multiple antigen epitopes (MAP) of the schistosome triose phosphatase isomerase (TPI) (McManus and Loukas, 2008).

2.16 CONTROL OF SCHISTOSOMIASIS

In the past decades, schistosomiasis control has switched from disease control to morbidity control. Numerous studies have identified schistosomiasis associated disease markers in order to assess the impact of treatment on morbidity. Not surprisingly, assessment of morbidity in genital schistosomiasis has completely been neglected (Poggensee *et al.*, 2001). This is so because despite successful control programmes that has taken place during the last 20years, the distribution of the disease is constant and increasing (Chitsulo *et al.*, 2000). Movement of people, increasing population, sanitary problems and the construction of irrigation plants and dams have resulted to the development and spread of schistosomiasis to new regions (King *et al.*, 2003; 2006; Spear *et al.*, 2004). Documented sequels of genital schistosomiasis such as infertility or ectopic pregnancy and the important role FUGS; assumedly, plays through interacting with other infectious diseases, make it essential to include genital morbidity into control measures aimed at morbidity reduction. So far, chemotherapy is currently the cornerstone of schistosomiasis control. The use of drug, for instance, has been reported to have led to a dramatic reduction of morbidity in endemic areas (King *et al.*, 2006; Magnussen *et al.*, 2003; WHO, 1999). In theory, schistosomiasis could be eradicated if excreted eggs are prevented from reaching freshwater bodies that harbor the intermediate snail hosts even if man was not the only definitive host (Fenwick *et al.*, 2003; Thors, 2006). Therefore, health education, installation of latrines, improvement of water supply, biological control of snails, good diagnostic tools, sanitation in combination with treatment have all been advocated for as good schistosomiasis control measures (King *et al.*, 2006; Magnussen *et al.*, 2003)

2.17 HUMAN IMMUNODEFICIENCY VIRUS (HIV) AND ACQUIRED IMMUNE DEFICIENCY SYNDROME (AIDS)

The Human immunodeficiency virus (HIV) infection is a progressive depletion of the CD4⁺ cells which results to a weakened immune system that is unable to control a range of endogenous and environmental pathogens. HIV infection causes Acquired Immunodeficiency Syndrome (AIDS) through the depletion and eventual exhaustion of immune responses, leading to clinical illness and eventually, death in most individuals (Fauci, 1988). AIDS was first reported in June, 1981 in Los Angeles USA (CDC, 1987; Gallo *et al.*, 1983). It was initially identified in homosexual men and was characterized by clusters of unusual diseases that had previously been extremely rare in young adults in the West (Essex and Mboup, 2002). This marked the beginning of HIV, the causative agent of AIDS whose epidemic has become the greatest public health challenge to mankind in recent years (UNAIDS, 2007; Gallo *et al.*, 1984). Further, in 1986, it became evident that there were two types of the virus; the predominant HIV type-1 (HIV-1) and HIV type-2 which is more restricted in distribution. HIV-1 was the first Lentivirus to be identified, followed by related viruses known as Simian Immunodeficiency Virus (SIV), which was discovered in monkeys. Soon after the identification of the SIVs, the HIV-2 was identified in populations of female commercial sex workers in West Africa (Dada *et al.*, 1993). HIV-2, according to Kanki *et al.*, (1997) is antigenically indistinguishable from SIVs but distinguishable from HIV-1, is less virulent than HIV-1 and can cause clinical AIDS that looks similar to that caused by HIV-1. In the same vein, HIV-2 was reported as a virus that does not spread so efficiently between people, either by sexual transmission or by transmission from infected mothers to their infants (Bulterys and Lepage, 1998; DeCock and Brun-Vezinet, 1989). Individuals with HIV-2 infection were reported to have initially come from West African Countries (like Senegal, Gambia,

Burkina Faso and Cote d'Ivoire) and now spread to East Africa, France, Asia and Latin America (Barre-Sinoussi *et al.*, 1996;), although, less common in Nigeria (Williams *et al.*, 1997). HIV type-1, from most sub-Saharan Africans, on the other hand, has been reported to have differences, in that the dominant HIV-1 of the West which is also quite different from the HIV-1 from Asia. The HIV-1 strains found in Africa are divided into three groups based on genetic diversity. The main group M had 10 subtypes A-I, the second group known as group O (outlier), represent a number of highly divergent strains while the third group are known as the N group (Janssen *et al.*, 1997; McCutchan, 2000). HIV infection is characterized by a chronic progressive destruction of the immune and neurologic system, leading to the development of multiple opportunistic infections (OIs) (Lindo *et al.*, 1998) and malignancies (Schulz *et al.*, 1996).

Although, on average, an infected individual spends several years without manifesting the disease, the time from infection to AIDS varies widely between individuals from a few months to as many as 20 years. Existing evidence also revealed that 50% of the individuals progress to AIDS in 10 years (<7 years in developing countries), which has been accepted as the incubation period of the virus (WHO, 1994). Furthermore, in 1999, the origin of HIV was traced to Chimpanzee of the West Equatorial Africa (*Pan Troglodytes*). Their investigations revealed that some of the virus sub-types was as a result of genetic recombination in the chimpanzees before they infected man (Sharp *et al.*, 1994). Many other studies alleged that HIV-1 was introduced to human population through exposed hunters via infected blood of the animals they killed (Weiss and Wrangham, 1999). Recently, the degree of mortality and morbidity resulting from HIV, the global impact on public health and its socioeconomic development throughout the world are already becoming unmanageable as the pandemic continues to grow (Erikstrup *et al.*, 2007; UNAIDS, 2007).

2.17.1 Mode of Infection

HIV virus primarily attaches to a subset of immune conscious, the CD4⁺ cells, or other host cells including T-helper cells macrophages and follicular dendritic cells in the lymph nodes. The glycoprotein's gp120 on the surface of the virus interacts and binds to the CD4⁺ antigen on the host cell while only the core of the virus containing the RNA core proteins and enzymes enters the host cell. Thus, the pathogen is intracellular and is maintained in every living blood and blood products in man (Barre-Sinoussi, 1996; Wolinsky, 1998; Idoko *et al.*, 2001).

2.17.2 Biology

HIV type-1 and type-2, like other retroviruses, (Fig. 3), contain a virus capsid; which consists of the major capsid protein, p24, the nucleocapsid protein p7/9, the diploid single-stranded RNA genome and three viral enzyme protease, reverse transcriptase and integrase (Weiss, 1996). The reverse transcriptase is capable of transcribing its genomic RNA into double stranded DNA (provirus). According to Levy, (1993), the provirus subsequently becomes a virion when matured and different genes encode the viral structural proteins. The group associated genes (*gag*), the protease, reverse transcriptase and integrase, *pol* encode the proteins, matrix proteins and p7/9. The viral protease is also required for the generation of individual proteins by proteolytic processing from the *gag* precursors and *gag/pol* precursor proteins, gp120 and gp41 encode the envelop precursor protein gp160 (Barre-Sinoussi *et al.*, 1996).

2.17.3 Classification

HIV is considered a member of the Lentivirus family of animal retroviruses on the basis of genetic sequence makeup, phenotype and life cycle (Fig 4). Lentiviruses constitute a separate genus of the retroviridae family, which includes a large number of

different viruses infecting diverse groups of animal species (Coffin, 1992). Retroviruses are single-stranded RNA-directed DNA-polymerase that possesses ribonuclease activity which enables the RNA of the virus to produce a DNA copy of itself in order to become integrated and replicated in host cells (Levy, 1993). The ability to transcribe DNA from RNA is a unique feature of retroviruses and gives them their name, because usually RNA is transcribed from DNA. Moreover, HIV is the first described human Lentivirus since most known lentiviruses infect animals, causing slow-progressing disease, including immunodeficiency in some primates (Schulz *et al.*, 1996). The lentiviruses are spherical particles 100-140nm in diameter with a spherical core (Coffin, 1992).

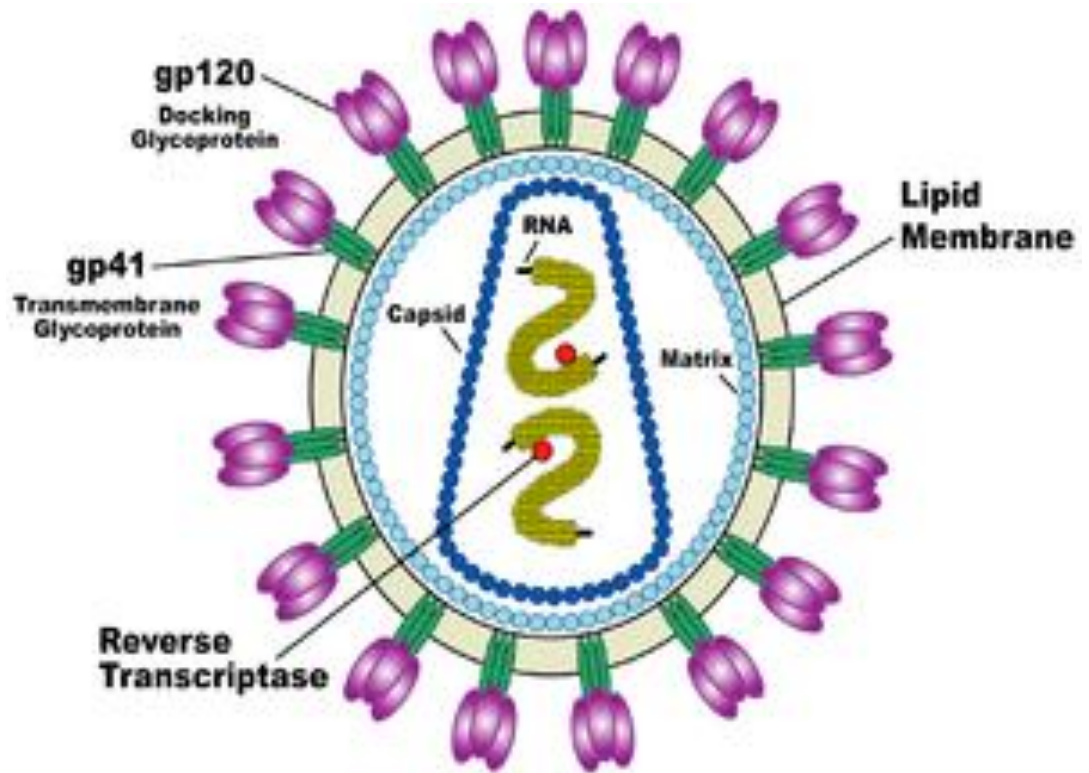


Fig. 3: Structure of an HIV Virion Particle (Barre-Sinoussi *et al.*, 1996).

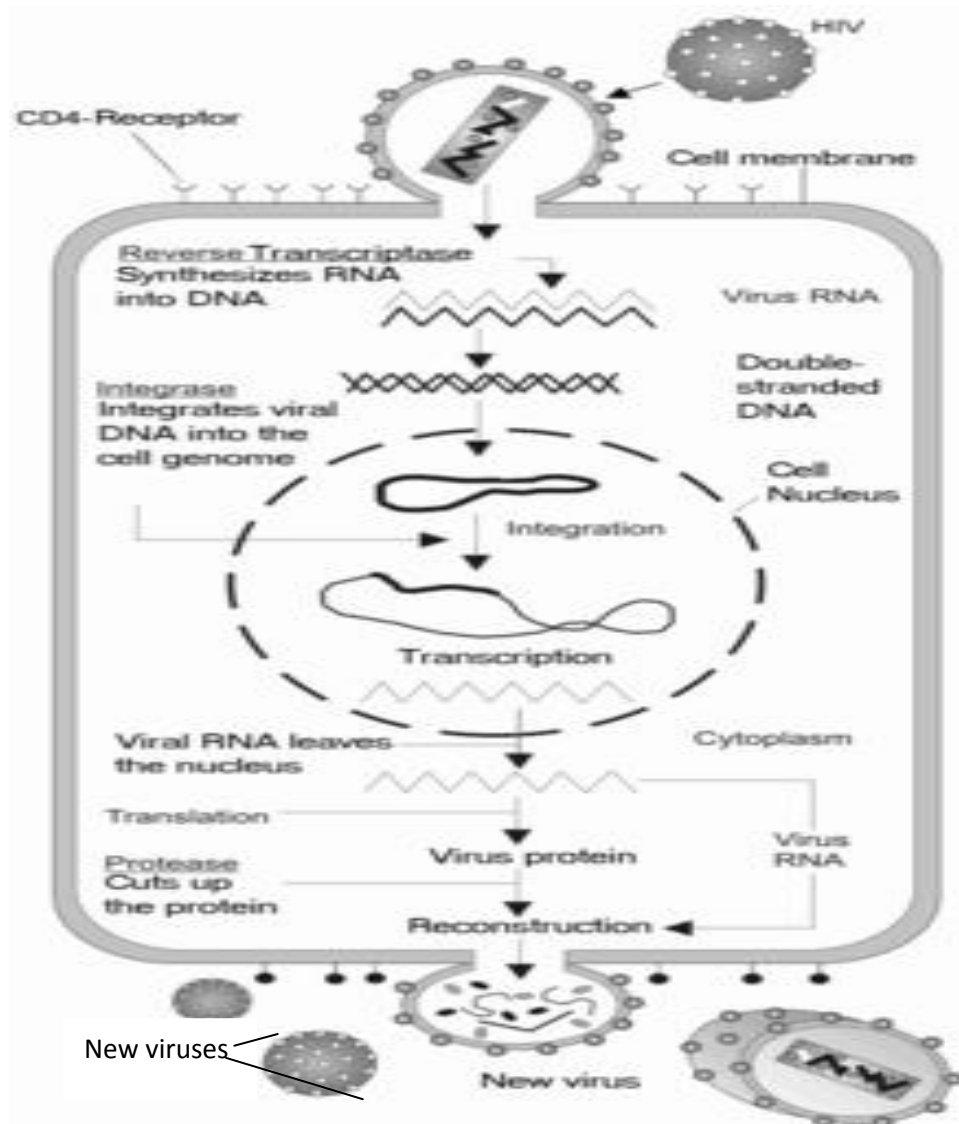


Fig.4: Life Cycle of Human Immunodeficiency Virus

HIV virus enters the host cells after binding to the cellular receptor, CD4⁺ and envelop protein complex mediated fusion of the viral lipid envelop with the cellular membrane. In the cytoplasm, where there is an initiation of reverse transcription, the pre-integration complex (proviral DNA) however, is targeted to the nucleus where the provirus is integrated into the host genome-the template for cellular DNA-dependent RNA polymerase in order to generate new viral RNA genome (Barre-Sinoussi *et al.*, 1996).

2.17.4 Burden of HIV Pandemics

More than two decades since the first case of AIDS was recognized, HIV has risen to the level of a global pandemic and has become a great concern to the world community. Although promising developments have been seen in recent years, in efforts to address the global AIDS epidemic, including access to effective treatment with management drugs and prevention programmes, the number of people living with HIV continue to remain high, as does the number of deaths due to AIDS (UNAIDS, 2006). This figure includes the estimated 4.3 million adults and children who were newly infected with HIV in 2006. Although about 21.8 million deaths have been reported to have occurred worldwide due to AIDS since the beginning of the pandemic, in 2006 alone about 2.9 million deaths were reported as against 2.7 million in 2004 (UNAIDS, 2007). The lengthy invisibility of HIV infection has made the condition easy to ignore, which has helped the epidemic to spread unchecked (WHO, 1996). Sub-Saharan Africa continues to bear the brunt of the global epidemic. About 70% all HIV globally live in sub-Saharan Africa (UNAIDS, 2007). The 1.6 million AIDS deaths in sub-Saharan Africa represent 76% of global AIDS deaths. Across this region, women were noted to bear a disproportionate part of the AIDS burden, because not only are they more likely than men to be infected with HIV. Furthermore, in many parts of the world, new HIV infections were noted to be heavily concentrated among young people 15 - 34 years. In 2007 alone, adults 15 years and older, accounted for 40% of new HIV infections (UNAIDS, 2007).

As at the end of 2003, Nigeria was adjudged to have the third highest burden of HIV in the world, after South Africa and India (UNADS, 2007). The HIV prevalence in Nigeria was reported to have been on a consistent increase from 1.8% in 1991 to 5.8% in

2001 before a decline to 5% in 2003 and 4.4% in 2005 (FMOH, 2006). From the results of the 2005 sentinel survey, it was estimated that about 2.9 million Nigerians are currently living with the virus (FMOH, 2006).

2.17.5 Human Immunodeficiency Virus in Nigeria

The first case of clinical AIDS in Nigeria was reported in 1986 in a 13-year old sexually active girl (Ani and Agwale, 1998). In the same year, reports of first cases of maternal HIV transmission and AIDS among commercial sex workers in Lagos and Enugu were made. In addition, cases of HIV seropositivity began to emerge from several parts of the country (Olaleye *et al.*, 1993; Harry *et al.*, 1993). No state of the federation is spared from the clutches of this endemic scourge (Sani-Gwarzo, 1998). However, reports from several studies of the 6 geo-political zones of the country on different aspects of HIV infection and implications now paint a hopeful future with the very positive impact antiretroviral therapy. The epidemic in Nigeria has since extended beyond the high risk groups to the general population. All the states of Nigeria could be said to have a generalized epidemic, which is described as heterogeneous with various communities in different stages, some declining and others still rising (FMOH, 2011, 2006). The north-central zone was considered to have the highest HIV prevalence with Benue State recording 10%. Plateau State, also located in this zone had a prevalence of 4.9% and it was considered to be low compared to a survey conducted in Jos where a HIV prevalence of 7.1% was recorded (Idoko *et al.*, 1998). The study further indicated that HIV was more pronounced among the 20-29 year olds especially those in the urban areas and also amongst persons with only primary and secondary education (FMOH, 2006). This report reaffirms the fact that no state or community is spared the epidemic, although there are wide variations in between the states, urban and rural areas across the

country. However, though there is a national decline in HIV spread from 2005 sentinel survey, it calls for no outright celebration or relaxation of intervention, since the survey may be inconclusive to make direct comparisons between aggregate figures obtained, due to differences in location and number of survey sites (FMOH, 2011).

2.18 HIV/AIDS IN AFRICA

HIV is responsible for the global pandemics of AIDS (UNAIDS, 1998, 2007). The epidemic has spread throughout the continents to devastating effect since it was first observed about 22 years ago in Africa (UNAIDS, 2007). Across sub-Saharan Africa, the average prevalence of HIV in adults aged 15-49 is 8.8%, while within sub-Saharan Africa, HIV is not uniformly distributed (NACA, 2012; Piot *et al.*, 2001). For instance, East Africa once had the highest infection rates on the continent but has now been overtaken by the southern cone. Among specific population groups that are disproportionately affected by the epidemic, there are a number of locations where HIV prevalence has been found to exceed 70%. This was observed among sex workers and people with sexually transmitted diseases (STDs) as well as occupational groups such as mine workers and military personnel (United States Bureau of the Census, 2000). Both HIV-1 and HIV-2 strains are spread in the same way and have the same AIDS causing consequences, though HIV-1 has a shorter incubation period of 7-10 years, compared to HIV-2, which is considerably longer and often less severe (UNAIDS, 2006; Barre-Sinoussi, 1996).

2.18.1 Routes of HIV Transmission

HIV is present in semen, vaginal/cervical secretions, blood and breast milk and these are the major routes by which the virus is transmitted. Some studies have reported that the virus may be present in saliva, tears, urine, stool, lymph, sweat, cerebrospinal

fluid and infected discharges, but are not the major routes by which HIV is spread (Siegal, 1981). Epidemiological evidence do not support transmission through water or food, sharing eating utensils, coughing or sneezing, toilets, swimming pools, insect bites, shaking of hands or other casual contacts, hence there is no public health reason for discrimination and/or restrictions. Transmission of the virus is generally through intimate sexual contact and contaminated blood. The syndrome was reported to have been observed first in bisexuals and homosexuals (Siegal, 1981), injection drug users and heterosexuals, hemophiliacs, blood and blood product recipients and infants through their mothers (Scarlati, 1996; Brookmeyer, 1991).

2.18.2 Risk Factors for HIV Transmission

Heterosexual transmission

Heterosexual intercourse forms the major portal of entry for HIV. In Africa alone, the predominant mode of HIV transmission is by heterosexual contact. This is due to the fact that 10-30% of seminal/vaginal fluid have transmissible virus (Royce *et al.*, 1997; Henin *et al.*, 1993). Thus, AIDS was associated with sexual route, with high prevalence in homosexual men. Subsequently, the pathogen became synonymous with heterosexual activity and is now responsible for the pandemic (Stoneburner *et al.*, 1990; UNAIDS, 1998). Epidemiological data unanimously agree on the point of entry which is through the vagina or the anal canal, implying that abrasions at these sites presumably would increase transmission. This has helped to explain the high level of heterosexual spread in sub-Saharan Africa and other developing countries where genital ulcers from venereal diseases (e.g. syphilis, herpes virus infections), genital inflammations and lesions due to STDs are associated with increased HIV seroprevalence (Gray *et al.*, 2001; UNAIDS, 1998a; Hook *et al.*, 1992). For example, higher probabilities of transmission were reported in Thailand and Kenyan for heterosexual sex with

commercial sex workers (Mastro *et al.*, 1994), possibly due to the presence of STDs, particularly those that cause genital ulceration (Gray *et al.*, 2001) or discharge (Quinn *et al.*, 2000).

Bouvet *et al.*, (1997) believes that vaginal pH neutralization by semen is a co-factor of HIV transmission. Heise *et al.*, (1991), in another study reported on how HIV could directly infect the bowel mucosa, and perhaps cervical epithelium without the need for ulcerations. This is where the relatively low risk of the mucosal lining of the foreskin, urethral canal and oral genital contact (though minimal) were all implicated (Winkelstein *et al.*, 1987). The risk of infection increases with the number of sexual partners, a person has. Although, exposure to HIV is needed for the induction of immune activity against infection, as observed in HIV resistant groups (Catalfamo *et al.*, 2008). This has not yet been validated in the general population.

Blood transfusion

Blood transfusion is the third most important mode of HIV-1 transmission in Africa. Although it's proportional significance has declined in comparison to the massive sexual transmission. Blood-borne HIV transmission is well known (just as pre-screening of donated blood for HIV antibodies reduces the incidence of transfusion associated AIDS) (Harry *et al.*, 1993). Records of transmission through other body fluids (e.g. saliva) strongly suggests that a rare source of viral infection (<10% of both free virus and infected cell) have been reported in saliva (Mortimer and Parry, 1994). Nonetheless, this fraction remains important in HIV spread. Urine, sweat, breast milk, amniotic fluid, feces and tears have also been shown to contain none or only low levels of infectious HIV (Dean *et al.*, 1988; Mundy *et al.*, 1987). Studies around the world have reported several cases of vertical transmission of HIV that is, from mother to child.

At the end of 2008, there were 2.1 million children living with HIV around the world. An estimated 430,000 children became newly infected with HIV in 2008. Most children living with HIV (9 out of 10), live in Sub-Saharan Africa, the region of the world where AIDS has taken its greatest toll. Around 90% of all children living with HIV acquired the infection from their mothers during pregnancy, birth or breastfeeding (UNAIDS 2009). In countries where antiretroviral therapy is available, the rate of transmission is 4% to 10% and has been reduced even further by frequent caesarian section (Luo, 2000). According to Bulterys and Lepage, (1998); the high rate of mother-to-child transmission in the sub-Saharan Africa was attributed to a lack of access for women to both HIV care and to ante- and postnatal care.

2.19 DIAGNOSIS OF HUMAN IMMUNODEFICIENCY VIRUS AND ACQUIRED IMMUNE DEFICIENCY SYNDROME

Serological assays have been an established method for the clinical diagnosis of HIV infection since the 1980s. These techniques for detecting HIV infection have also been fundamental to the screening of blood donations and blood products and to the epidemiologic monitoring of the severity and extent of the AIDS epidemic worldwide.

HIV detection methods are based on the identification of various markers. The indirect or serologic detection is based on the identification of specific antibodies to HIV, while direct detection is based on the identification of whole viral particles, circulating antigens, or viral nucleic acids in biologic samples. Examples of assays that utilize each of these detection methods include the Enzyme-Linked Immunosorbent Assay (ELISA), the most common serologic or indirect assay; and the Polymerase Chain Reaction (PCR), the most common direct detection assay. However, the genetic variability of HIV can reduce the efficiency of various HIV assays including that of antibody tests and viral nucleic acid tests (Weiss and Goodnough, 2005; Kanki *et al.*, 1997).

2.19.1 HIV Screening Assays

Enzyme-linked immunosorbent assay (ELISA)

The enzyme-linked immunosorbent assays are useful in many settings because they are simple to perform, relatively inexpensive, standardized, reproducible, highly sensitive and able to test large numbers of samples simultaneously. The First generation ELISA tests made use of disrupted whole virus that was obtained from purified HIV virus grown on cell culture. The second generation ELISA tests makes use of recombinant proteins or synthetic peptides for higher specificity while the third generation ELISA tests are based on the sandwich format; though the various ELISA tests use enzyme conjugates bound to specific antibodies (HIV, Schistosoma) -which , are bound to colorimetric substrates that produce measured color in a reaction catalyzed by enzyme conjugate.

Simple tests

These tests are often based on agglutination and can also be performed without instrumentation, but will take longer than 30 minutes to perform. The solid phase could be red blood cells, latex particles, or gelatin. Simple tests cost less than rapid tests (FMOH, 2006, Kanki *et al.*, 1997).

2.19.2 Confirmatory Assays

Western blot analysis

The use of Western blot Assay is to confirm a serum as positive for antibodies to HIV (Williams *et al.*, 1997). The assay is known as the gold standard of HIV testing, is based on electrophoretic migration of a viral lysates on a denaturing polyacrylamide gel to separate viral proteins according to their molecular weight. The assay also known as immunoblotting is highly specific and detects antibodies against the complex mixture of antigens found in HIV infection (Constantine, 1993). While ELISA and other related screening tests rely on color change or a measure of optical densities (OD) of such color,

western blot immunoassay relies on reactivity observed in any of the following major products of structural genes *gag* (*p24*) and *env* (*gp120/160*; *gp41*) (WHO, 1991). It also allows for identification of specific antibodies bound to their corresponding proteins. In interpretation of the western blot assay, the procedure is highly technical and requires well-trained personnel. The results are interpreted by criteria that vary according to the lab performing the test. The bands must have a score of one band positive (+) or greater, while a blot without any HIV-1 specific bands are considered negative. Indeterminate results also do occur with western blot when only one or more viral specific bands observed are insufficient to call positive or reject as negative (Anglaret *et al.*, 1997; Williams *et al.*, 1997; Sharp *et al.*, 1994).

Polymerase chain reaction (PCR)

The polymerase chain reaction detects proviral DNA in lymphocytes. Its value is mainly in the early detection of HIV-1 infection. The PCR technique, however, is difficult, expensive and requires the facilities of specialized HIV testing laboratory. It cannot be performed in a clinical diagnostic laboratory for routine diagnosis. It is currently only in use in advanced research laboratories (Young *et al.*, 2000). The PCR-based detection systems are some of the most sensitive tools developed for clinical and research applications. In addition to diagnosis and clinical assessment of HIV-infected individuals, PCR-based technologies have been fundamental to a large number of discoveries that have expanded our understanding of HIV biology, pathogenesis, variation, evolution, and epidemiology.

2.20 FEMALE UROGENITAL SCHISTOSOMIASIS AND HIV/AIDS

Pathophysiological, immunological and epidemiological evidence suggests that female urogenital schistosomiasis (FUGS) is a risk factor for the transmission of agents of sexually transmitted diseases (STDs) but presumably also alters the natural history of

such infections of which the Human Immunodeficiency Virus (HIV) is one. Schistosomiasis of the lower reproductive tract has been reported to interfere with the natural history of HIV infections at various levels starting from an impaired barrier function of genital epithelium to the deleterious modulation of protective immune responses (Shattock *et al.*, 2000). With the disruption of the epithelial surface, the ability of the epithelium to act as an effective physical barrier to any viral transmission is diminished (Feldmeier *et al.*, 1994; Royce *et al.*, 1997; Maybey, 2000). It has been convincingly demonstrated that breaks in the integrity of the mucosal barrier due to either trauma or sexually transmitted ulcerative diseases are associated with an increased risk for HIV transmission (Rebbapragada *et al.*, 2007; 2008; Kaul *et al.*, 2008).

Subsequently, FUGS of the lower reproductive tract does not present itself as a simple localized sore as in other sexually transmitted ulcerative diseases, rather various lesions co-exist surrounded by an altered epithelium (Poggensee *et al.*, 2001). Thus, thinning, erosion and ulceration of the epithelium are typical findings of urogenital schistosomiasis (Poggensee *et al.*, 2000; Kjetland *et al.*, 1996). More studies have observed that women with genital schistosomiasis often had lesions on their vulva, vagina and cervix (Poggensee *et al.*, 2001; Midzi *et al.*, 2003). Not only do these lesions erode the physical protection provided by an intact epithelial layer, but they also recruit activated lymphocytes that may become targets for viral infection at the site of viral exposure (Leutscher *et al.*, 1997; Mutapi *et al.*, 2008). According to Kjetland *et al.*, (2006), schistosomiasis of the cervix is characterized by sandy patches, contact bleeding and neovascularization of the epithelium which provides direct access to the systemic circulation of the HIV virus during sexual intercourse. Similarly, Shattock *et al.*, (2000) noted that since vaginal and cervical lesions tend to bleed easily, HIV present in semen of an infected man could easily have a direct access to the blood circulation of the

woman via ulcerated lesions. In addition, HIV in semen could have easier access to a deeper genital cell layers in women with urogenital schistosomiasis because of the friable, eroded epithelium or through broken vessels during coitus, thus, creating direct points of contact between HIV and the receptive cells in the genital tissues (Feldmeier *et al.*, (1994). These pathological alterations have also been suggested to facilitate as well as propagate high-risk HPV infection (Feldmeier *et al.*, 1997). This was confirmed in a case report by Petry *et al.*, (2003). Similarly, a study on Tanzanian women confirmed FUGS of the cervix with significant clinical signs of erosion, ulcerations, sandy-patches and leukoplakia (Kjetland *et al.*, 1996; Feldmeier *et al.*, 1994). In another study on Zimbabwean women, urogenital schistosomiasis was strongly associated with homogenous yellow sandy patches, pathologic vessel morphology and bleeding (Kjetland *et al.*, 2005). Vaginal washes from women with genital *S. haematobium* infections have been observed to contain elevated levels of eosinophil cationic protein; suggesting that the lesions provide a Th2 environment, which perhaps could even further increase susceptibility to HIV-infection. The regional lymph nodes are also highly suitable for viral replication, early onset of infection (Mutapi *et al.*, 2008).

Schistosome eggs on the other hand, evoke a complex cellular and humoral immune response. There is increasing evidence that schistosomiasis modulates the transmission of HIV infection in several deleterious ways (Poggensee *et al.*, 2000; 2001). First, granulomata formation around schistosome eggs are as a result of delayed type hypersensitivity reactions mediated through a cell mediated immune response to soluble egg antigens (Rumbley and Phillips, 2000). These granulomata are composed of activated lymphocytes, macrophages, epithelioid cells and Langerhans giant cells, which are cell types that express CD4 cells, the receptors used by HIV-1 to enter the host cell (Harms and Feldmeier, 2002). These cells which are abundant within egg granulomas

and adjacent areas, all combine to alter the pattern of cytokines secreted locally during the inflammatory response (Helling-Giese *et al.*, 1996; Rumbley and Phillips, 2000). Secondly, immune cells in the genital lesion or in adjacent areas may provide an alternative route for HIV infection (Fincham *et al.*, 2003; Sheffield *et al.*, 2007). Inflammation around these egg-associated lesions recruit activated immune cells expressing the CD4 and higher expression of the CCR5 receptor on the surface of T-cells, increasing the risk and opportunity for HIV to bind (Secor *et al.*, 2003; Poggensee *et al.*, 2001). In fact, immunologically activated areas in the genitals have been noted to be easy points of entry for the HIV virus to attach to (Wald, 2002; Rebbapragada *et al.*, 2008; Wira and Fahey, 2008).

This was obvious when secretion of pro-inflammatory cytokines such as interferon-gamma, interleukin-2 (IL-2), Tumour Necrosis Factor (TNF) and the generation of reactive oxygen intermediates, all triggered the replication of the HIV virus (Moriuchi *et al.*, 1996) by stimulating the production of NF_{kappa}B (Bentwich, 2003, 1999). It also enhanced the susceptibility of different cell populations to HIV infection and increases the permeability between epithelial junctions (Shattock and Griffin, 1996). Rumbley and Phillips (2000), further explained that the concentrations of cytokines released when new granulomas build up, are high and the resultant cell inflammation increases the Human Leukocyte Antigen (HLA) expressions on the epithelial cells which potentially enhances the ability of such cells to readily bind to the HIV infected CD4⁺ cells (Weiss, 2000).

Thirdly, eosinophils represent almost 50% of the granuloma cells (Weinstock *et al.*, 1988). In other words, when put together the abundance of CD4⁺ receptor bearing cells within the confines of the granuloma and in adjacent areas, make a rapid binding of

the virus after penetrating through the friable and eroded epithelium very likely (Poggensee *et al.*, 2001). Fincham *et al.*, (1999) however, gave some evidence that eosinophils express the CD4⁺ receptor, which may constitute a compartment that is susceptible for HIV infection. More so, the immunological sequence after the integration of HIV into appropriate cells in the sub-epithelial tissues, in part determines the latter clinical course of the infection. The picture that emerges is one of battle during the initial weeks after the HIV infection, between the virus trying to replicate in CD4⁺ cells and the CD4⁺ cells trying to respond to sites of viral replication. Losing this essential battle, in the early phase of HIV infection, results in a rapid propagation of HIV (Del Amo *et al.*, 1998; Levy, 1993, Brown *et al.*, 2006). Conceivably, the early bursts of viral replication as well as the attempt of the immune system to control the infection are both altered in the presence of urogenital schistosomiasis, based on the assumption that induction of the transcription factor NF_{kappa}β through the cytokines released from the granuloma may augment the early viral burst (Feldmeier *et al.*, 1998). In addition, due to identical binding sites for NF_{kappa}β in the host DNA and the enhancer region of HIV, increase in the transcription will also augment the replication rate of HIV considerably (Moriuch, *et al.*, 1996; Hu and Temin, 1990).

In the initial phase of HIV infection, protective immune mechanisms assumedly are mediated by cytotoxic T cells (CTL) and the NK cells (Takeda *et al.*, 2000, Chen and Paul 1997). In other words, experimental evidence has proven that schistosomiasis impairs the CTL response to retrovirus infection (Actor *et al.*, 1993) thereby, reducing the number of circulating NK cells as well as their efficiency to kill (Poggensee *et al.*, 1999). After the spread of HIV to the systemic circulation, its replication is said to be limited by the few activated lymphocytes and differentiated macrophages present in the blood stream (Shaunak and Teo, 1996).

Nonetheless, in patients with FUGS, panels of immunological alterations have been observed to facilitate rapid replication of HIV in the systemic circulation (Bentwich *et al.*, 1995; 2003). First of all, the number of activated T cells expressing HLA-Dr. and HIV co-receptors were observed to be elevated (Hirayama *et al.*, 1999). Secondly, HIV replicates preferentially in activated T cells, especially those with a Th2 and Th0 phenotype (Shaunak *et al.*, 1999, Koot *et al.*, 1996) and these Th2 cells are usually abundant in FUGS infected individuals (Bentwich *et al.*, 1999). Thirdly, peripheral blood mononuclear cells of patients with helminthes infections have been reported to be significantly, more susceptible to HIV infection than their uninfected counterparts (Modjarrad, 2005; Pearce *et al.*, 1991; Brown *et al.*, 2005).

Consequently, the early presence of the IL-4 which is the most potent stimulus for Th2 differentiation is elevated. The inducing effect of the IL-4 dominates over other cytokines so that if IL-4 levels reach a certain threshold, differentiation of the TH cell into the Th2 phenotype ensures (Fincham *et al.*, 2003). And finally, these elevated IL-4 levels, characteristic for the Th2 type of immune response in helminthic infection, down regulates the Th1 cells, inhibit macro activity and impair the cytotoxic T-lymphocyte response (Bundy *et al.*, 2000).

The assumption that immune dysregulation associated with chronic helminthic infections alters the course of HIV infection however, were sustained by a field study from Ethiopia, whereby the HIV load was significantly higher in individuals with various helminthic infections, in particular FUGS, than in individuals without and it also correlated positively to the parasite load (Bentwich *et al.*, 2000; Crump *et al.*, 2000). Furthermore, the viral load decreased after elimination of the worms by anti-parasitic treatment (Bentwich *et al.*, 2000). Taken together mucosal transmission is a relatively inefficient mode of HIV transmission compared with intravenous inoculation but with FUGS, it is much more efficient (Poggensee *et al.*, 2001) and this explains why women

with FUGS would have a higher per episode risk. Thus, the immunological characterization of schistosomiasis in general, and the clinicopathological characteristics of female urogenital schistosomiasis in particular make infection of women with HIV and rapid propagation of the infection likely especially since urogenital schistosomiasis seems to favor the initiation of virus replication in sub-epithelial tissues in order to increase the initial viral burst replication (Poggensee *et al.*, 2001). Hence, a faster progression from symptomless HIV infection to full-blown AIDS becomes more pronounced in FUGS women (Morgan and Whitworth, 2001).

CHAPTER THREE MATERIALS AND METHODS

3.1 ETHICAL APPROVAL AND INFORMED CONSENTS

The ethical committee of Plateau State Specialist Hospital (PSSH), Jos granted approval for this study. Official permission was sought and obtained from community leaders, principals or administrative heads of schools together with written informed consents from parents of volunteer students and volunteer subjects that participated in the study.

3.2 DESCRIPTION OF STUDY AREA

Jos, capital of Plateau State is a City located in the middle belt zone of the country, and in the North east area of North Central Nigeria. It lies between latitudes 70° and 110° north and longitudes 70° and 25° east in the Middle Belt of Nigeria. It is known for its waterfalls, mining and dairy industries. It is situated almost at the geographical centre of Nigeria and about 179 kilometers (111 miles) from Abuja, the nation's capital; Jos is linked by road, rail and air to the rest of the country. The city has a population of about 900,000 residents based on the 2006 census. [Jos crisis-Judicial Enquiry, 1994].

Jos consists of deeply eroded remnant volcanic rocks with an average maximum temperature, of 28°C-30°C, an average minimum temperature of 11°C, and a mean annual rainfall of 1,300mm, with the rain lasting between 6 and 7 months. Jos lies at an elevation of about 1,238 meters or 4,062 feet high above sea level and is noted for her savannah surroundings, relatively cool climate and high altitude qualities. Jos is an important national administrative, commercial, and tourist centre. The city is divided into 3 local government areas, which are Jos north, Jos south and Jos east. Jos is the industrial centre of Plateau State due to the presence of industries like the NASCO

group, Standard Biscuits, Grand Cereals and Oil Mills, aluminium roofing industries, Jos International Breweries among others. This "melting pot" of race, ethnicity and religion makes Jos one of the most cosmopolitan cities in Nigeria. Tin mining has led to the influx of migrants from all over the country.

Mining activities for over a century now, has brought to the Plateau, many expatriates and Nigerians from other parts of the country with easy access by road, air and rail links. Today this has resulted in high number of recreational, tourists, institutions of higher learning and research (National Institute of Policy and Strategic Studies (NIPSS), the University of Jos; Jos University Teaching Hospital, JUTH; National Veterinary Research Institute, etc). It is formed on a basement of complex rocks, which have produced the characteristic iceberg landscape. The Plateau highlands stand at an average height of 1200 meters above sea level with peaks like the famous Sherri Hills, rising over 5000 meters above sea level. The highlands are slightly undulating and rise from the steep escarpments of the river line plains of the River Benue and descend towards Bauchi State.

Their temperature climatic conditions are greatly influenced by their strategic location on the Plateau, making Jos climate the nearest equivalent to the temperature climate in Europe and America. The months of December through February are particularly cold due to the dry harmattan winds. This peculiar climate of Jos has endeared it to people of different cultures and tribes. Due to these attributes, Jos is noted as a favourite holiday location for both tourists and expatriates based in Nigeria and other countries (Plateau State Lands and Survey, 2008; Fig. 5 and 6).

3.3 SOCIOECONOMIC INFORMATION AND ACTIVITIES

Plateau State has over 50 ethnic groups including the Dass, Ngas, Berom, Taroh, Jarawa (Afizere), Ganowuri, Irigwe, Pan, Rukuba, Goemal, Anaguta and Pakara. These

ethnic groups are known with diverse cultural festivals. Tin mining; predominated economic activities in this area; but is now giving way to subsistence agriculture. Due to its climate, crops such as Irish potatoes, millet, guinea corn, maize, beans, wheat and groundnut are produced. These cereals and food crops form major source of local brews which includes; Gosklolo, conventional alcohol (including beer and hot drink of assorted brands and grades) Pito, Burukutu, Mos, Kunnu to mention but a few. . The area is also known as a city of commerce with other local enterprises such as food processing, beer brewing, and the manufacture of cosmetics, soap, rope, jute bags, and furniture. Livestock like cattle, goats, sheep and pigs are also kept. Heavy industry produces cement and asbestos cement, crushed stone, rolled steel, and tire retreads. In addition, subsistence farming is also practiced in these areas. Crops like maize, cocoyam, sweet potatoes, Irish potatoes, cabbage, tomatoes and other crops were planted, generally, for both domestic and commercial purposes.



Figure 5: Map of Nigeria Showing Jos in Plateau State

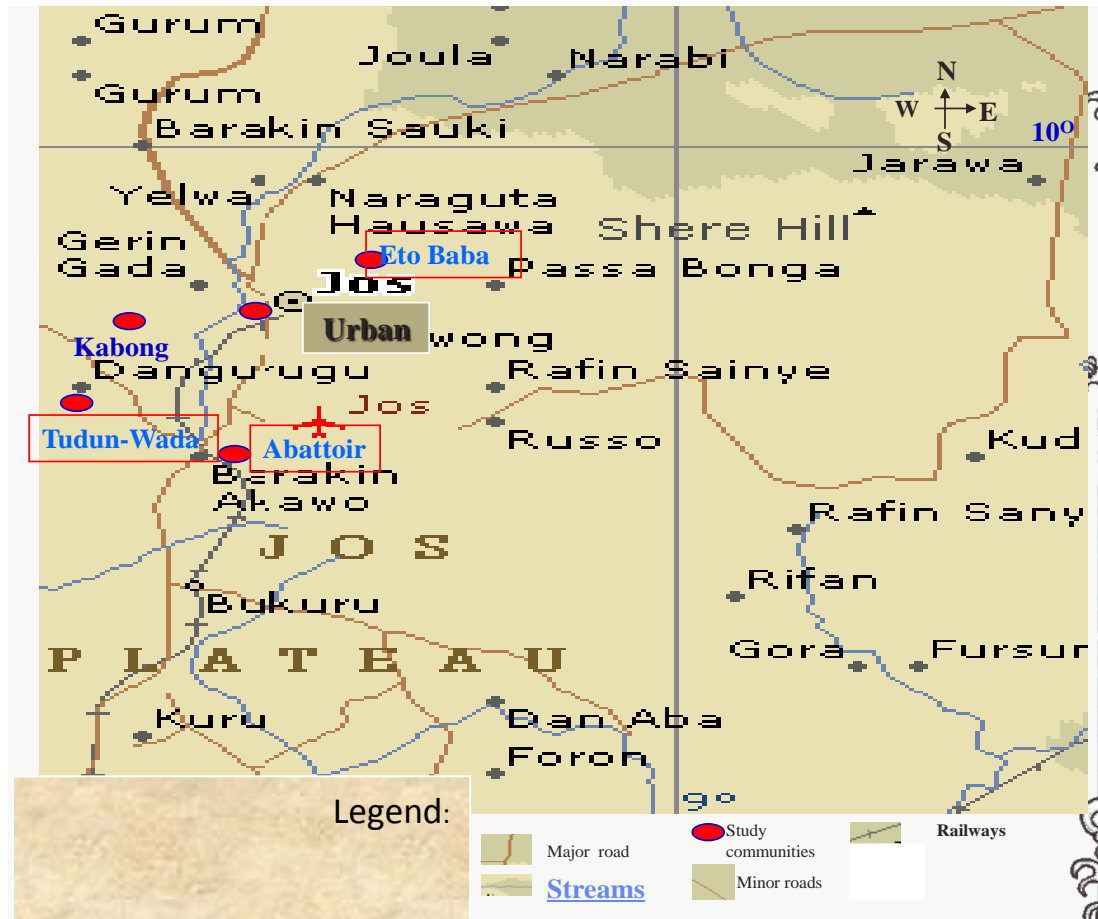


Fig.6: Map of Jos, Plateau State Showing the Study Area Communities and Streams
(Plateau State Lands and Survey, 2008)

3.4 SELECTION OF STUDY SITES AND STUDY POPULATION

The choice of study area was based on several factors which included, records of *Schistosoma* infections reported by other studies (Mafuyai *et al.*, 2006; Cater Center, 2007), hospital and primary health care centers located in the areas, the indiscriminate and rampant defecation observed along the shores of the streams, in the streams and around the houses of the residents, and the lack of pipe borne water for drinking and domestic chores. Due to the location of the study sites and the formation of rocks that are part of its edaphic nature, perennial shallow freshwater, were observed flowing from these rocks throughout the communities. Several canals dug in order to irrigate the farms during the dry periods and also to collect water during the rains were located in the areas. These canals contained water and together with the perennial shallow freshwater from the Rock Springs; created permanent shallow freshwater channels that promoted the growth of aquatic plants which harbored various schistosome snail host. Major human Water Contact Activities (WCAs) observed in these areas included, bathing, playing, fishing, washing of vehicles, fetching water for household chores, fetching water for block molding, fetching water for crop watering and irrigation farming, etc. Although limited alternative water sources such as boreholes and pools of water that were gathered during the rainy seasons existed, the residents still preferred the customary water contact points at the streams. Sometimes they bath due to the hot weather conditions experienced in the dry season. Thus, a total of 3 major streams with up to six snail sampling sites were monitored. They were selected based on observed human water contact activities.

Based on these factors, a study on the prevalence of urinary schistosomiasis and HIV was conducted between 2006 and 2009. The study population were mainly females- school children and adults (from ages 5years and above), from Abattoir, Tudun-Wada, Kabong (Nabong) areas of Jos and Jos urban communities (i.e., referrals

from out-patient and In-patient clinics of several urban and rural health centers to Plateau State Specialist Hospital (PSSH) which were routinely collected three times a week at PSSH for 1 year). Urine samples of both adults and school children from the endemic communities were conducted for 9 months. All samples were stored at -40°C and positive samples for urinary schistosomiasis and HIV were noted and classified for further analysis.

3.5 EPIDEMIOLOGICAL STUDY

3.5.1 Sampling of Snail intermediate hosts

The snail species were collected with scoop nets (Plate 4) from the underside vegetations surrounding the streams, along the banks of streams, around irrigated farms and stagnant water bodies found in the study areas. Collected snails were washed and later transferred to the laboratory, where they were separated by species according to Danish Bilhaziasis Laboratory reference key as described by Christensen and Frandsen (1985) and screened for cercarial shedding (Plate 5). Ten snails were placed in glass vials containing clear, filtered water, exposed to indirect sunlight for a maximum duration of three hours. Snails that did not shed cercariae on the first exposure were re-exposed on the second day. For every 10 snails, thousands of cercariae were shed, categorized either as those of *S. haematobium* or *S. mansoni* (Christensen and Frandsen, 1985; plate 6). Cercarial densities were calculated from weekly and monthly filtrations. Snail infection rate was determined by the percentage number of snails shedding cercarial over total number collected per species. Snail shells were later preserved for further identification studies.

3.5.2 Observation of Water Contact Patterns

The direct observation method as described by WHO (1979, 1987); was used to investigate water contact activities; which exposes subjects to urinary schistosomiasis

infection. Three research assistants were recruited and trained to assist in the monitoring and recording of the water contact behaviors of the population. Water contact behaviour was observed twice weekly at six sites of three major local streams, which had most of water contact activities (WCA) of interest, including the irrigation farming fields, laundry sites and community contacts for domestic, recreation and economic uses (block industries, processing of cattle horns and bones). Age, type of activity and the contact site used were recorded.



Plate 4: Field Sampling of *S. haematobium* Snail Intermediate Hosts



Plate 5: *Bulinus* species from schistosomiasis endemic communities of Tudun –Wada, Nabong and Abattoir



x2 Megapixel
(*Microscope Digital Camera Eyepiece,*
CMOS Micron MT9P001)

Plate 6: *Schistosoma haematobium* cercariae observed on slide in lugol iodine

3.6 SPECIMEN COLLECTION FOR PARASITOLOGIC, URINALYSIS AND IMMUNOASSAY STUDIES

3.6.1 Sample Collection

Urine specimens of consented subjects were collected after a 20-30 minute brief physical exercise between 10:00 and 12:00hrs. Each participant was given a pre numbered sterile wide-mouthed plastic bottle in the field with the name and age of the person entered against the appropriate number on a form kept for further analysis. Sampled subjects were also interviewed on other clinical signs of the disease. The signs included cystitis, haematuria, dysuria, tender abdomen, leukocyturia and supra-pubic tenderness. Their urine samples were screened for urinary schistosomiasis using the Urine Syringe Filtration Technique as described by WHO (1983). Furthermore, 5ml of venous blood was obtained by venipuncture from consented individuals and transferred immediately into a vacutainer. The serum was later derived from each blood sample in the laboratory and stored at -40°C until assayed. All samples collected (i.e. urine and blood) were replicated twice and screened later in the Laboratory for AIDS and Leishmaniasis Research, Department of Zoology, University of Jos. HIV serological testing by the use of parallel testing was routinely done on plasma specimens of individuals.

For follow-up studies, urine and plasma (derived from blood) of randomly selected volunteer subjects (537) were screened. The physical and chemical indicators of the urinary tract pathology of positive urogenital schistosomiasis persons were assessed and measured using the urine reagent strip. This test showed the chemical/physical profile of individuals' urine tested as well assesses urogenital pathology after physical observation of all urine samples was conducted.

Among the 381 urinary schistosomiasis positive subjects, only 94 volunteered to continue and met the criteria for follow up study (number still varied due to participants' dedication, absenteeism in school and Jos City crisis).

3.6.2 Assessment of FUGS Immunopathology using Cytokines and T Cells

Urine and plasma of apparently healthy, urogenital schistosomiasis/HIV co-infected volunteers were collected at intervals of 3, 6, 9 and 12 months after baseline. Commercial enzyme linked immunosorbent assay (ELISA), were used to assess the presence and level of pro-inflammatory cytokines and; while commercial CD4 count kits were used to measure the CD4 levels of the study population.

3.6.3 Treatment

Plateau State Human Virology and Research Center (PLASVIREC) situated inside PSSH, provided care, treatment and management for HIV infected individuals in the study through the antiretroviral therapy team. All these benefits provided an added advantage in the recruitment of subjects for the study, especially for those that constituted the urban communities. HIV serostatus and history of diagnosis were recorded. Other hospital information on associated infections were also obtained from volunteer patients' records. Treatment was also provided for individuals that were found infected with urogenital schistosomiasis. The drug of choice, Praziquantel was given in a standard dose of (40mg/kg) according to body weight to all the individuals that tested positive. In addition, an interview technique for collecting vital environmental, socio-demographic and medical data as was described by (Dakul *et al.*, 1997) was adopted. Reports from these interviews were used for further analysis of the study outcome.

3.7 LABORATORY STUDIES

3.7.1 Determination of *Schistosoma haematobium* egg in Subjects by Parasitology

Visible haematuria was determined using one strip of commercially prepared reagent strip comb 19 (Macherey-Nagel, Ch. B Lot 32225), dipped into each urine sample and the color change was matched with standard colors by the side of the container of the reagent strips. The protocol was adopted after Poggensee *et al.* (2000b). In addition, visible haematuria based on the appearance of bloody urine was also recorded.

For parasitological screening of urinary schistosomiasis and Female Urogenital Schistosomiasis (FUGS); *S. haematobium* eggs were detected in the urine samples of the subjects by the Urine Syringe Filtration Technique (USFT) method as described by WHO (1983). Here, 10ml of urine was collected into a syringe from sampled subjects. Nylon filter was screwed onto the head of a plastic filter chamber and capped with the plastic filter support cap. The urine in the syringe was homogeneously mixed and filtered through the chamber with sediments left on the nylon filter paper. The nylon filter was placed on glass slide with forceps, stained with Lugol's iodine and examined under the x40 objective of a microscope for *S. haematobium* eggs. All infected urine samples were stored in cryovials at -40°C for further analysis.

3.7.2 Determination of *Schistosoma haematobium* egg in Subjects Genitalia Using High Vaginal Swab

To establish *Schistosoma* egg deposition on the genitalia; long plastic swab sticks were used by health workers to collect exudates from the lower genital tract of consented females. The swabs were washed into normal saline solution and screened using the Urine Syringe Filtration Technique (USFT) method. Infection was confirmed

by the presence of *S. haematobium* eggs in both the high vaginal swab (HVS) specimens and by USFT method. Those found positive were also treated with Praziquantel and included in follow-up study where both urine and blood samples were collected quarterly from consented volunteers (Plate 7).



Plate 7: Laboratory Screening of High Vaginal Swab (HVS) Specimens

3.7.3 Screening of Human Sera for HIV Infection

For serodiagnosis of HIV antibodies, in plasma of blood collected from individuals were performed using parallel testing algorithm for HIV testing (rapid ELISA technique). The algorithm was applied according to FMOH (2007) and WHO, (2006). Confirmation of each specimen was done using the double ELISA. Where discordant results occurred, a third test (tie-breaker) was used to identify the differences and confirm infection or no infection of HIV accordingly. The following were used according to availability of supplies *viz*: Capillus (Cambridge Diagnostics, Ireland), Bundi Rapid HIV-1/-2 (Bundi International Diagnostics Ltd., Nigeria); Determine (Abbot Laboratories, Germany).

ELISA procedure using capillus

- i. All reagents and frozen sera were first allowed to reach room temperature of 18°C - 25°C before use.
- ii. The latex reagents were mixed very well before use and all subjects' identification numbers recorded.
- iii. 120µL of the latex reagent was drawn onto the slide and 10ul of subjects sample dispensed directly into the latex solution.
- iv. A thorough mixing of both the test sample and the latex solution was done at least 5x using pipette so as to ensure free capillary flow.
- v. The latex mixture was then allowed to flow for 3-7 minutes through the entire capillary channel and into the viewing window before the interpretation of results.

vi. Interpretation of Results:

Samples observed through the viewing windows, which demonstrated any latex aggregation, were considered to be reactive (that is positive). While samples that

showed no aggregation were interpreted as non-reactive or negative (Cambridge Diagnostics, Ireland)

3.8 IMMUNODIAGNOSIS OF FEMALE UROGENITAL SCHISTOSOMIASIS

To establish the level of pathology in the study population, urine and plasma (derived from blood) of 537 volunteer subjects were randomly selected.

3.8.1 Laboratory Studies of FUGS Pathology

In order to assess the level of pathology, urine of volunteer subjects were examined using an x500 and x2Megapixel digital imaging microscopy (Micron MT9P001)

3.8.2 Laboratory Studies of Urinary Tract Pathological Indicators

The chemical/physical profile of the urinary tract of consented individuals was measured using the urine reagent strip test kit (Hemastix, Bayer, UK).

Urine Reagent Strip Test Procedure

1. Fresh urine collected from subjects was brought to room temperature.
2. One reagent strip was removed and container cap replaced immediately so as to minimize the exposure of the remaining test strips to air and light.
3. The strip reagent pads was completely immersed in the urine sample and removed immediately in order to avoid dissolving out the reagent pads.
4. The edge of the strip was run against the rim of the specimen container in order to remove excess urine.
5. The strip was then held in a horizontal position to prevent possible cross contamination of chemicals located in adjacent reagent pads.

6. The color change on the reagent pads were compared to the corresponding color chart on the bottle label.
7. Results were read according to the chart's time frame for each panel tested. Note: all results were read within 2hours.

3.8.3 Circulating Cathodic Antigen in Urine

For the detection of circulating cathodic antigens, 197 APH, urinary schistosomiasis positive persons, and HIV/urinary schistosomiasis co-infected persons consented to their urine being screened. The principle of the test is an antigen-capture assay. That is, by mixing the sample and the carbon-conjugate containing buffer, the *Schistosoma* CCA is bound to the antibody carbon particles. Lateral flow transports the CCA-carbon complex through the strip, which if CCA is present will bind to the anti-CCA antibody at the test line, showing a black carbon precipitation while excess carbon conjugate is caught by the control line.

Preparation

First the CCA buffer was prepared by adding 10ml of distilled water (MilliQ-distilled) was added to 1 tube of lyophilized CCA buffer. The dissolved suspension was then mixed until a complete homogenous mixture was gotten and 75 μ l of the CCA buffer was used per sample (note: 1 tube is enough for 100 samples). Standards: All standards (10000, 1000, 100, 10 and 0) was run as a quality control for every 100 urine samples tested. The standard 100 was the cut-off sample. The CCA strip is considered negative when the test line is weaker than the standard 100 and positive when the test line is stronger than the standard 100.

Procedure:

1. For every sample, 75 μ l of CCA IDIS buffer was added into a CCA IDIS carbon tube.

2. Then 25µl urine (or standard) was added into the buffer, mixed by pipetting up and down for at least 5 times until the suspension is homogenous.
3. The CCA strip was inserted on the sample pad side (i.e., shortened side), down into the 100µl mixture of urine/buffer/carbon. After which it was incubated for 40 minutes at room temperature. Results were then read against the cut-off sample standard 100.
4. Interpretation of Results: The test was considered positive when the test line is visible and stronger than the standard 100. While the test was considered negative if the test line is not visible or is weaker than the standard 100.

3.8.4 Measurement of Immunological Markers of FUGS

For the measurement of morbidity in the genital tract of urinary schistosomiasis infected females, immunological markers (cytokines) specific for urogenital schistosomiasis was used. The cytokine levels in plasma of study population was evaluated and assessed. The subjects were grouped into four, namely: group1 = control (urinary schistosomiasis/HIV negative individuals) individuals, group2 = urinary schistosomiasis positive/HIV negative, group3= HIV positive/urinary schistosomiasis negative subjects and group4= urinary schistosomiasis/HIV co-infected individuals. Fifty individuals consented to their blood being screened at baseline, but only 33, 31, 28 and 28 completed the post treatment study of 3, 6, 9 and 12 months respectively.

Estimation of levels of inflammatory cytokines: interferon gamma (IFN), tumor necrosis factor (TNF) and interleukin (IL-4) in study population

Cytokine levels in subjects' plasma were measured by ELISA and were expressed in picogrammes per milliliter by interpolation from standard curves. All ELISA tests and cytokine measurements were run in duplicate and mean concentrations were calculated.

Enzyme immunoassay (EIA) for interferon gamma (IFN- γ)

The IFN- γ of subject's plasma was determined using the IFN- γ enzyme-linked immunosorbent assay (ELISA) kit (Human IFN- γ ELISA kit, BD) which applies a technique called a quantitative sandwich immunoassay. The microtitre plate provided in this kit was already pre-coated with IFN- γ specific monoclonal antibodies.

Procedure

1. The Wash Buffer (1X) and IFN- γ Standards was prepared before starting assay procedure.
2. 100 μ L of Standard or Sample was added to the appropriate well of the antibody pre-coated Microtitre Plate and mixed by gently tapping the plate. The microtitre plate was then covered and incubated for 1 hour at room temperature.
3. All the wells were aspirated and washed 5x using wash buffer (1x). After final wash, plate was inverted and blotted dry by hitting plate onto absorbent paper or paper towels until no moisture appeared.
4. 100ul of conjugate was dispensed into each well, mixed well, covered and incubated for 1 hour at room temperature.
5. Substrate Solution was prepared 15 minutes before end of second incubation.
6. The microtitre plate was again as described in Step 3.
7. 100 μ L of Substrate Solution was added into each well, covered and incubated for another 15 minutes at room temperature.
8. Then 100 μ L of Stop Solution was added to each well and mixed.
9. The Optical Density (OD) was then read at 450 nm using a microtitre plate reader within 30 minutes.
10. Calculation of Results

In order to measure the concentration, the standard curve was generated and used to determine the amount of IFN- γ in an unknown sample. The standard curve was generated by plotting the average OD (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding IFN- γ concentration (pg/mL) on the horizontal (X) axis.

1. First, we calculated the mean OD value for each standard and sample. All OD values were subtracted by the value of the zero-standard (0 pg/mL) before result interpretation. Then the standard curve was constructed using statistical software.
2. To determine the amount of IFN- γ in each sample, first we located the O.D. value on the Y-axis and extended a horizontal line to the standard curve. At the point of intersection, a vertical line was drawn to the X-axis and the corresponding IFN- γ concentration read. Samples that generated values higher than the highest standard, was diluted with the appropriate Calibrator Diluent and assay repeated. The concentration read from the standard curve was multiplied by the dilution factor.
3. The IFN- γ cut-off was $0.78\text{pg/ml} = (2\text{S.D} + \text{mean O.D of 18 persons})$. All samples above the cut-off were considered positive. These were confirmed with ELISA LogIt 2005 v2.5 software used in OD transformation.

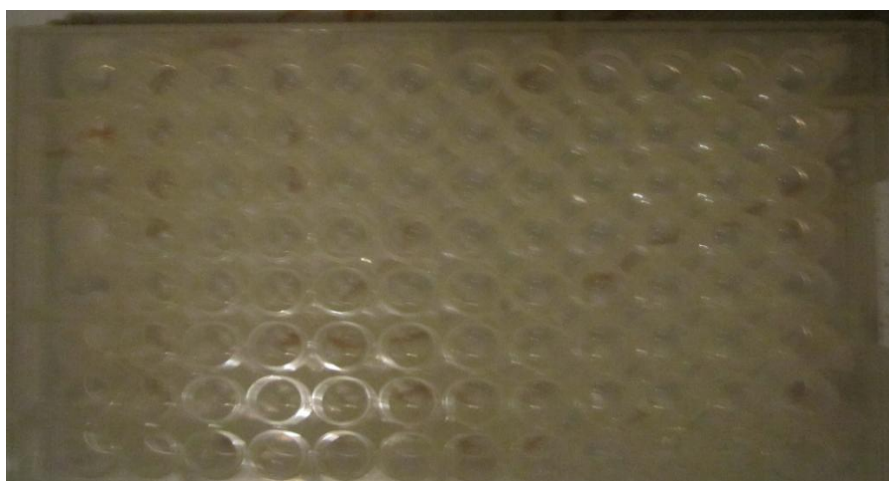


Plate 8: A Microplate ELISA assay with sample preparation

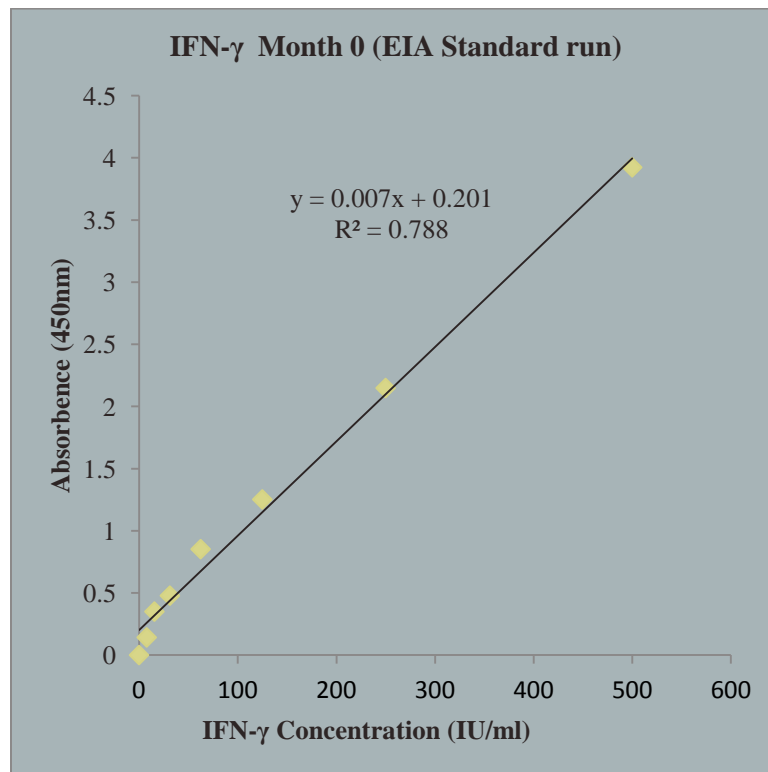


Fig. 7: Control Standard values against known Control of IFN- γ EIA run
(This was replicated in all ELISA assays for IFN, TNF and IL-4)

Enzyme immunoassay (EIA) for TNF- α in the study population

For the quantitative determination of human tumor necrosis factor alpha (TNF- α) concentrations in serum, the Human TNF- α ELISA Kit (Pharming, BD) was used.

Assay Procedure

1. Wash Buffer and TNF- α Standards were prepared before starting assay procedure.
2. 50 μ L of Biotin Conjugate was added to the antibody pre-coated Microtitre Plate.
3. 100 μ L of Standard or Sample was added to the appropriate wells, Mixed well, covered and incubated for 1 hour at room temperature.
4. All wells were aspirated and microtitre plates washed FIVE **times** using Wash Buffer (1X). After final wash, plate was inverted and blotted dry by hitting plate onto absorbent paper or paper towels until no moisture appeared.
5. 100 μ L of Avidin Conjugate was added to each well, covered and incubated for another 1 hour at room temperature.
6. Substrate Solution was prepared 15 minutes before end of incubation and wash procedure repeated as in Step 4 again
7. Repeat wash procedure as described in Step 4 again
8. 100 μ L Substrate solution was added into each well covered and incubated for another 20-30 minutes, at room temperature.
9. Add 100 μ L of Stop Solution was then added to each well and mixed and Optical Density (O.D.) read at 450 nm using a microtitre plate reader within 30 minutes.
10. Calculation of Results

The standard curve was used to determine the amount of TNF- α in an unknown sample.

The standard curve was generated by plotting the average OD (450 nm) obtained for

each of the standard concentrations on the vertical (Y) axis versus the corresponding TNF- α concentration (pg/mL) on the horizontal (X) axis.

1. First, calculate the mean OD value for each standard and sample was calculated by subtracting all OD values from the zero-standard (0 pg/mL) before result interpretation. Then the standard curve was constructed using statistical software.

2. To determine the amount of TNF- α in each sample, first we located the OD value on the Y-axis and extended a horizontal line to the standard curve. At the point of intersection, a vertical line to the X-axis was drawn and the corresponding TNF- α concentration value read. Samples that generated values higher than the highest standard was diluted further and assay repeated. The concentration read from the standard curve was multiplied by the dilution factor. The TNF- α cut off was 0.53pg/ml = (2S.D + mean OD of 18 persons) and values above the cut-off were considered positive.

Estimation of levels of interleukin (IL) -4 in the study population

Interleukin (IL) -4 levels, a measure of Th-2 immune response was estimated in plasma of study subjects (Sarquin Pelikines; human IL-4 ELISA: Amsterdam, The Netherlands). Protocol was followed strictly as described by manufacturer. A lower detectable limit was 0.3pg/ml. i.e. (3S.D + mean O.D of 18 persons) Therefore all samples above the lower detectable limits were considered positive and compared to standard and control group results.

3.9 CD4 COUNT DETERMINATION

The CD4 counts of the subjects whose serum was assayed for cytokine measurement was determined serologically using the ParTec kit (ParTec GmbH, Gorlitz, Germany).

Procedure

1. Parameter histogram was displayed and log 3 for fluorescence selected.

2. TRIGGER = florescence parameter was selected and a dilution factor to 1 was set (for CyFlow Counter).
3. The Count Check Bead bottle was shaken thoroughly and a sample of 850µl taken and put into a sample tube in order to avoid air bubbles.
4. Then the tube was plugged to the sample holder of the Flow Cytometer and the peak adjusted to position 100 by modifying the gain value. The speed 4.0 was used and the system left to run in COUNT mode until it stops automatically. Then the measurement was taken. Note: After setting the range between 10 and 800, another cycle can be run.

3.10 STATISTICAL ANALYSIS

Data analyses, using expressive percentage, parametric and non-parametric tests were used to assess the significance of varied observations. Univariate and multivariate analyses were carried out using 2-Way analysis of variance with replication (2-ANOVA) to assess for potential statistical associations between infection status and the different UTPs investigated (urinary tract pathologies). 2-Way analysis of variance without replication (2-Way ANOVA) was also used to assess any association between disease burden and treatment in the follow-up examination. For each variable, P-values < 0.05 was considered indicative of statistical significance in SPSS version 15.1 and all statistics; while Optical Density values were transformed by ELISA LogIt 2005 software.

CHAPTER FOUR RESULTS

In this study, a total of 1,245 persons (Group A) were screened for *S. haematobium* infection, of which 1,007 specimens were from apparently healthy individuals and the remaining 238 samples were from hospital population.

For follow-up study, 537 randomly selected females (Group B) were studied of which 381 of them had detectable *S. haematobium* eggs in their urine. From the 381 *S. haematobium* positive persons, the worm burden of 197 subjects (Group C) were studied using circulating cathodic antigens (CCA) while cytokine levels of only 50 subjects (Group D; which still varied due to specific criteria met by the subjects) were studied to ascertain FUGS pathology, characteristics and its relationship with HIV pathogenesis and progression.

4.1 PREVALENCE OF UROGENITAL SCHISTOSOMIASIS

In this study, 1245 persons submitted their urine samples for *S. haematobium* screening, of which 1007 specimens were from apparently healthy individuals while the remaining 238 samples were from hospital populations. Out of the 1007 apparently healthy individuals, only 396 subjects and all symptomatic hospital population (that is, 238), were screened serologically for HIV. While another one thousand, three hundred and seventy-six persons were observed for different risk activities which exposed the subjects to urogenital schistosomiasis infection.

The prevalence of urogenital schistosomiasis using presence of *S. haematobium* eggs in urine of apparently healthy populations was 265(26.3%). Females 11-20 year old accounting for higher infection (28.27%), followed by 21-30 years (21.84%). The least was 12.5% recorded in 0-10 year-old. A Chi-Squared Analysis (χ^2 -test) showed a significant difference ($P < 0.05$) among the different age groups (Cal $\chi^2_{0.05} = 40.944 >$

Tab $\chi^2_{0.05, df.5} = 11.07$). This meant that age is associated with risk factors in the prevalence of urinary schistosomiasis (Table 6, Plate 9). When compared to the hospital populations, a 21.43% prevalence was obtained with subjects aged 11-20 years (29.63%) again having the highest infection, followed by 31-40 year-old (20.63%). Invariably, more than 50 (i.e. ≥ 50) year olds (6.67%) accounted for the least infection. Using a Chi-Squared analysis, in the symptomatic persons, urogenital schistosomiasis among hospital populations were not dependent on age ($P > 0.05$, df_5 ; Cal $\chi^2 = 8.316 < \text{Tab } \chi^2_{0.05} = 11.07$) (Table 7 and 8).

In relation to signs and symptoms associated with urogenital schistosomiasis, 61(4.9%) subjects exhibited haematuria in their urine, 273(21.9%) had proteinuria; 108(8.7%) persons had both haematuria and proteinuria, while 804(64.6%) persons had neither haematuria nor proteinuria (Fig.8).

Table 6: Parasitological Prevalence of *Schistosoma haematobium* in Apparently Healthy Adults and School-Age Children in Study Communities

Age Group (Yrs.)	No Screened	Prevalence of urogenital schistosomiasis	
		No infected with <i>S. haematobium</i>	Prevalence percentage (%)/ Confidence Interval [95% CI]
0 – 10	16	2	12.5 [10.4-14.6]
11 – 20	774	218	28.3 [25.5 – 31.1]
21 – 30	87	19	21.8 [19.2 –24.4]
31 – 40	66	13	19.7 [17.2 – 22.2]
41 – 50	49	9	18.4 [16.0 – 20.8]
≥51	15	4	26.7 [23.9 – 29.5]
Total	1007	265	26.3 [23.5 -29.1]

(Cal $\chi^2_{0.05} = 40.944 > \text{Tab } \chi^2_{0.05, df_5} = 11.07$) (P < 0.05)

Table 7: Prevalence of Urogenital Schistosomiasis in the Hospital Populations

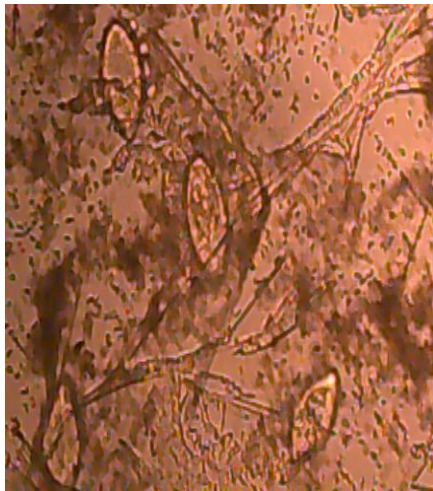
Age Group (Yrs.)	Urine Analysis		
	No screened	No Positive	Percentage (%) [95% CI]
0 – 10	12	2	16.7 [11.9 – 21.5]
11 – 20	27	8	29.6 [23.7 – 35.5]
21 – 30	73	15	20.5 [15.3 – 25.7]
31 – 40	63	13	20.6 [15.4 – 25.8]
41 – 50	48	9	18.8 [13.7 – 23.9]
≥51	15	4	6.7 [3.5 – 10.0]
Total	238	51	21.4 [16.1 – 26.7]

CI: Confidence Interval (95% CI)

(Cal $\chi^2_{0.05} = 8.316 < \text{Tab } \chi^2_{0.05} = 11.07$ at df_5) ($P > 0.05$)

Table 8: Prevalence of Female Urogenital Schistosomiasis in Hospital Population Using High Vaginal Swab

High Vaginal Swab (n = 74)			
Age Group (Yrs)	No Screened	No Positive	Percentage (%) [95% C.I.]
0 -10	32	1	3.1 [1.0 – 7.2]
11 - 20	17	3	17.7 [8.9 – 26.6]
21 - 30	4	0	0 [0.0 – 0.0]
31 - 40	14	0	0 [0.0 – 0.0]
41 - 50	4	1	25.0 [15.0 – 35.1]
≥51	3	0	0.0 [0.0 – 0.0]
Total	74	5	6.8 [0.9 – 12.7]



X500 pixel



×2 Megapixel

Plate 9: *Schistosoma haematobium* eggs with its typical terminal spine, observed in urine of an infected girl (Microscope Digital Camera Eyepiece, CMOS Micron MT9P001).

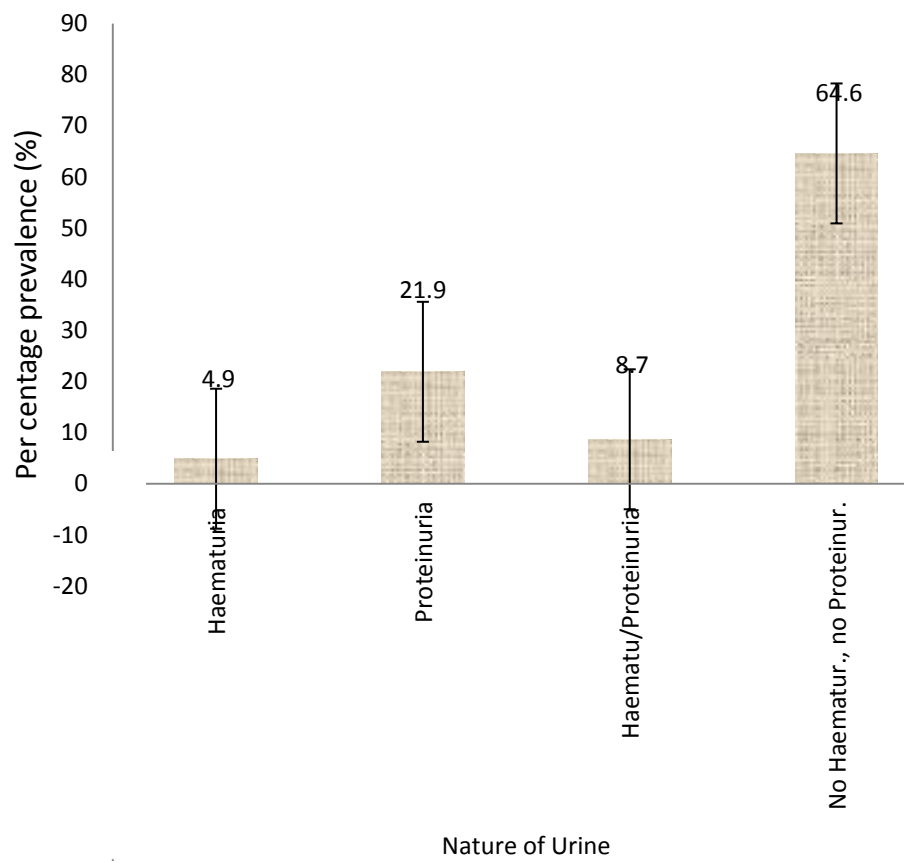


Fig. 8: Symptoms Associated with Urogenital Schistosomiasis in the Study Area



Plate 10: An infected child in Tudun Wada community showing clinical symptom (hepatosplenomegaly) and impact of urogenital schistosomiasis in children

Majority of the houses in the study communities lacked good sewage system while some did not have any. Consequently, it was noted that two-thirds of the randomly selected houses, the occupants indulged in defecating indiscriminately in and around their houses, along river banks and some into the river (Plate 11). These activities we believe continued to ensure re-infection by schistosomiasis in the communities. Furthermore, lack of pipe-borne water or even bore hole in these communities has resulted to the inhabitants depending on the streams and rivers that flowed along their villages for their domestic chores, bathing, drinking and irrigation practices. The farmers irrigated their farms during the dry seasons, thereby creating permanent shallow freshwater channels that promoted the growth of aquatic plants which harbored the *Schistosoma* snail intermediate hosts.

Along the banks of these streams were located dense vegetations which included aquatic plants that haboured schistosome snail host, such as the *Bulinus* species and *Biomphalaria* species, which are snail hosts of *S. haematobium* and *S. mansoni* respectively. The snail hosts breed/feed on these aquatic plants. Inhabitants of these communities defecate and urinate in/around the river banks (Plate 11). The eggs from infected persons, under conducive environment, hatch and in the water bodies. Here they are ingested by the snail hosts where further process of the life cycle continues until the cercariae are released into the water. Individuals get infected when they engage in any form of water contact activities.

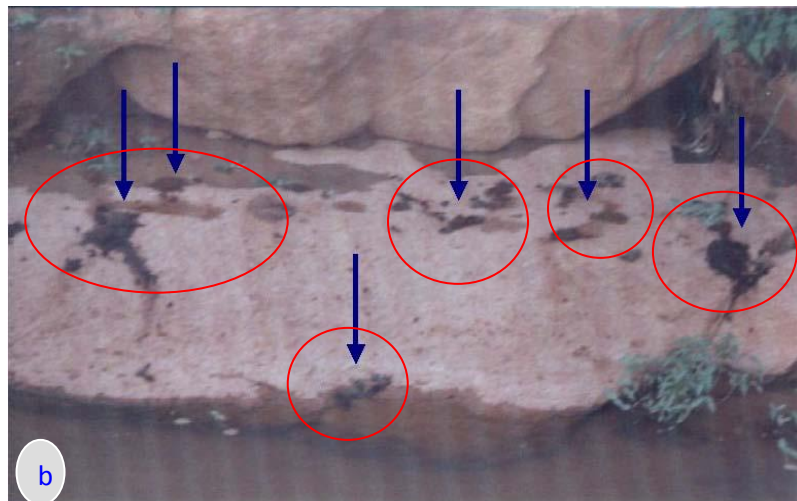


Plate 11: Sewage and Human Waste Disposal Systems Leading to Water Contamination

- a. Sewages wastes disposal strategies leading to water contamination
- b. Fecal waste disposal leading to stream contamination in study communities

4.2 DISTRIBUTION OF SNAIL HOST SPECIES IN THE STUDY AREA

Four species of snails, *Bulinus globosus*, *Bulinus truncatus*, *Biomphalaria pfeifferi*, *Lymnaea natalensis*, were common in all the study areas. *B. globosus*, *B. pfeifferi* and *L. natalensis* in particular were found in large numbers near aquatic vegetations found along and inside the streams, around irrigated canals and rain-filled pools in the Tudun Wada and Abattoir vicinity, while *B. truncatus* was found in abundance in the Nabong polluted (stagnant) streams. Along the banks and on the surface of these shallow perennial streams were horizontal vegetations which included the water lilies (*Nymphaea spp.*) and slimy vegetations (the spirogyra). Grasses were also seen growing along the banks of the streams, some in the irrigated canals and pools of stagnant waters. Most of the snails were observed resting on the underside of these oxygen-rich vegetations (due to photosynthesis). These vegetations provide egg-laying surfaces, shelter from the sun and predators. They also serve as food for many snail species.

Bulinus globosus was the most common snail species in this study area, accounting for 41.3% of all snails collected (Fig.9), followed by *Biomphalaria spp.* (24.7%), the intermediate host for *S. mansoni*. *L. natalensis* (15.6%) had the least percentage population diversity. *L. natalensis*, though the intermediate host of *Fasciola hepatica* was also collected in the study. They could easily be mistaken for *B. globosus* except for their larger size and curving of their trunk which is left-handed compared to that of *B. globosus* whose trunk is curved right-handed. In relation to the study communities, *B. globosus* was more common in Tudun Wada area compared to the other two communities. On the other hand *B. truncatus* was in abundance in the Nabong streams than in the other communities. While *B. pfeifferi* together with *B. globosus*, were in abundance in the Abattoir area (Table 9; Fig.9).

Using a 2-Way Analysis of Variance, no significant difference was observed. Hence it was evident that the abundance of the different snail species in a particular community did not really matter, as far as the infective intermediate host for *S. haematobium* was found in such environment. Treatment (Snail species): Cal. $F = 6.87 < \text{Tab. } F_{0.01, df_{3,6}} = 9.78$; $P > 0.05$ (not significant). Block (Different communities): Cal. $F = 0.04 < \text{Tab. } F_{0.01, df_{2,6}} = 10.92$; ($P > 0.05$).

Percentage cercarial shedding of the *Bulinus* species (*B. globosus*, intermediate host of *S. haematobium*) indicated a higher percentage cercarial shedding, 42.2 vs. 36.5% during the dry and rainy seasons respectively. Similar results were also obtained for *B. pfeifferi* (intermediate host of *S. mansoni*); which accounted for *S. mansoni* cercariae shedding, during the dry and rainy seasons (45.5% vs. 34.5%). Dry season study of intermediate host snails sampled from Abattoir water sites had more *S. haematobium* infection (64.4%). This was followed by Tudun-Wada (57.1%).

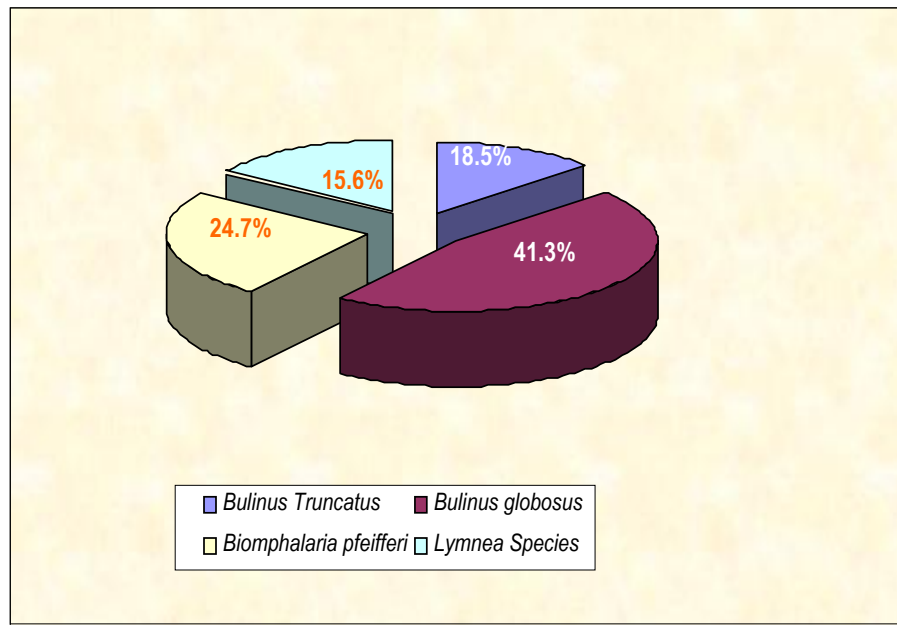
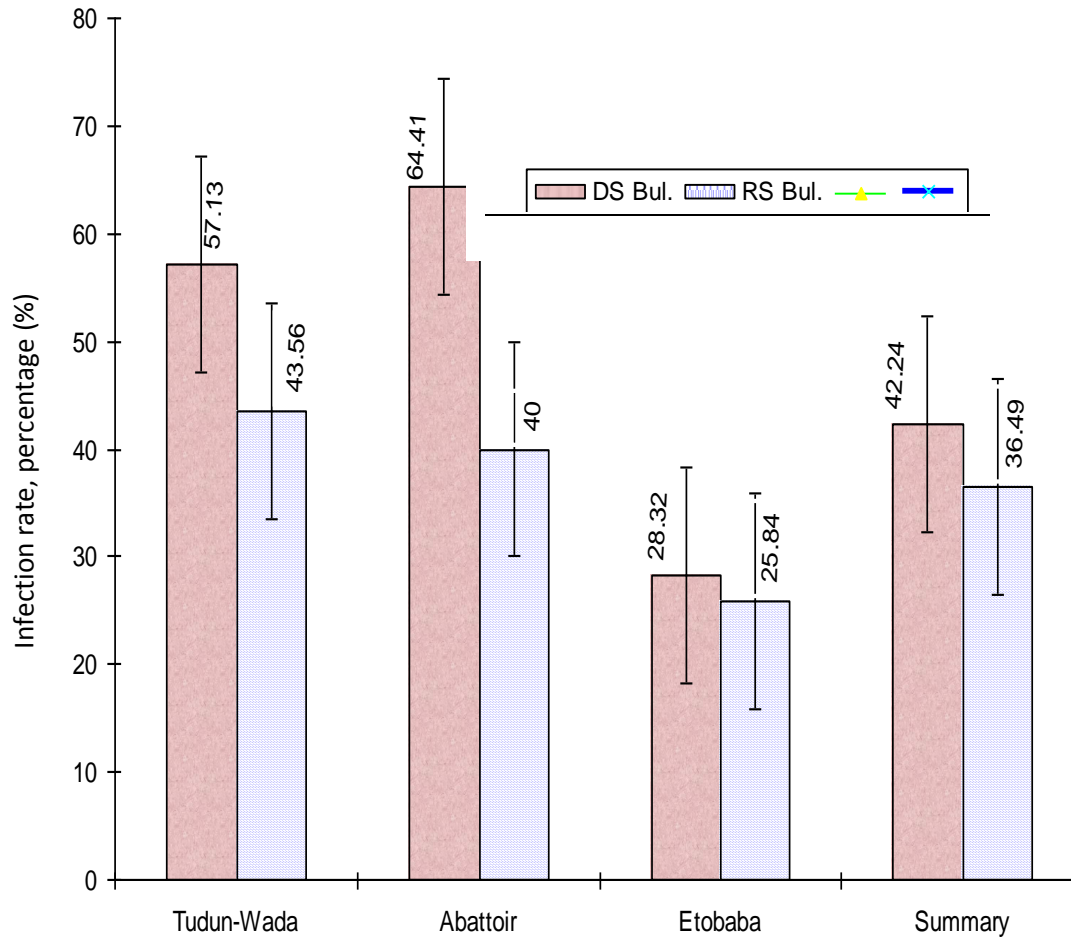


Fig.9: Snail Intermediate Host Species Population Diversity

Table 9: Distribution of Snail Intermediate Host Species in Frequently Used Streams of Study Communities.

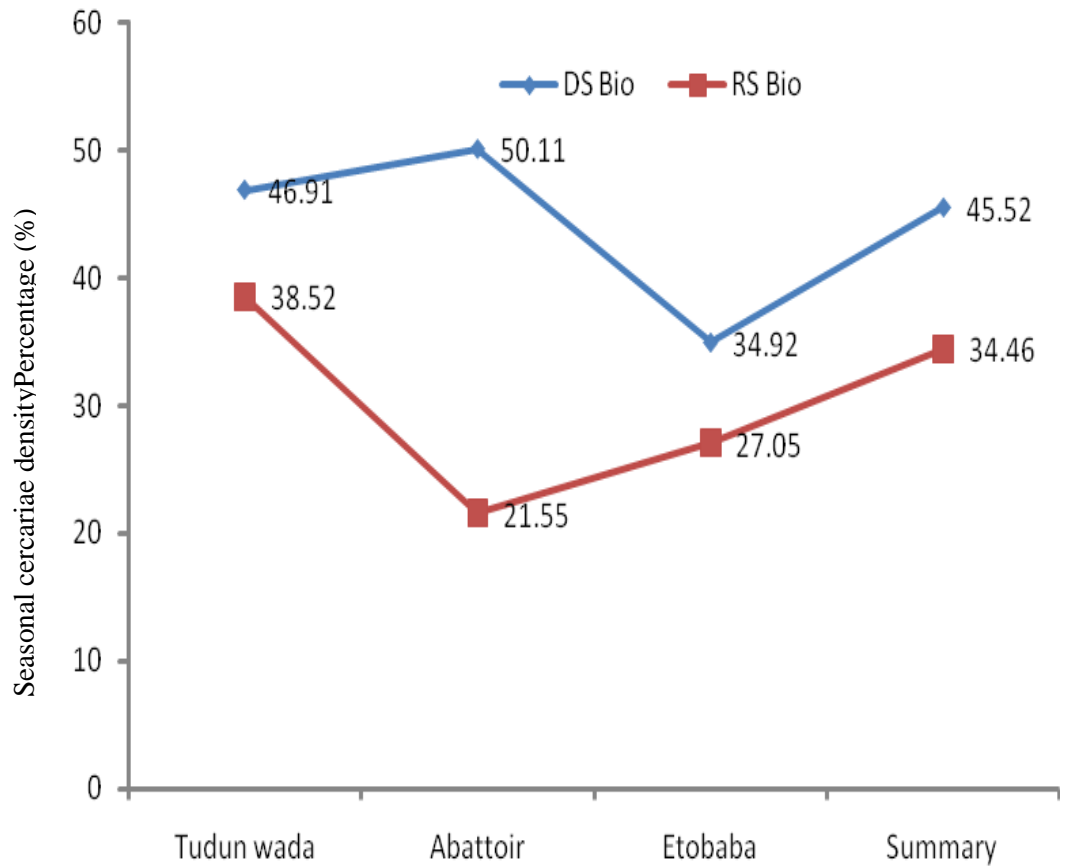
Snail Species	Study Communities		
	Tudun-Wada (n=3698)	Abattoir (3683)	Nabong (3447)
<i>Bulinus globosus</i>	2124(57.4%)	1439(39.1%)	905(26.3%)
<i>Bulinus truncatus</i>	200(5.4%)	297(8.1%)	1503(43.6%)
<i>Biomphalaria pfeifferi</i>	812(22.0%)	1234(33.5%)	628(18.2%)
<i>Lymnea natalensis</i>	562(15.2%)	713(19.4%)	411(11.9%)

While during the rainy season, Tudun-Wada recorded the highest snail infection with 43.6% followed by Abattoir with 40% (Fig.10). In a comparative assessment of *B. pfeifferi* for *S. mansoni* cercariae infection (Fig.11), similar pattern was observed. A 2-Way ANOVA showed no significant difference in seasonal abundance of snails during the dry or rainy seasons among the study communities. Treatment (Different communities): Cal. $F = 1.9120 < \text{Tab. } F_{0.05, df_{2, 2} = 19.0}$; (i.e., not significant, $P > 0.05$). However, a significant difference in cercarial abundance was recorded for dry and rainy seasons respectively. Block (Dry season and rainy season): Cal. $F = 20.9271 > \text{Tab. } F_{0.05, df_{1, 2} = 18.5}$; $P < 0.05$.



Key: DS – Dry Season, RS: Rainy Season, Bul.: *Bulinus* species.

Fig.10: *Schistosoma haematobium* Cercariae Infection Rate of *Bulinus* Spp. in Relation to Season.



Key: DS – Dry Season, RS: Rainy Season, Bio: *Biomphalaria pfeifferi*

Fig.11: *Schistosoma mansoni* Cercariae Infection Rate of *Biomphalaria* Species in Relation to Season

4.3 DISTRIBUTION OF RISK ACTIVITIES IN THE STUDY COMMUNITIES

At each community, the risk activities embarked by the subjects in the reams streams were studied. Though children accounted for more recreational activities; on the average adults had more WCA for economic and domestic activities. Using a 2- Way Analysis of Variance (ANOVA), age was not the determinant factor in engaging in WCA (Cal. $F = 0.04 < \text{Tab } F_{0.05; df_{1, 9} = 5.12}$; $P > 0.05$. Conversely, this means that age is not a key factor in risk exposure through WCA. However, there is a significant difference; between the frequency of different WCAs (Cal. $F = 6.13 > \text{Tab } F_{0.05; df_{.9, 9} = 3.23}$; $P < 0.05$). (Table 10, Plate 12-13).

Table 10: Distribution of Risk Activities for the Transmission of *S. haematobium* Infection in the Study Communities

Major Activities	Children		Adult		Total Contact	
	No of Persons (n=671)	Contact percentage (%)	No of Persons (n=705)	Contact Percentage (%)	No of persons (n=1376)	Percentage (%) [95% CI]
Recreational	92	13.7	8	1.1	100	7.3[6.0 - 8.0]
Bathing	22	23.9	8	1.1	30	30.0[20.8 - 39.2]
Playing	70	76.1	0	0.0	70	70.0[60.8 - 79.2]
Domestic Needs	144	21.5	241	34.2	385	28.0[26.0 – 30.0]
Defecation	30	20.8	13	5.4	43	11.2[8.0 - 14.4]
Washing (Utensils & Laundry, etc.)	75	52.1	217	90.0	292	75.8[73.6- 78.0]
Fetching water for Household chores/other uses	39	27.1	11	4.6	50	13.0[9.6 - 16.4]
Economic	435	67.9	456	73.3	891	64.8[62 - 67.0]
Fetching for Block industry	33	7.6	22	4.8	55	6.2[4.6 - 7.8]
Farming activities such as: i. Soil preparation/planting	99	22.8	83	18.2	182	20.4[17.7 - 23.1]
ii. Irrigation	69	15.9	97	21.3	166	18.6[16.0 - 21.2]
iii. Weeding	11	2.5	31	6.8	42	4.7[3.3 - 6.1]
iv. Crop watering	223	51.3	223	48.9	446	50.1[46.8 -53.5]

All activities included involved water contact *Fetching water for household and domestic uses, block industries. ** Wadding on the infected irrigation canals during working



Plate 12: Risk Activities Leading to *Schistosoma* Infection

- a. fetching water from cercariae-infested water for irrigation agriculture
- b. irrigation agriculture and canal practice for water retention
- c. a collection of Irish potatoes, proceeds of irrigation agriculture
- d. potato irrigation farm with a gully for water retention during the rains

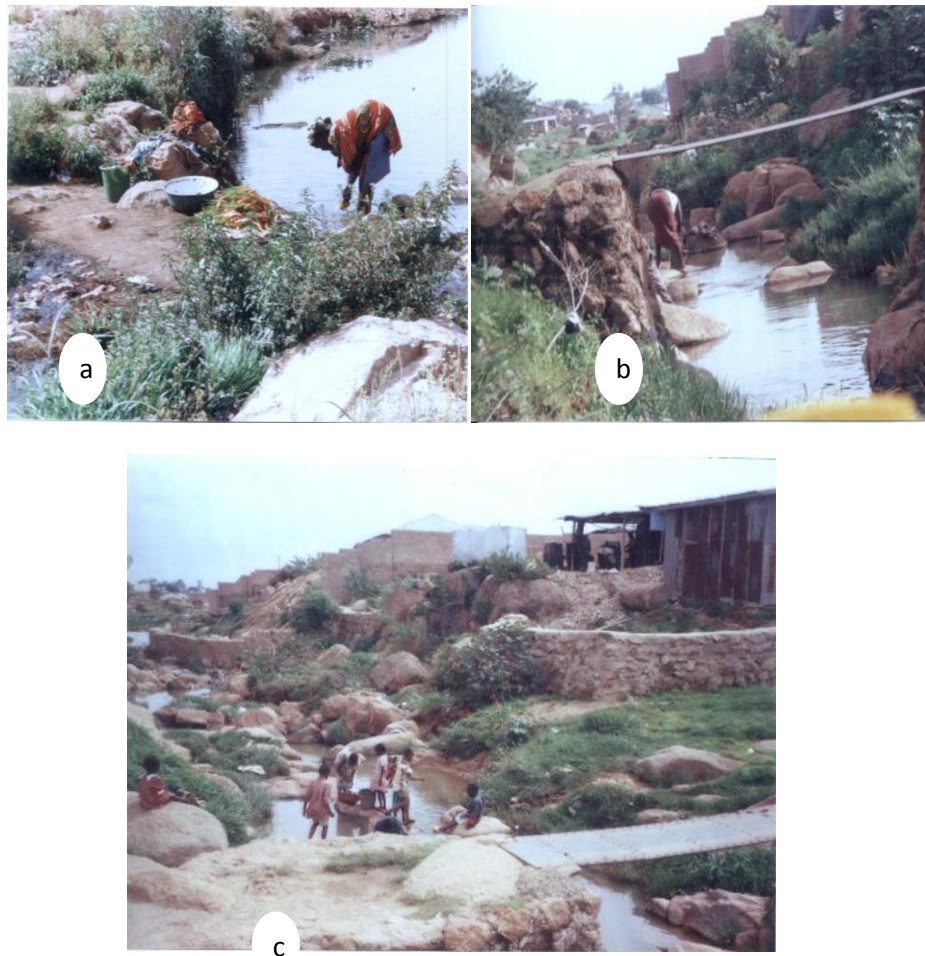


Plate 13: Risk Activities that Expose Residents to Schistosomiasis

- a. woman washing carrot in an infested stream
- b. woman washing clothes in another infested stream
- c. Children playing in water

4.4 SEROPREVALENCE OF HIV IN THE STUDY COMMUNITIES

Blood specimens of 396 individuals who allowed the use of their blood for HIV screening had 27 (6.8%) positivity. Females aged 21-30 years had the highest infection of 9.4%, while the least infection was recorded among ≥ 51 year olds (3.7%). On Analysis of the result using chi-squared (χ^2) test, HIV infection varied significantly ($P < 0.05$) among the different age groups (Cal $\chi^2 = 4.98 > \text{Tab } \chi^2_{0.05, df_{10}} = 3.94$) (Table 11). In relation to occupation, students had the highest infection (39.7%), followed by farmers (15%) while housewives represented the least infection with 1.3% (Fig.12).

Table 11: Age Related Seroprevalence of HIV in Jos Sub-urban Communities

Age Group (Yrs.)	Nabong			Abattoir			Tudun Wada			Total		
	No Screened	No Infected	Percentage (%)	No Screened	No Infected	Percentage (%)	No Screened	No Infected	Percentage (%)	No Screened	No Infected	Percentage [95% CI]
0-10	7	1	14.3	4	0	0.0	3	0	0.0	14	1	7.1[4.5 - 9.7]
11-20	27	2	7.4	20	1	5.0	15	1	6.7	62	4	6.5[4.0 - 9.0]
21-30	91	9	9.9	28	2	7.1	20	2	10.0	139	13	9.4[6.5 - 12.3]
31-40	44	3	6.8	33	1	3.0	14	1	7.1	91	5	5.5[3.2 - 7.8]
41-50	46	3	6.5	13	0	0.0	4	0	0.0	63	3	4.8[2.7 - 7.0]
≥51	27	1	3.7	0	0	0.0	0	0	0.0	27	1	3.7[1.8 - 5.6]
Total	242	19	7.9	98	4	4.1	56	4	7.1	396	27	6.8[4.3 - 9.3]

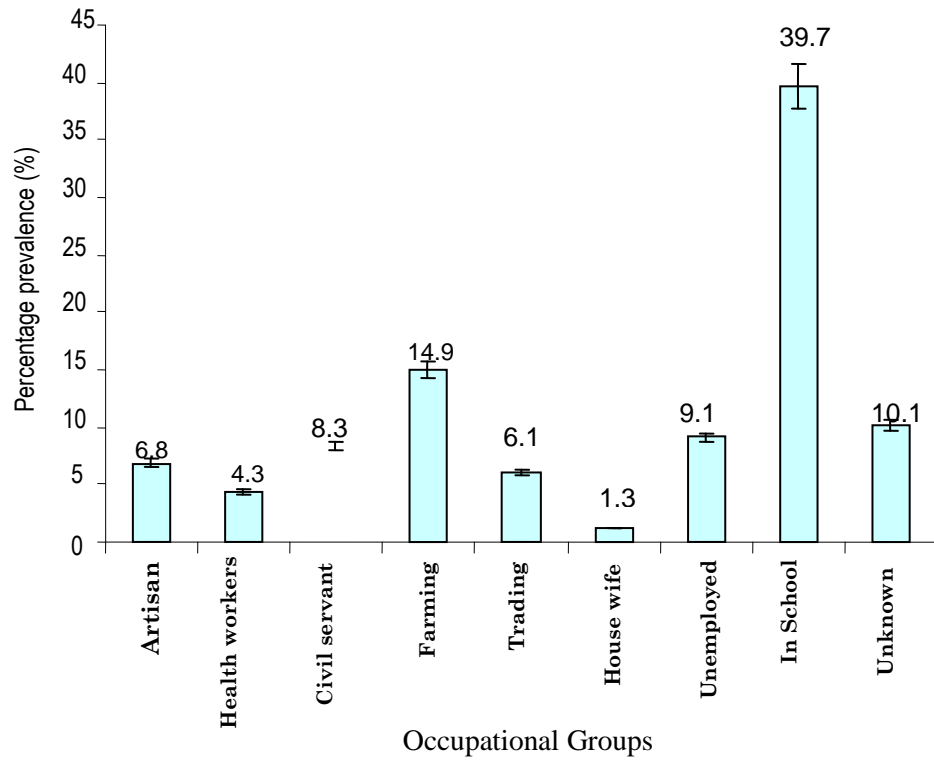


Fig. 12: Occupational Distribution of Study Population Screened for Human Immunodeficiency Virus.

4.5 PREVALENCE OF UROGENITAL SCHISTOSOMIASIS AND HIV COINFECTION IN THE STUDY COMMUNITIES

To understand the impact of schistosomiasis on HIV and *vice versa*, we identified the 265 individuals who were already infected with urogenital schistosomiasis and had their samples concurrently screened for HIV infection. Of this number, 14(5.3%) were infected with both *S. haematobium* and HIV. Individuals aged 21-30 years (42.2%) had the highest infection while the least infection was recorded among the 11-20 year olds (1.4%). Very importantly, individuals 11-40 years had 1-42% concurrent infection. HIV infection alone was also high among this same age group, and ranges from 6.5% at age 11- 20 year olds through 9.4%: 5.5% in those 21-30 and 31-40 years olds respectively (Table 12).

Table 12: Age-Related Prevalence of Urogenital Schistosomiasis and HIV Coinfection in the Study Population

Urogenital schistosomiasis/HIV Co-infection				
Age Group	No. Screened	No. Infected	%age of Schist./HIV co-infection	Confidence Interval (95% C.I.)
0 – 10	2	0	0.0	0.0 - 0.0
11 – 20	218	3	1.4	-0.0 - 2.8
21 – 30	19	8	42.1	36.0 - 48.2
31 – 40	13	2	15.4	11.0 – 19.8
41 – 50	9	1	11.1	7.2 - 15.0
≥ 51	4	0	0.00	0.00 – 0.00
Total	265	14	5.3	2.6 – 8.1

4.6 PATHOLOGICAL MARKERS OF FEMALE UROGENITAL SCHISTOSOMIASIS IN THE STUDY POPULATION

4.6.1 Indicators of Urinary Tract Pathology (UTP)

Among five hundred and thirty-seven females who were randomly selected, three hundred and eighty-one (70.9%) were urogenital schistosomiasis positive. In the positive individuals, $\leq 10 - 24$ yr. olds (teenagers/young adults) expressed higher nitrate concentrations of the UTPs (54.1%), compared to 45.4% observed in the $25 - \geq 40$ yr. olds. In these two age categories; it was Blood (55.9% vs. 44.1%), protein (45.4% vs. 39.6%) and leucocyte (58.0% vs. 41.2%) concentrations; among other major indices, of urinary tract morbidity, varied significantly between the infected and uninfected individuals. Statistically, age: determinant of urogenital Cal. F at $df_{1, 20} = 3.10 < \text{Tab. F}$ at $df_{1, 20} = 5.12$ ($p > 0.05$) Block (U/pathology/morbidity indicators): Cal. F at $df_{9, 20} = 2.54 > \text{Tab. F}$ at $df_{9, 20} = 2.45$ (SG, $p < 0.05$) (Table 13)

Table 13: Age Related Prevalence of Urinary Tract Pathological Indicators of Positive and Negative Urogenital Schistosomiasis Females in the Study Communities

Pathological Indicators	Urogenital Schistosomiasis Positive Individuals (n=381)		Urogenital Schistosomiasis Negative Individuals(n=156)	
	Age Group		Age Group	
	≤ 10 – 24 yrs.	25 - ≥ 40 yrs.	≤ 10 – 24 yrs.	25 - ≥40yrs
Glucose	13 (3.4%)	28 (7.3)	63 (16.5)	78 (50.0)
Bilirubin	89 (23.4)	99 (26.0)	9 (5.7)	13 (8.3)
Nitrate	206 (54.1)	173 (45.4)	11 (7.1)	15 (9.6)
Urobilinogen	55 (14.4)	73 (19.2)	5 (3.2)	7 (4.5)
Protein	173 (45.4)	151 (39.6)	23 (14.7)	21 (13.5)
pH	155 (40.7)	137 (36.0)	42 (26.9)	69 (44.2)
Blood	213 (55.9)	168 (44.1)	31 (19.9)	27 (17.3)
Specific Gravity	111 (29.1)	121 (31.8)	56 (35.9)	64 (41.0)
Ketone	97 (25.5)	113 (29.7)	51 (32.7)	83 (53.2)
Leukocyte	221 (58.0)	157 (41.2)	105 (67.3)	53 (34.0)

Treatment (Age): Cal. F at $df_{1,20} = 3.10 < \text{Tab. F at } df_{1,20} = 5.12$ (NS, $p > 0.05$)

Block (Urinary pathology/morbidity indicators): Cal. F at $df_{9,20} = 2.54 > \text{Tab. F at } df_{9,20} = 2.45$

($p < 0.05$)

Urogenital pathology *with* typical destruction of tissues and blood cells were observed around eggs of *S. haematobium*, in urine of an infected girl using high resolution Digital Microscope photometry (Digital microscope Camera Eyepiece; CMOS Micron MT9P001), with U-Lead photographic software. Destroyed red blood cells, from feeding and spine burst of the blood cells and lesions of the cervix/vagina and nodules from the genital organs were evident (Plate 14).

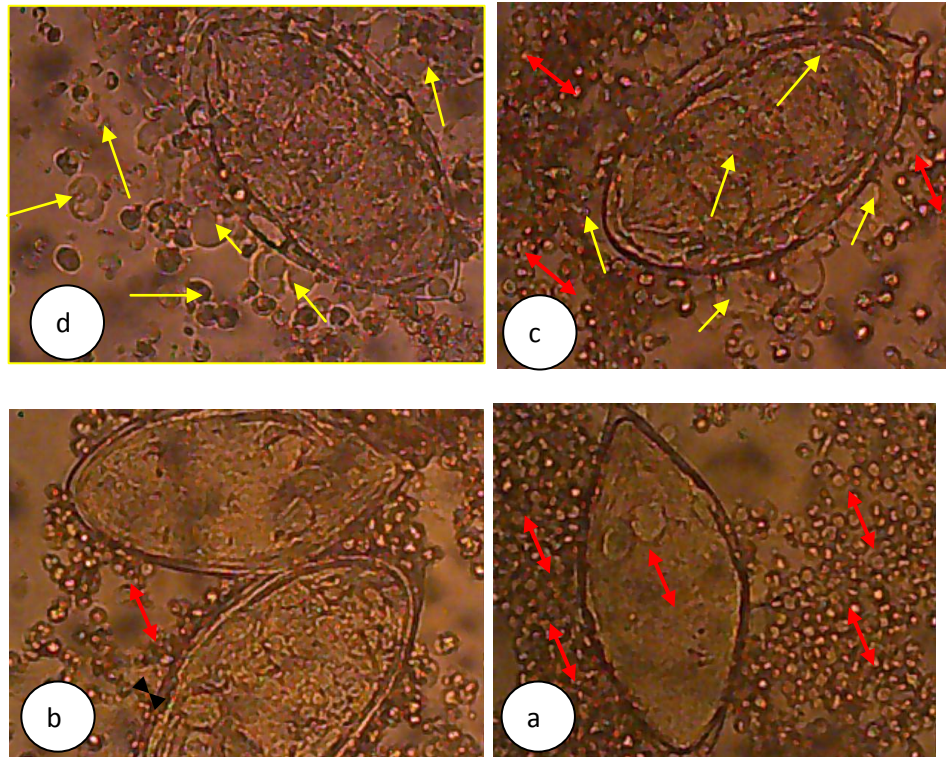


Plate 14: Urogenital pathology with typical destruction of tissues and blood cells observed around eggs of *S. haematobium*, in urine of an infected girl

Legend: ↔ Destroyed red blood cells, from feeding and spine burst of the blood cells
→ Lesions of the cervix/vagina and nodules from the vulva and other genital organs.

4.6.2 Occurrence of Circulating Cathodic Antigen (CCA) in Study Population

In this study, the urine of 197 APH, HIV negative/urogenital schistosomiasis, and HIV/urogenital schistosomiasis Co-infected individuals were screened for CCA antigens. The results obtained in the study showed a high prevalence of 127 (67.8%) for HIV negative/urogenital schistosomiasis positive individuals and 6 (41.2%) for HIV/urogenital schistosomiasis co-infected subjects. In relation to age, the 11-20 year olds (84.6%) and the 21 -30 year olds (66.7%) had the highest prevalence for both HIV negative/HIV positive urogenital schistosomiasis subjects respectively. Statistically, using chi-squared (χ^2) test, the presence of circulating cathodic antigens (CCA) did not vary among the HIV negative/HIV positive urogenital schistosomiasis infected individuals [Cal $\chi^2 = 6.79 < \text{Tab } \chi^2_{0.05, df_4} = 9.49$; $P > 0.05$: not significant] (Table 14)

Table 14: Prevalence of Circulating Cathodic Antigen (CCA) in Apparently Healthy, Urogenital Schistosomiasis and Urogenital Schistosomiasis/HIV Dual Infections

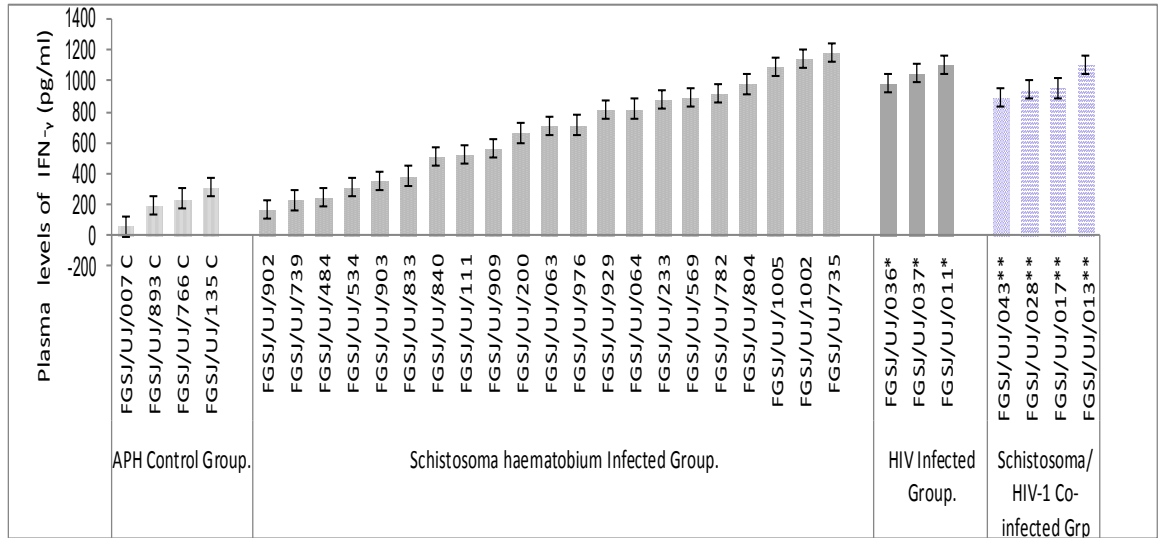
Age Group	HIV-Negative + Urogenital schistosomiasis			HIV-Positive + Urogenital schistosomiasis (N = 6)		
	No. Sampled	No. Positive	Percentage positive (%)	No. Sampled	No. Positive	Percentage positive (%)
≤ 10	47	24	51.06	0	0	0.00
11 – 20	78	66	84.62	7	3	42.8
21 – 30	20	12	60.00	3	2	66.7
31 – 40	28	19	67.86	3	1	33.3
≥41	11	6	54.55	0	-0.0	0.00
TOTAL	184	127	67.76	13	6	41.15

[Cal $\chi^2 = 6.79 < \text{Tab } \chi^2_{0.05, df_4} = 9.49$; $P > 0.05$: not significant]

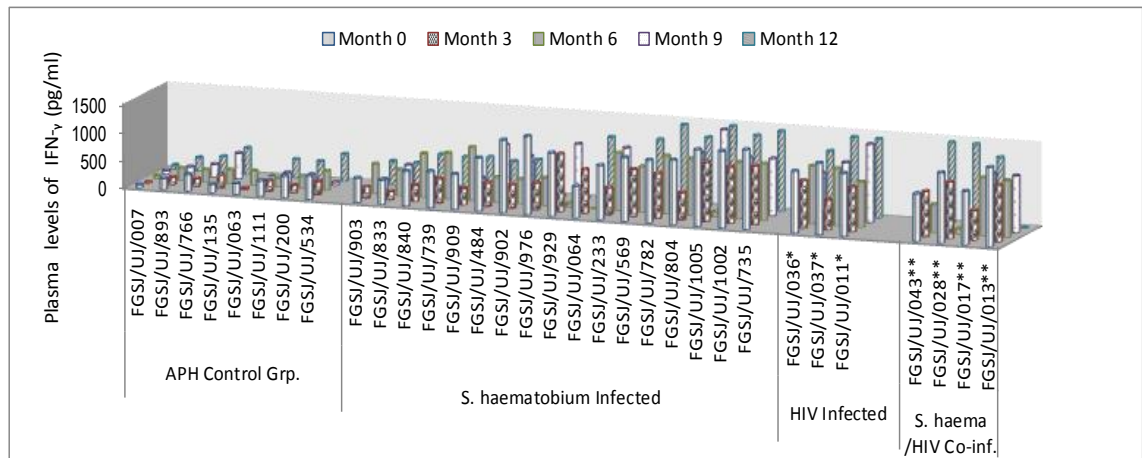
4.7 CYTOKINES, IMMUNOLOGICAL MARKERS OF FEMALE UROGENITAL SCHISTOSOMIASIS IN THE STUDY POPULATION

Immunological studies to provide evidence of urogenital schistosomiasis (FUGS) morbidity and establish the presence of immunopathology and other markers of FUGS in our study subjects were carried out - using important regulatory T- cells and important cytokines involved in cellular and humoral immune response. These were used to establish the status of immunopathological indices of urogenital schistosomiasis morbidity (FUGS) in the study population.

About 79.3% schistosomiasis and HIV infected (single and dual) groups expressed high level of IFN- γ (5-fold) (Tab. F $df_{27, 108} = 1.39 < Cal. F = 2.41$; Tab. F $df_{4, 108} = 2.52 < Cal. F = 108.36$ ($p < 0.05$). However, it was only 4 fold increase in schistosomiasis infected group, compared to the five fold in HIV infected and HIV/schistosomiasis co-infected persons (772: 1046: 975 pg/ μ L; Cal. F = 2.41 > Tab. F = 1.39). Tumor necrosis factor-alpha (TNF- α) revealed elevation in 58.0% of the schistosomiasis, HIV and HIV/schistosomiasis co-infected study subjects when compared to control (Tab. F at $df_{4, 108} = 2.53 < Cal. F$ at $df_{4, 108} = 5.87$ ($p < 0.05$), while schistosomiasis infected group had as high as 7-fold plasma TNF, compared to only a 2-fold obtained in co-infected subjects. Interleukin (IL) - 4 presented more irregular interaction with a 42.3 fold elevation observed in HIV infected group compared to 23.1 and 15.3 fold plasma elevation recorded in schistosomiasis infected group and HIV/schistosomiasis co-infected (Fig. 13 – 15).



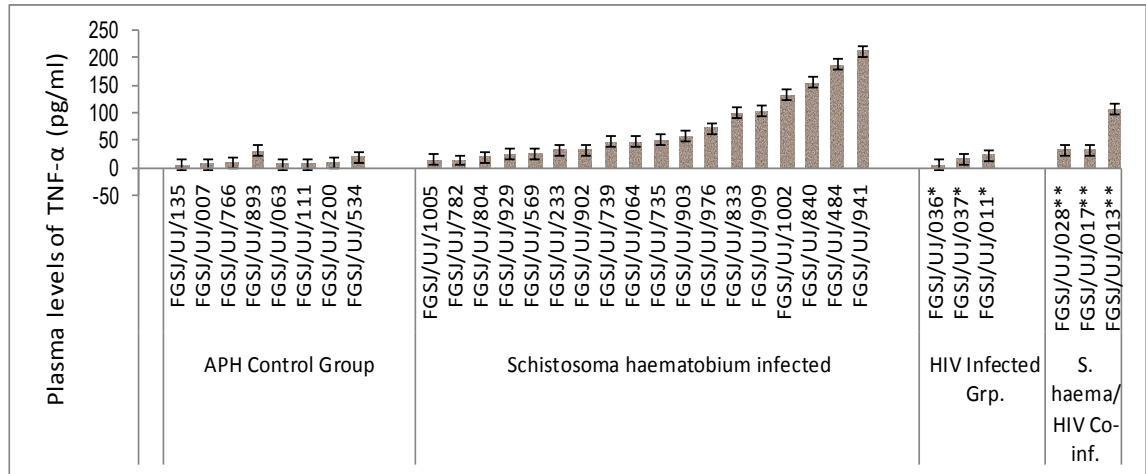
(a)



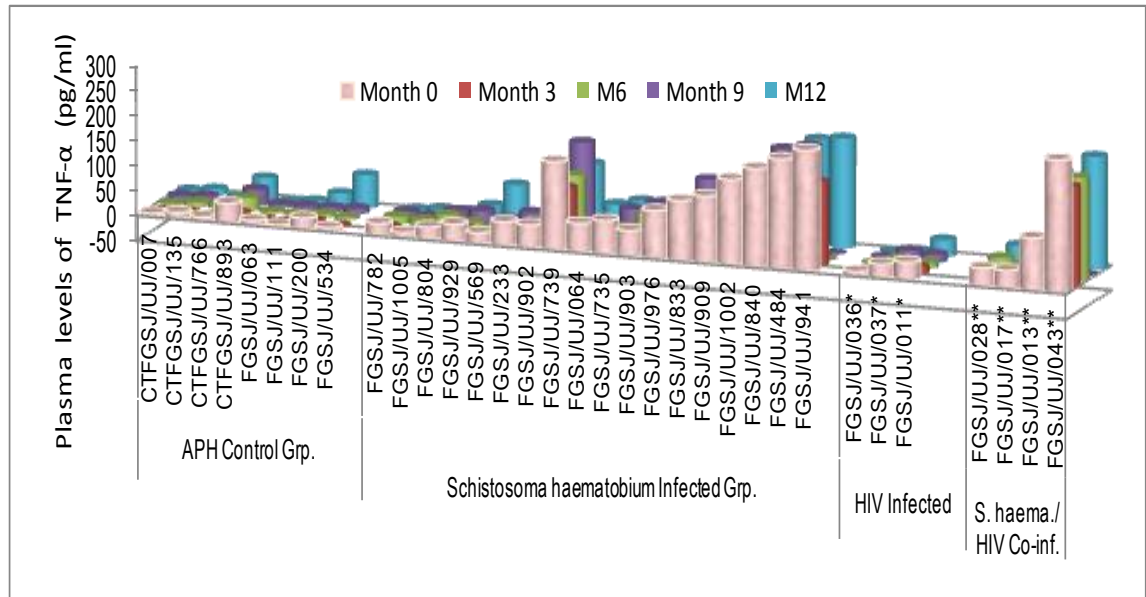
(b)

Fig 13: Plasma levels of IFN-γ in study population

- a): Mean plasma levels of IFN-γ in control and infected subjects,
- b): Sustained plasma levels of IFN-γ during 12 months (Cal. F = 2.41 > Tab. F = 1.39; Cal. F = 108.4 > Tab. F = 2.53: p < 0.05)



(a)



(b)

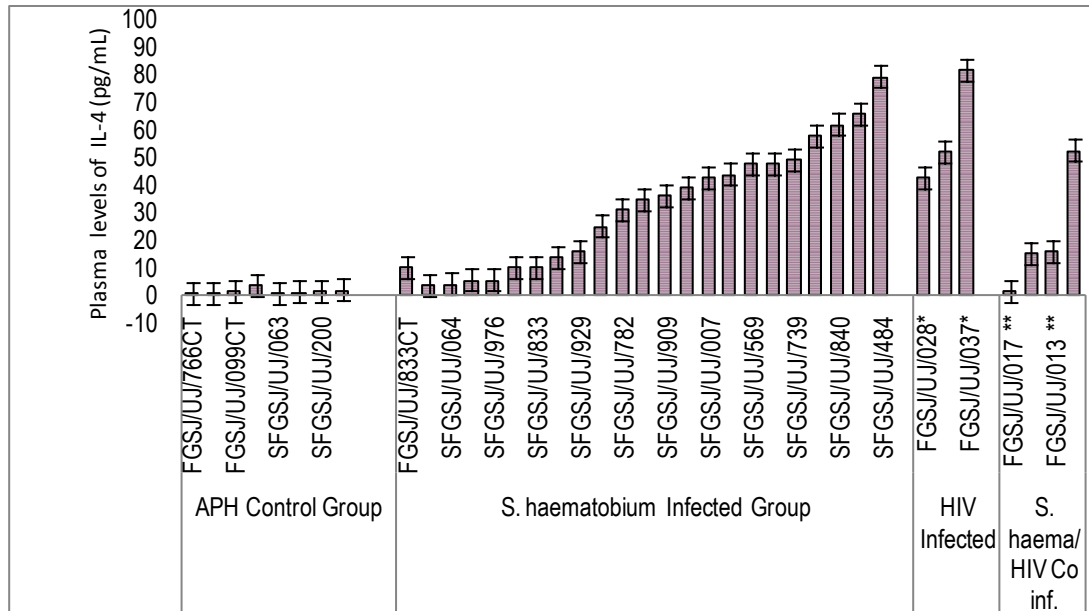
Fig 14: Plasma levels of TNF-α of study subjects

a) : Mean plasma levels of TNF-α

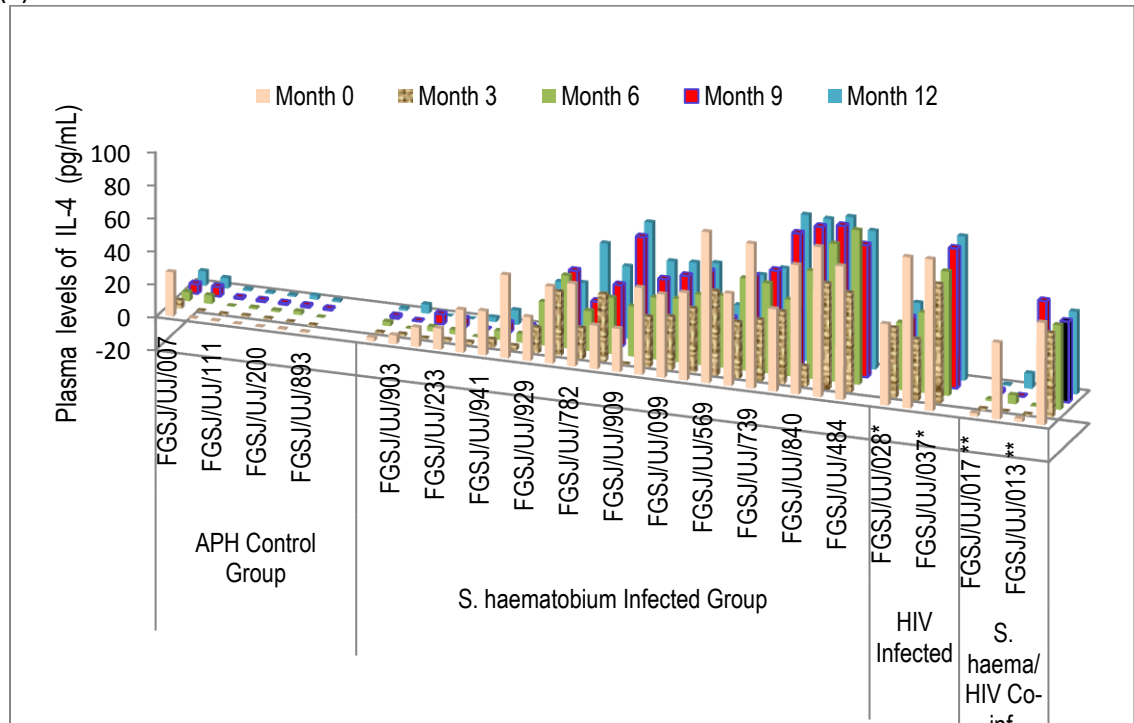
b) : Sustained plasma levels of TNF-α during 12 months

(Cal. F = 6.89 > Tab. F = 1.39 at P < 0.05; Cal. F = 5.87 > Tab. F = 2.53: P < 0.05)

(all significant)



(a)



(b)

Fig 15: Plasma levels of interleukin four (IL-4) in study subjects

a) : Mean plasma levels of IL-4.

b) : Sustained plasma levels of IL-4 in apparently healthy, single and dual infection of HIV and urinary schistosomiasis in female population in Jos, Plateau.

In all, changes in cytokines were marked with a general inconsistency across all groups (Fig. 16). This demonstrates urogenital schistosomiasis and HIV associated immunopathology and inflammatory reaction to *Schistosoma* infection. Rises in the levels, TNF- α increased in schistosomiasis infection and the increases in TNF- α and IL-4 levels appended to schistosomiasis morbidity by up regulation of immunopathology of schistosomiasis and HIV replication which contributed to cytokine disorder. The CD4 count of the study population revealed a no significant difference in APH and schistosomiasis infected groups (mean CD4 count: 373 – 1006 cells/ μ L: 322-976 cells/ μ L). There was however, a significant difference observed in the mean CD4 T-Cell counts in the HIV infected alone and HIV/urogenital schistosomiasis co-infected subjects (Fig. 17)

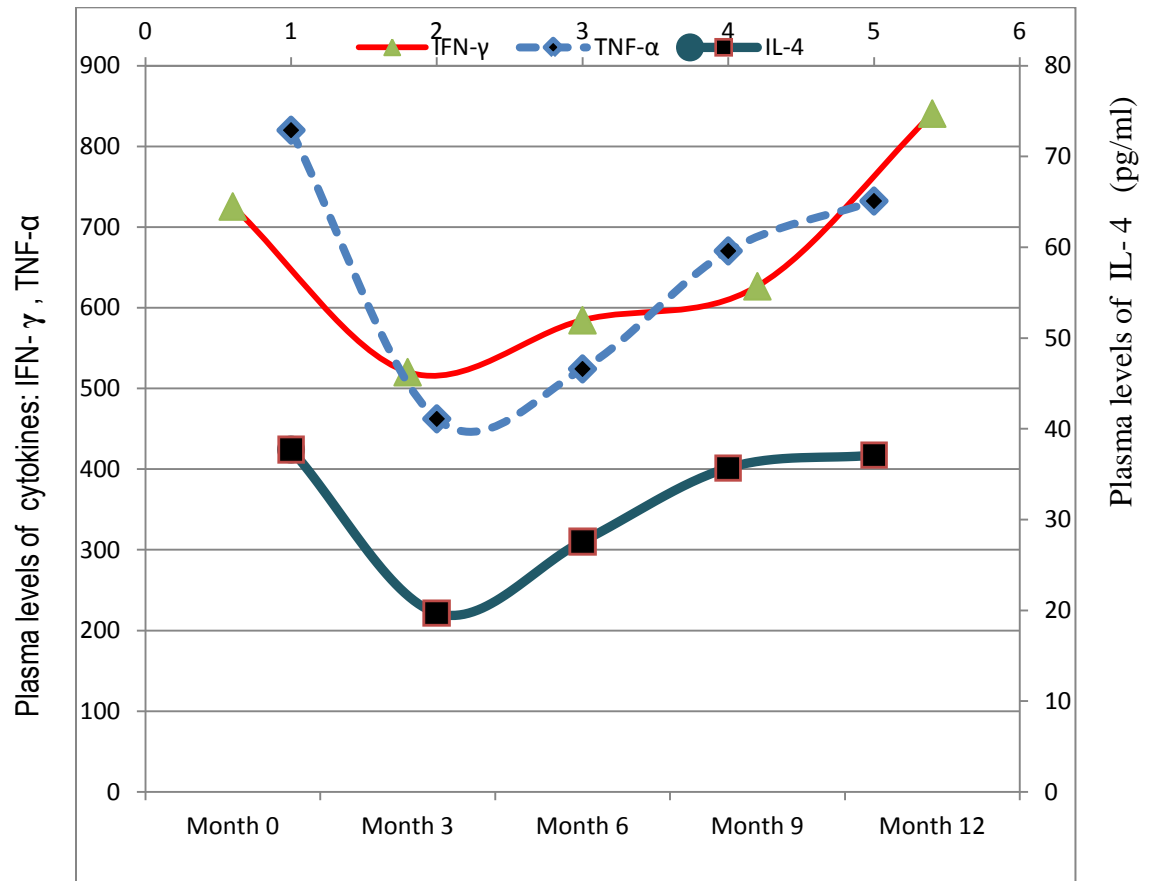


Fig 16: Plasma levels pattern of three relevant Cytokines (IFN- γ , TNF- α , and IL-4) in apparently healthy, HIV infected individuals from urinary schistosomiasis endemic female population in Jos Plateau

4.8 Clone Differential Four (CD4) T - Lymphocyte Cell Levels in Apparently Healthy, HIV infected and FUGS/HIV Co-infected females from Study Populations

The CD4+ cells count of both the HIV infected and HIV-negative schistosomiasis individuals were evaluated and measured. The CD4 count of the study population revealed a no significant difference in APH and schistosomiasis infected group (mean CD4 count: 373 – 1006 cells/ μ L: 322-976 cells/ μ L). There was however, a significantly different observed mean CD4 T- Cell count in the HIV infected alone and HIV/urogenital schistosomiasis co-infected subjects (Fig 17).

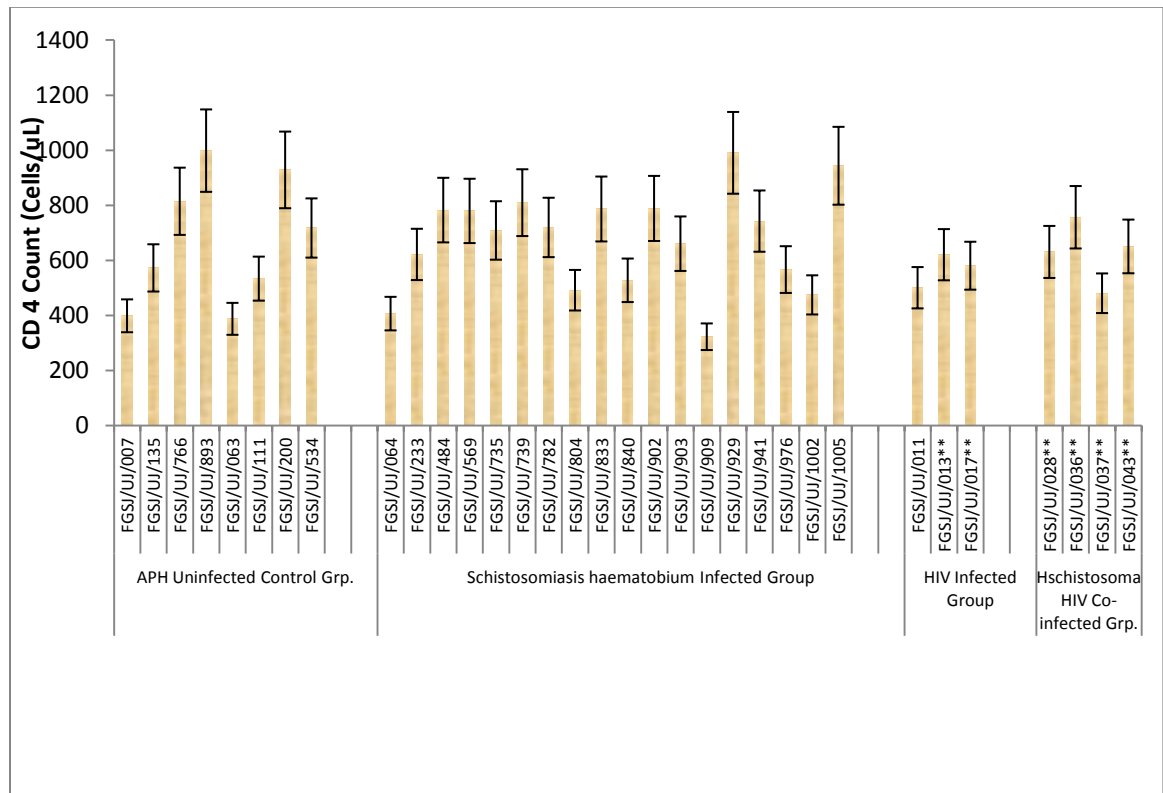


Fig. 17: Mean CD4 T Cell Levels of Study Subjects

CHAPTER FIVE DISCUSSION

5.1 PREVALENCE OF UROGENITAL SCHISTOSOMIASIS IN STUDY POPULATION

The result of the present study showed that urogenital schistosomiasis is endemic in Jos, Plateau State. These results show moderate infections of schistosomiasis in the study communities and these agree with earlier studies in Jos, and Toro in Bauchi State, where 20.5% and 21.4% were recorded respectively (Njoku *et al.*, 2004). Similar results were also obtained in endemic communities of Edo and Ebonyi States with 22.9%; 22.1% prevalence respectively (Nmorsi *et al.*, 2001a; Anosike *et al.*, 2006). A much higher prevalence of 32.6% was reported in some parts of Edo State (Nmorsi *et al.*, 2001b); 42.3% in the north-central zone of Abia State (Anosike *et al.*, 2001); 47.8% in a surveillance study of some local government areas of Plateau State (Akufongwe *et al.*, 1996); 66% and 65.0% in Delta and Edo States (Ukwandu and Nmorsi, 2004; Nmorsi *et al.* 2005;) and 83.3% in Port-Harcourt (Agi and Okafor, 2005). Other studies recorded a 100% (hospital-based study) in Malawi; 42% prevalence in Tanzania, and 76% infection rate in Madagascar respectively (Kjetland *et al.*, 1996, Poggensee *et al.*, 2000a, Leutscher *et al.*, 1998). However, in contrast to these moderate and high occurrences, Okpala *et al.*, (2004) reported a low prevalence of 0.67% in Apata and Laranto areas of Jos. While in the urban city of Port – Harcourt, Arene *et al.*, (1989) obtained 5.7% schistosomiasis prevalence.

The percentage prevalence observed in this study however, reflects the level of *S. haematobium* transmission in the study areas. Interviews conducted, revealed that the study communities lacked pipe-borne water or good alternative water sources; which

resulted in a high dependence on the cercariae infested perennial and seasonal streams located in the areas. These streams, which are natural freshwater sources, distributed over the communities are the main transmission points by which the inhabitants are infected. The streams also provide ideal aquatic vegetations and environment required for the breeding of the *Bulinus* and other snail intermediate hosts species. This then serves as a meeting point for the *Schistosoma* parasites, their intermediate hosts and the susceptible individuals. Thus, the high dependence of the inhabitants on these streams for their daily domestic chores, recreational and economic needs, is believed to be responsible for the moderate or high prevalence recorded in the study. This further, ensured that the subjects are continuously being infected and re-infected since no intervention strategy has been carried out in the area nor will be in the near future.

In addition, the cluster of houses, non-availability of household sanitary facilities and indiscriminate defecation around the houses, inside and along the stream banks confirms the filthy environment in which the subjects are exposed to, and the deficit of personal hygienic practices. This is obviously responsible for maintenance of the vicious cycle of human infection – contamination of the freshwater stream – infection of snail hosts and human infection through water contact. Although, infections varied among subjects in the various communities, it was not statistically significant ($P > 0.05$). This was expected since the communities had similar environmental, ecological and edaphic features (rocky valleys with infested shallow freshwater streams, pools of seasonal streams and irrigation canals).

Stratifying the present result by age, the prevalence of infection was highest among the 11-20 year olds followed by those aged ≥ 50 and 21–30 year-olds respectively. A Chi-Squared Statistical Analysis of the results revealed that *S.*

haematobium schistosomiasis is more common to some age groups ($P < 0.05$). The high prevalence observed among the 11-20 years is suggestive of the risk of exposure within this age group, who spend more time in infected water for either domestic, recreational or economic activities. This agrees with Anosike *et al.*, (1992); Nmorsi *et al.*, (2005); Anosike *et al.*, (2006) who recorded similar findings among the same age groups, although, they attributed the variation of infection to different water contact pattern.

The high prevalence observed among the ≥ 50 year olds disagrees with several other studies where low prevalence have been maintained. This observation, we believe could have been as a result of the number examined and the frequency of contact by these group of individuals with the cercariae infested water bodies. The low prevalence reported by other studies, however, was attributed to a combined function of acquired immunity and frequency of water contact (Nwoke *et al.*, 2004; Anosike *et al.*, 2003). Obviously, they believed that older persons delegate their younger ones to carry out domestic chores. This reduces the exposure of older ones to infested waters. Akufongwe *et al.*, (1996), reporting on studies from rural communities in Plateau State, observed 51.3% in 21-24 age group. They believed in this case, that higher contact with cercariae-infested water was responsible, since the villagers had no alternative water sources and used the streams all the time for all activities.

In relation to signs and symptoms associated with urinary schistosomiasis, 8.7% had clinical symptoms of schistosomiasis while low prevalence was recorded for haematuria and proteinuria, respectively. The prevalence of haematuria was low compared to other studies where prevalence as high as 40-86% have been reported (Ahoskie *et al.*, 2006; Norse *et al.*, 2005; Kwando and Norse, 2004; Dakar *et al.*, 1997; Kwando and Buck, 1996). The low haematuria prevalence reported in this study is suggestive of acquired immunity, developed by repeated subjects' multiple exposure in

the course of advancement in age. Haematuria and proteinuria according to Marieke *et al.*, (2004), has been used as a predictive tool to the prevalence of *S. haematobium* infection in an endemic area and Poggensee *et al.*, (2000a) also associated pathology/morbidity of *S. haematobium* infection as indicative.

5.2 PARASITOLOGICAL PREVALENCE OF FUGS

Parasitological diagnosis of *S. haematobium* infection by urine analysis and high vaginal swab methods in this study were low compared to the 40% positivity by urine analysis and 23% by HVS, obtained by Poggensee *et al.*, (1998) in a study in Tanzania. The occurrence of *S. haematobium* eggs using the HVS method confirmed FUGS prevalence parasitologically. This is so because according to Mosunjac *et al.*, (2003), FUGS had been detected frequently in women with scanty or even no egg excretion in their urine. Poggensee *et al.*, (1998) in another study also confirmed this as a highly sensitive urine examination technique when applied on a population-based study. They observed that 23% of the women who did not excrete *S. haematobium* eggs in their urine were classified as uninfected. A multiple occurrence of *S. haematobium* eggs (0.6%) in both the urine analysis and HVS methods confirmed FUGS. The FUGS prevalence obtained in this study is in the same range with earlier studies by Njoku *et al.*, (2004). Although low, Poggensee *et al.*, (1998) and Kjetland *et al.*, (1996) obtained an FUGS parasitological prevalence of 55-65% in women with diagnosed urogenital schistosomiasis, while Szela *et al.*, (1993) reported 46% FUGS in Ghana. This suggests that in the diagnosis of FUGS, urine analysis sensitivity and specificity may not be as high as HVS method. Thus, HVS method may be more specific in determining FUGS parasitologically.

5.3 SEROPREVALENCE OF HIV INFECTION IN THE STUDY POPULATION

A prevalence of 6.8% (27/396) HIV infection was recorded in apparently healthy (AH) population of this study. The prevalence observed was attributed to the difficulty in changing human behaviors which supports the persisting increase of HIV infection in the society. Despite the enlightenment campaigns, awareness programmes to improve on the denial/ignorance of the disease, the attitudes of the populace and habits of indulging in illicit sexual activities suggests a persisting rise of HIV prevalence in the healthy population, in this study. This agrees with the FMOH, (2006) report where prevalence of between 5.5%-10% have been recorded in north central Nigeria.

In relation to the study communities, HIV prevalence recorded in this study agrees with Idoko *et al.*, (1998) who observed a similar prevalence in Abattoir community of Jos, Plateau State. In the present study, sociocultural and economic factors were attributed to the variations in HIV infection in the different communities. These observations agree with UNAIDS, (2007), which reported similar observations as a contributing factor to the 1.8% HIV prevalence recorded in Benin Republic and 18.8% in South Africa respectively.

In relation to age, HIV infection was high among the 0-40 year-olds with adults 21-30 year olds accounting for the highest proportion of infection. A significant difference of HIV infection observed in the different age groups, confirmed age as a confounding factor in HIV epidemiology. The percentage prevalence observed for these age groups is low compared to other studies where as high as 18.5% - 44.4% had been reported among

the 20-40 year olds (Njoku *et al.*, 2004; Ouma *et al.*, 2000). Other studies in Botswana, Lesotho, Swaziland and Zimbabwe, reported 20%-36% HIV prevalence among females aged 15-49 years (CDC, 1997; UNAIDS, 2002).

The observed prevalence in these age groups was linked to various socio-economic, behavioral and cultural factors, which serve as risk socio-epidemiological factors. In this respect, most of those infected were young single girls, married/separated and widowed women. These adults are known to be the most sexually active groups, who, coupled with economic demands on them, indulge in sex trade as a means of survival and sex satisfaction especially with men of better economic advantage. In Nigeria and most parts of Africa, similar reports had been made. High HIV prevalence was seen as a common phenomenon, where cultural norms favor polygamy and multiple sex partnership for the male folk with the attendant liberty to indulge in illegitimate sex with many sex partners (Agwale *et al.*, 2001; Nnatu *et al.*, 1993; Olaleye *et al.*, 1993).

HIV prevalence in children (0-10yrs) (7.1%) and the elderly (≥ 50) (3.7%) however, is low in the study areas. The low prevalence observed in the children was perhaps due to the fact that children mostly acquire the virus from their mothers. While in the elderly, perhaps due to old age, and reduced sexual activity (Njoku *et al.*, 2004; Fylkesnes *et al.*, 1997; Olaleye *et al.*, 1993). Occupationally, students had the highest infection (39.7%), followed by farmers (15%) while housewives represented the least infection with 1.3%. The high prevalence recorded among the students is in line with (FMOH, 2007) who observed them as the most active sexual group who will indulge in any form of sexual act as a means to generate income since most of them come from very poor homes. This is consistent with reports by Ani and Agwale, (1998),

emphasizing the group as high risk group for HIV infection, especially in the light of the economy of the country.

5.4. DISTRIBUTION AND PREVALENCE OF *S. haematobium* INTERMEDIATE HOSTS IN THE STUDY COMMUNITIES.

The findings in this study show that *Bulinus* spp. (*Bulinus globosus* and *Bulinus truncatus*), the intermediate hosts of *Schistosoma haematobium*, *Biomphalaria* and *Lymnaea natalensis* were the most common snails found at the community streams of the areas studied. This is consistent with other studies where the abundance of different snail species, which included *B. globosus*, *B. truncatus*, *B. senegalensis*, *Biomphalaria pfeifferi*, *Melanoides tuberculata*, *Lymnaea natalensis*, *Physa waterloti*, and *Gyraulus* spp. were reported (Anosike *et al.*, 2006). *Bulinus globosus* accounted for more than two-thirds of all species collected, followed by *B. truncatus* and *Lymnaea natalensis*. In similar observations, Kariuki *et al.*, (2004) reported *B. nasutus*, *Lanistes purpureus* and *B. forskalii* as the most common snails in Kenya, with *Melanoides tuberculata*, and *Pila ovata*, that were occasionally recovered from the rice farms in that area.

In the present study, *Bulinus* species were found to occupy a wide diversity of habitats such as vegetations in and around shallow perennial fresh water streams, irrigation canals, seasonal pools and stagnant water bodies. The abundance of *B. globosus* in particular was more pronounced in the shallow flowing sections of the streams that flow directly from the rocks in the study area. These study sites harbor aquatic vegetations and grasses from which the snails feed on. Similarly, many *B. globosus* were also recovered from underneath vegetations and grasses that grew in the irrigated canal and rain-filled pools as was observed in the Tudun Wada and Abattoir

community sites. *B. truncatus* on the other hand, thrived more in the Nabong (stagnant) sewage polluted stream. Here, the stream is fed by three major pollution sources which are the refuse dump of the old legislative quarters, the community and the automobile wastes of the mechanic village which all empty directly into the stream. The stream bed also provides the best flat rocky basements for laundry, from where detergents are very common.

Biomphalaria species thrived more in the Abattoir small freshwater habitats. *Lymnaea natalensis* (the intermediate host of *Fasciola hepatica*), which could easily be mistaken for *B. globosus* thrived in similar habitats as *B. globosus* but is more tolerant of both pollution and temperature variations. Thus, the snail density and species diversity in this study could likely have been as a result of the chemical, physical and biological factors to be found in a particular water body at a given period of time. Secondly, the presence of horizontal lilies (*Nymphaea spp.*) and slimy vegetation (spirogyra) seen growing in and around streams was also associated with snail density. Most snails were seen resting on the underside of these oxygen rich vegetations (due to photosynthesis) which also provide egg-laying surfaces and shelter from both sun and predators (O'Keefe, 1985a, 1985b). For food, the snail feed on the micro flora and decaying plant matter of the lilies. This is in conformity with studies by Kariuki *et al.*, (2004) and Sturrock *et al.*, (1990), who reported similar findings. They further, associated the abundance of *Bulinus* species to water temperature and unseasonable rains during the cooler months.

There was no significant variation from site to site, perhaps, due to similar water, vegetation and edaphic features of all study communities. Thus, the common occurrence of known schistosomiasis snail intermediate hosts (*Bulinus* and *Biomphalaria*) snail

species in the water bodies and streams of the study communities predisposes the population to the risk of *S. haematobium* and *S. mansoni* infections.

The relatively high infection rate of the *Bulinus Species*, it is believed, was responsible for the high human cases of urinary schistosomiasis in the studied communities. Infections in snails were sustained throughout the seasons with snail infection rate higher in the dry season (42.2%) than the rainy season (36.5%). These along with the high level of human activities in and around the water bodies exposed the inhabitants of these communities to infection (Akunfogwe *et al.*, 1996; Anosike *et al.*, 1992). In the same vein; Kariuki *et al.*, (2004), associated low *S. haematobium* cercarial shedding to heavy and extensive rainfall and Anosike *et al.*, (2006) in a study in Ezza, Ebonyi State, associated low infection rate of *B. globosus* to low prevalence and intensity of urinary schistosomiasis in the studied communities. Impliedly, they agreed that *S. haematobium* infection is a factor of community schistosomiasis prevalence and snail infectivity.

5.5 RISK ACTIVITIES ASSOCIATED WITH SCHISTOSOMIASIS IN STUDY COMMUNITIES

In this study, several risk activities were engaged by the subjects, which exposed them to *S. haematobium* infection. These risk activities involve contact with water for different economic, recreational or domestic activities. The streams, irrigation channels, seasonal pools of water gathered during the rainy seasons served as the main transmission points. Water contact for economic and domestic nature resulted in more frequent and intensive contact with infected water bodies than that for recreational purposes. Water contact for economic need (irrigation farming) was exhibited on different daily and seasonal periods in particular during the hot dry seasons. The frequency of contact with these cercariae infested water bodies either during crop

watering or irrigation farming placed the subjects constantly at risk of infection. Secondly the edaphic nature of the farms which are very often adjacent the *Schistosoma* infested streams, make it very difficult for the soil to retain water even during the rainy season because of the strong run-off at the hillside farms. Thus, this necessitated the creation of irrigation channels and blocking of ridge valleys during the rainy seasons. These channels made good breeding grounds for the snail intermediate hosts and also provides the inhabitants the opportunity for transmission of schistosomiasis through the wadding in these pools, during weeding and harvesting of crops. The channels on the other hand, are often more than 4ft deep, providing immediate reservoir for watering of crops at the onset and main dry seasons until the water level get very low for a shift into the adjacent stream. At all points in this particular activity, these individuals are exposed to high concentrations of *Schistosoma* cercariae.

In addition, in this part of the country, irrigation farming is practiced as many time as possible before the rains because of the beneficial costs of dry season crops and vegetables (such as potatoes, tomatoes, carrots, cabbage, etc.) which encourages the most of water contact activities despite the risk. Furthermore, during the dry months of the year, alternative sources of water supply dry up in the absence of pipe-borne water and residents are forced to depend on the *Schistosoma* -infested streams for the supply of water for their laundry and irrigation. As a result, they get infected. The observed percentage frequency for this particular water contact activity, in this study, is however, in contrast, with some studies where the most frequent water contact reported was that for recreational purposes (swimming, playing) (Kapito-Tempo *et al.*, 2009; Opara *et al.*, 2007; Kloos *et al.*, 2006). Akufongwe *et al.*, (1995) in another study reported a higher contact among subjects who were involved in laundry, bathing, and fetching of water for domestic chores. The high level of exposure to contaminated water was noted among

adults with a percentage contact of 73.3% compared to the school aged children (67.9%). This is consistent with reports of Poggensee *et al.*, (2002) and Nmorsi *et al.*, (2001b) where 20-30yrs had a more frequent contact while involved with washing, practicing irrigation farming, fetching water for daily routine household chores, fermentation of cassava tubers or even recreation. Ofoezie *et al.*, (1998) and Kapito-Tembo *et al.*, (2009) on the other hand, had a contrary view. They observed a more frequent contact among the 6-14 year olds, although water contact for economic nature was higher in 20-30 year olds.

In the case of water contact for domestic needs, the female adults (34.2%) also had a higher contact than the children (21.5%). The reason being that these women, due to educational and economic backwardness, who are also known as the keeper of their homes in our African setting, spend more time on domestic chores (such as washing utensils, laundry) and washing of their farm products like carrots, potatoes for their local markets, thus exposing themselves to infection (Etim, 1995). This is contrary to Anosike *et al.*, (2006), who observed a higher contact among the children/adolescence in a study among Ezza people in Ebonyi State. They attributed their findings to young people's habit of spending more time in washing, fishing or playing. In addition, he also observed changes in water contact habits of the older adults. Here they rather delegate the young ones to carry out their domestic chores than being exposed to the infested water. There was no significant difference ($P>0.05$) in WCA of children and adults. This is likely because while children assist their parents in their irrigation and domestic activities in the water; adults are actively engaged in these activities themselves.

It has been observed that decrease in water volume during the dry seasons and the scorching heat encourage children to play, bath or swim as a way of cooling off,

hence increasing the frequency of infested water contact (El-Khoby *et al.*, 2000). In this study however, the water contact for recreational activities (i.e. swimming, bathing /playing) scored lowest, 7.3% when compared to other studies where as high as 86% have been reported (Akufongwe *et al.*, 1996). The low frequency of WCA recorded was attributed to the unattractive edaphic nature of the small rocky streams and the low water volume, which did not encourage much swimming or playing. Other studies on the other hand, implicated water contact for recreational purposes as the major source of contracting urinary schistosomiasis (Satayathum *et al.*, (2006); Kapito-Tembo *et al.*, (2009); Ndyomugenyi (2001); Kloos *et al.*, (2006); Ofoezie *et al.*, (1998). They linked this recreational water contact behaviors to hot seasons, high volume of water and consequently, to higher frequency of contact to infested water. They further suggested that all these activities by virtue of keeping the subjects much longer in water contributed to the high prevalence and intensity of *Schistosoma* infection reported in the communities. In relation to age, children and adolescence were observed to exhibit more of these water contact behaviors than the adults. Interestingly; although age was not a determinant of risk exposure through WCA, there was a significant difference between the frequency of different WCAs ($P < 0.05$).

Other water-related activities, with their percentage frequencies include, fetching of household water for domestic purposes (3.6%), molding of block (4.0%) for the block industry. The low percentages obtained could be attributed to the fact, that these children do not fetch the water at all times and even when they do, they do not dip their legs into the waters to fetch the water. On further enquiry, we observed that the block industries have support water pumping machines which assist them to mechanically pull water from the streams. This limits their frequent fetching contact although; they may still get exposed during block watering process. In addition, machine

breakdown or gas conservation (which cost is at the moment cost intensive) often increase WCA. Many households are also limiting their use of the contaminated water through storage of harvested rain water and shallow wells in their homes. All these are helping to reduce the frequency of water contact with infested streams. However, a few accept that they have been educated on the dangers of the small snails, but said they have no immediate alternative. While some did not believe it is as dangerous, others believe that the medicines they get (from the Carter Center) can take care of everything. Perhaps this is may be encouraging but need to be supported by the State and Federal Ministries of Health, to increase the benefits to endemic communities.

5.6 STREAMS CONTAMINATED WITH FAECAL WASTES

In this study, we observed that the semi-urban communities are very poor and that majority of the houses lacked sanitary facilities. Rampant defecation around individual homes of inhabitants and water bodies were more pronounced during the dry season probably due to the stable low water level. The increases in water level during the rains obviously wash the feces into the stream, while rodents and livestock activities assist in pushing the feces into the stream. This favors water contamination with eggs and miracidia with consequent snail infection. Those that lived very near the streams build their suck-away facilities or sewage tanks near the stream banks and made them to unhygienically empty directly into the stream. The stools and urine wash directly into these rivers or streams contaminating them and continuing the vicious cycle of infection from homes to snails in the waters, from where infective cercariae emerge to infect the population through WCAs.

5.7 PREVALENCE OF UROGENITAL SCHISTOSOMIASIS AND HIV IN THE STUDY POPULATIONS

In this study, a concurrent association between *S. haematobium* and HIV was observed. A 14(5.3%) *S. haematobium*/HIV co-infection was obtained with as high as 42.1% co-infection in the 21-30 years individuals. These results confirm *S. haematobium* and HIV co-infections in some endemic communities in Plateau State. Similar observations have been noted elsewhere and linked to FUGS (Erikstrup *et al.*, 2007; Secor *et al.*, 2003; Bentwich *et al.*, 1999). *Schistosoma* worms according to Kallestrup (2003) secrete substances that induce a panel of immunological alterations which often facilitate and propagate rapid replication of HIV individuals. This according to the study is because *S. haematobium* eggs or worms in the female genitalia causes disruption in the subjects' genitalia and recruits activated lymphocytes which are targets for viral infection at the site of viral exposure. Adverse and novel events have been observed in concomitant infections of urinary schistosomiasis with HIV and other opportunistic infections (Crump *et al.*, 2000). Here, many studies have shown that CD4⁺ cells from patients with active schistosomiasis express higher levels of the chemokine receptors/HIV-1 co receptors CXCR4 and CCR5, than do cells from patients who previously had schistosomiasis but had been treated (Secor *et al.*, 2003). This is because HIV-1 replicates more readily in activated T cells (Kullberg, *et al.*, 1992, Mossman *et al.*, 1989,). Secondly, they observed an increased production of the type Th2 cells, which favors the replication of HIV virus and impairs the cytotoxic T-lymphocyte response (Bundy *et al.*, 2000). This results in elevated IL-4 levels, which are characteristic of the different types of immune response in helminthic infections (Bentwich *et al.*, 1995). Findings in this study, suggest interplay of the immune status, effects of the pathogens in the host's immune environment as well as the stage of the HIV infection in the individual. This means that urinary schistosomiasis could be a

strong co-factor in promoting HIV-1 replication and progression especially in the presence of FUGS.

5.8. INDICATIONS OF UROGENITAL PATHOLOGY IN *S. haematobium* INFECTED FEMALES IN JOS PLATEAU

In this study, immunological indices of urogenital pathology were observed among the urinary schistosomiasis infected females compared to the uninfected ones. These pathological indices were more common among *S. haematobium*, HIV positive and in dual infected individuals. This suggests that these indicators, is a reflection of the level of disease morbidity in the study population. Secondly, the measurements of these pathological indices show evidence of infection, intensity of disease burden and level of pathology on the urinary tract of infected hosts. This is consistent with studies by AL-Sherbing *et al.*, 1999 and Marieke *et al.*, (2004) who reported that the detection of pathology/morbidity due to *S. haematobium* showed a clear consistent association with prevalence of infection. Other studies also explored association between prevalence of infection and haematuria measured by reagent strip (Lwambo *et al.*, 1997) and found patterns similar to the current study. Many other studies observed that ultrasound detectable pathology correlated well with other measures of morbidity which included urinary egg count, haematuria, proteinuria and leukocyturia (Hatz *et al.*, 1998; Doehring and Kardoff, 1995; Hatz *et al.*, 1990; Heurtur *et al.*, 1986; Doehring *et al.*, 1985;). Figueiredo *et al.*, (2009) in his study observed that a raised urine-albumin level was found to be associated with the presence of micro-haematuria in school-children, whereas in adults a raised urine-albumin level was found to be significantly associated with the presence of leukocytes and higher urine's specific gravity (1.02 g/ml).

Although ultrasound does not detect the early inflammatory reactions or early pathological changes of the bladder wall (Burki *et al.*, 1986) and despite the fact that

intensity of infection is a major factor responsible for development of pathology, disease *sequelae* between individuals with the same intensities may vary (Smith and Christie, 1986). Furthermore, our methodology offers a closer look at the test characteristics of the different urinary tract indices and its biological relationships to urinary tract morbidity. This is made possible since the risk of both schistosomiasis and urinary tract infection will probably be higher in environments with no sanitary facilities available. In addition, individuals living in poor rural areas are likely to have nutritional deficiencies making them more susceptible to acquiring the disease.

In relation to age, higher descriptive percentage was recorded in the young adults/teenagers ($\leq 10 - 25$ yrs) compared to the older adults ($26 - \geq 40$ yrs); statistically (Cal F = 48.7 > Tab F. = 5.12; df_{1, 20}) a significant difference was observed suggesting that although the disease cuts across all ages, the young adults were more exposed than the older subjects. In this study, however, the intensity of urinary schistosomiasis egg-burden as was observed (in earlier studies) was rather more pronounced in the young adults/teenagers who were associated with frequent visits to the cercariae infested waters for their economic and domestic needs. In the same vein, the older adults frequent this same streams but with their young ones to assist them during their economic/domestic visits to the streams. Thus, reducing the frequencies of direct contact with the cercariae infested water bodies but exposing their young ones instead. Therefore, contact then is made only when they help in the daily domestic/economic (i.e. during irrigation farming, washing clothes, fetching of water for domestic purposes) visits to the cercariae infested waters. Therefore, infection of both the young and the old adults is made possible. Similar studies were recorded in Zanzibar, where higher prevalence was observed in adults males (64.4%) compared to the children (39.4%). Marieke *et al.*, (2004) in another study also observed higher prevalence in adult (24 vs. 16%). On the

contrary, Woolhouse *et al.*, (1991) reported higher prevalence and intensity of infection in children than adults. He attributed the cause to this difference, to other causes such as urinary tract infection, sexually transmitted diseases and menstruation which of course are observed more in adults than children.

5.9 THE CIRCULATING CATHODIC ANTIGEN MEASURES IN STUDY POPULATION

In this study, the detection of circulating cathodic antigens (CCA) was used as an additional measure of urinary tract morbidity assessment. It was also used as secondary measure of patients' infection status in both HIV infected and non-infected genital schistosomiasis patients. The results obtained in this study, shows that the CCA test could serve as an alternative non-invasive diagnostic method for urinary schistosomiasis. With the increased sensitivity of the assay, one can determine early infection and worm burden of infected subjects with higher accuracy. In addition, the use of this method highlights the value of integrating immunodiagnostic tools and clinical findings, since the clinical findings (hematuria, leukocyturia) have been significantly associated with egg-positive urinary schistosomiasis patients and urinary tract pathology. The results from this study is consistent with other studies by Van Dam *et al.*, (2004); Van Lieshout *et al.*, (2000) and Polman *et al.*, (1995) who all described the sensitivity and specificity of CCA test as 96% - 100% which is very high compared to the parasitological methods. They also ascribed the CCA test as a very sensitive indicator of level of worm burden and active infections. Al-Sherbing *et al.*, (1999) in their study, noted that the urine-CCA assay identified 52 (78%) out of 67 cases of urinary schistosomiasis.

However, in another study, Stothard *et al.*, (2006) observed that the CCA dipstick could not detect the circulating antigens for *S. haematobium* infections even though that of *S. mansoni* was detected. The reason, they attributed to the CCA strip stoichiometry of the antigen-antibody interaction having a greater affinity to intestinal schistosomiasis than urinary schistosomiasis. Karanja *et al.*, (1998) in a study in Western Kenya, reported high prevalence in both HIV infected and uninfected urogenital schistosomiasis car washers. Furthermore, he observed that at six months, following Praziquantel treatment, the levels of CCA in these individuals was moderately lower than the initial CCA levels. He attributed this observation to the fact that the subjects became re-infected after being treated due to their nature of work which constantly exposes them to *Schistosoma* infections. Even those that were not excreting eggs at four weeks post-treatment, showed renewed egg excretion. Secondly, the fact that Praziquantel treatment does not clear the infection completely, only suggests that some of the worms that might have survived the therapy, emerge to continue their destructive act on the individuals tissues thus, increasing disease burden and morbidity. In addition, we believe that the participants in this study might have been exposed to re-infection since they continued with their daily water contact activities of economic/domestic needs.

5.10 OBSERVED IMMUNOLOGY INDICES IN STUDY POPULATION

Urogenital schistosomiasis has mainly depended upon finding ova in the positive persons' tissues, wet smears, pap smears and cytology or by ultrasound (Kjetland *et al.*, 2005; Poggensee *et al.*, 2001 and Kjetland *et al.*, 1996). Immunological testing has been used to enhance our ability to detect the disease morbidity. A variety of cell types including hepatic satellite cells, activated macrophages and regulatory T-cells have also been implicated in the pathogenesis of schistosomiasis (WHO, 2009). Feldmeier, (2009)

had noted that women develop genital schistosomiasis, lesions develop in the vagina and in the cervix and that the type of the lesions make the epithelium very thin and vulnerable. This, he suggests could be agent of sexually transmitted diseases particularly HIV.

5.10.1 Impact of Schistosomiasis on Cytokine Elevation

Sequestered schistosomiasis eggs have been shown to evoke a complex cellular and humoral immune response in tissues (Mbabazi *et al.*, 2011). The eggs of *S. haematobium* can induce areas of inflammation or of large granulomata in the females' genital tract with recruitment of plasma cells, lymphocytes, granulocytes, macrophages and eosinophils to the site (Helling-Giese *et al.*, 1996).

One important risk factor for development of morbidity is the severity and nature of host granulomatous inflammation (Burke *et al.*, 2009; Warren, 1982a, 1982b). Studies primarily from mice, have shown that the granuloma is a cellular immune response to antigens secreted by viable ova that recruits CD4⁻, $\alpha\beta^+$, and major histocompatibility complex II-dependent T cells. The immune response is often controlled by counter-regulating cytokines, chemokines, and cell adhesion molecules and by expression of their associated receptors, which successively initiate, maintain, and immunomodulate the granuloma (Cheever and Yap, 1997). In this study, however, the Th1 parasite specific plasma cytokines such as IFN- γ and TNF- α and the Th₂ cytokine IL – 4 levels were measured in HIV infected and non-infected *S. haematobium* positive subjects. At baseline and one year follow up examinations, significant differences in the cytokine levels of the four groups of individuals screened was observed. However, whether low

or highly elevated, the cytokine levels measured clearly shows that an inflammatory process involving the cytokines was occurring.

Immunological response in patients with chronic schistosomiasis has been characterized by decreased IFN- γ , TNF- α and enhancement of Th-2 cytokines such as IL-4 (Araujo *et al.*, 1996; Jesus *et al.*, 1993). According to some studies, the high IL-4 produced was induced by the parasite antigen itself for host defense mechanism against *S. mansoni* infection (Ribeiro *et al.*, 2000; Correa-Oliveira *et al.*, 1989 and Dessein *et al.*, 1988).

In the present study, the presence of pathologic changes detected by the levels of cytokine measured was significantly associated with the intensity of infection. This was obvious from the results obtained from the genital schistosomiasis positive/HIV negative individuals (that is, group 1), which show high concentration levels of IFN- γ , IL-4 and TNF- α at base line compared to the levels obtained in normal persons which is below 25pg/ml for IFN- γ and below 0.4pg/ml for IL-4 (Secor *et al.*, 2006). This result reflects the possibility that the intensity of urinary schistosomiasis could have increased the cytokine levels which of course would lead to increased urinary tract morbidity. This could be true since the intensity of schistosomiasis infection has been marked as a major factor responsible for the development of pathology (Mott *et al.*, 1983; Abdel-Salam and Elsan, 1978; Cheever *et al.*, 1978). This is consistent with other studies where high levels of IFN- γ and TNF- α have also been reported (Mwatha *et al.*, 1998; King *et al.*, 2001). Although Mwatha *et al.*, (1998) in his study of Kenyan schoolchildren associated the increased schistosome antigen driven TNF- α and IFN- γ production, elevated levels of soluble TNF- α receptors along with a reciprocal decrease in IL-5, with an increased risk for hepatosplenic schistosomiasis with *S. mansoni* infection.

The high concentration levels of TNF- α , IFN- γ and IL-4 observed in this study, we believe, represents some pathological changes occurring in the immune system of infected individuals which is suggestive of the nature and stage of urinary schistosomiasis infection in them. Interestingly, more studies have shown that increased cytokine and cytokine receptor levels could lead to increased urinary tract morbidity. This is because elevated production of TNF- α has been associated with the activation of NF- κ B cells; which aids in immune activation and viral replication (Erikstrup *et al.*, 2008). Schistosomiasis has also been implicated as the major contributor (Kalinkovich *et al.*, 1998; Bentwich *et al.*, 1996) to immune activation.

On the other hand, elevated IL-4, being one the characteristic of TH₂ immune response has been reported to be abundant in schistosomiasis infected persons and significantly susceptible to HIV infection (Bentwich *et al.*, 1998; Shapira-Nahor *et al.*, 1998) Taken together, it is believed that the presence of the *Schistosoma* pathogen could have sensitized the activation and proliferation of IL-4, thus ensuring the production of high levels of IL-4 while for TNF- α , the act of trying to repair the damaged tissues could have led to excess TNF- α been produced which induces deleterious effects on the immune system of infected schistosomiasis individuals. The different levels of cytokine obtained also indicate that the intensity of the disease burden could not have been the only factor influencing the inflammatory response of these individuals to schistosomiasis disease. Other factors such as the locality of the egg deposition in the tissues, immune status, physical variations and genetic factors of the host suggests probable factors influencing the individuals' response to urinary schistosomiasis infection, development of pathology and the production of high levels of TNF- α , IFN- γ and IL-4 observed in the study. This implies that the levels of the cytokines in an individual might be a more stable marker of morbidity. The notion that these cytokines

could be a more accurate maker of bladder morbidity was further supported by Reimert *et al.*, (2000) who described it as a better discriminating marker between the pathological classes than egg counts in general and single egg counts in particular. This could be true since cytokine levels have been reported to reflect the chronic inflammatory response of the host (Cheever *et al.*, 1978) when compared with egg output that may vary during the day. Urogenital schistosomiasis being a disease of morbidity has been reported to produce high th1 cytokines which has been linked to increased lesion (Mbabazi *et al.*, 2011). Generally, TNF- α which is known as a pleiotropic cytokine that can induce disease through TNF- α toxicity (tissue injury, catabolic illness and mediating shock) and improve host defense mechanisms by stimulating inflammation and increasing immune cell function (Ijerman, 1989), has been associated with deleterious effects on the human immune system when produced in excess. Some studies have implicated highly elevated levels of TNF- α as one of the factors contributing to the progression of *Schistosoma* disease with carcinoma of urinary bladder (Raziuddin *et al.*, 1993), while high *S. haematobium* induced urinary tract morbidity was linked to an increased TNF- α production and development of an exaggerated granulomatous response to ova trapped in the bladder wall of children and adolescents with associated urinary tract pathology (King *et al.*, 2001). Many people die annually from a complication of infectious disease called septic shock syndrome which is triggered by TNF- α . In many cases, elevated TNF- α serum levels predict a higher mortality (Grossberg, 1989), thus, in the future, therapies may be developed by blocking TNF- α harmful effects and enhancing TNF- α beneficial effects.

In addition, in *S. haematobium* infected individuals, studies have shown that the increased risk for bladder pathology in urinary schistosomiasis persons was not clearly associated with a shift to Th1 type immune response and away from a Th2 like

immunity to egg antigens, as has been suggested for disease associated with *S. mansoni* infection (Mwatha *et al.*, 1998). But evidence from mice studies, has shown that TNF- α plays a critical role both in the granulomatous response to eggs trapped in tissues and in morbidity. In an IL-4 deficient mice, studies have shown that the mice eventually die of TNF- α mediated cachexia and intestinal pathology (Brunel *et al.*, 1997), just as elevated serum TNF- α levels observed in IL-10/IL-12 knockout mice contributed to the increased morbidity observed in the animals (Hoffman *et al.*, 2000). Other morbidity associated with chronic urogenital schistosomiasis (e.g. Impaired growth and development), - have also been linked with excess TNF- α production (Assis *et al.*, 1998).

However, in African studies both peripheral blood mononuclear cell (PBMC) cultures and whole blood cultures stimulated with SWA (soluble worm antigen) have been shown to release higher levels of the Th2 cytokines; IL-4, IL-5 and IL-13 than cultures stimulated with SEA(soluble egg antigen) (Joseph *et al.*, 2004, Roberts *et al.*,1993). These *S. mansoni* studies in Africa suggest that SWA even in chronically infected individuals stimulates a measurable Th2 response while the Th2 response to SEA is down regulated to a much greater extent. In the same manner, many studies have led to the consensus that *Schistosoma* specific T helper 2 responses (IL-4, IL-5) are associated with and perhaps directly involved in human age-dependent immunity to reinfection after treatment. This suggests that a Th2 immune response to parasite tegumental antigen may contribute either directly or indirectly to immunity in humans (Dunne and Mountford, 2001).

Although among this same group of individuals, some of the subjects expressed low levels of TNF- α , IFN- γ and IL-4 respectively. This observation may be related to some inherent/genetic factors of the hosts as well as other opportunistic infections (like

malaria, hepatitis) which though were not investigated could have influenced these individuals' response to urinary schistosomiasis infection. Consistent with this observation was a study by Imai *et al.*, (2011) on children who were concurrently exposed to both helminthes and *Plasmodium* infection. He further explained that the production of low levels of these cytokines exacerbated *Plasmodium*-related pathology.

Among the Co-infected individuals (genital schistosomiasis /HIV positive individuals) in this study, higher concentration levels of IFN- γ and TNF- α were recorded with a low mean concentration level of IL-4. The low levels of IL-4 were due to the fact that the presence of IL-4 which is usually abundant in chronic schistosomiasis infection (Quinn *et al.*, 2000) induces and recruits HIV receptors which have been reported to replicate preferentially (Shaunak *et al.*, 1999, Koot *et al.*, 1996) in the activated Th2 T cells (IL-4). This promotes and increases the pathogenesis of HIV by increasing the progression of cell to cell spread of HIV virus, infecting and destroying the IL-4 as well as lowering their numbers in the immune system of co-infected hosts. Consequently, more Th1-type cytokines which have been reported to be down-regulated (Quinn *et al.*, 2000) by the IL-4 and are also known to contain HIV infection at the early stage are produced in excess and up-regulated. Therefore, the high concentration levels of IFN- γ and TNF- α as is observed in these individuals, induces the production of inhibitory cells which would help to reduce HIV pathogenesis and replication in the study group. Consistent with this observation are findings by Porto *et al.*, (2004) who reported higher IFN- γ production (1182 \pm 1785pg/ml) in patients co-infected with HTLV-1/*S. mansoni* than in subjects infected with only *S. mansoni*. They associated the high IFN- γ production in these individuals, to a reduced clinical disease and fibrosis resulting from schistosomiasis. High IFN- γ production and decreased type 2 immune response; according to Wynn *et al.*, (1994) and Rezende *et al.*, (1997) may have prevented the

development of fibrosis. In contrast, helminthes have been shown to induce regulatory activities to inhibit inflammatory responses. These regulatory activities according to (Brown *et al.*, 2006; Maizels, 2005; Maizels and Yazdanbkhsh, 2003), may not work in an HIV infected host. Thus impaired production of cytokines among HIV infected persons could lead to advanced disease and increased mortality (Ostrowski *et al.*, 2003).

For example, in a study with *S. mansoni/Plasmodium* co-infected Kenyan schoolchildren, the level of SEA-specific Th2 (that is IL-4) responses were low as they were also found to be in a Ugandan study (Joseph *et al.*, 2004; Wilson *et al.*, 2008). Similarly, in murine studies, mice co-infected with *S. mansoni/Plasmodium chabaudi* (Helmbj *et al.*, 1998; Yoshida *et al.*, 2000) and *S. mansoni/P. berghei* (Abdel-Wahab *et al.*, 1974) released significantly lower levels of IL-4 and IL-5, than mice infected with only *S. mansoni*, suggesting that Th2 responses to SEA may be down regulated by concomitant malaria infection and this may also result in poor eosinophil recruitment to the egg granuloma. While on the other hand high levels of TNF- α were produced in SEA stimulated cultures (Wilson *et al.*, 2008).

Mwinzi *et al.*, (2001) in a study, reported lower levels of Th₂ type cytokines in *S. mansoni*/HIV co-infected individuals, than those without HIV, implying that HIV-mediated Th2 type CD4⁺ T cell destruction. Consistent with studies in T cell deficient mice, Doenhoff *et al.*, (1986) observed that schistosomiasis patients with HIV-1 infections and reduced CD4⁺ T cell levels excreted fewer eggs than HIV-1 negative persons despite having comparable circulating antigen levels (an indication of having similar numbers of adult worms, Karanja *et al.*, 1997). This is in contrast to the study by Leutsher *et al.*, (2005), who reported higher level of IL-4, IL-6, IL-10 and TNF- α in the semen of HIV infected/ urogenital schistosomiasis co-infected men. According to him,

these cytokines induced viral replication and increased the concentration of HIV virus in semen. Six months after anti schistosomiasis treatment, the concentration of seminal lymphocytes and eosinophils were lower and the levels of cytokines reduced. However, chronic parasitic co-infection has been postulated to increase the rate of progression to AIDS in sub-Saharan Africa (Borkow and Bentwich, 2004; Bentwich, 2003; Fincham *et al.*, 2003), although other studies disputed this observation (Brown *et al.*, 2004; Elliot *et al.*, 2003). Nonetheless, multiple studies have also shown that the plasma HIV RNA levels are predictive of both HIV disease progression and risk of transmission of HIV to sexual partners (Quinn *et al.*, 2000; Mellors *et al.*, 1996). If the hypothesis is correct that schistosomiasis increases the HIV RNA levels in co-infected individuals, then treatment for schistosomiasis could delay the development of AIDS and decrease the spread of HIV in sub-Saharan Africa.

In the HIV-1 positive/urinary schistosomiasis negative individuals, highly elevated TNF- α , IFN- γ and IL-4 concentration levels were observed (Fig. 9, 10 & 11). Consistent with this observation are studies by Shapshak *et al.*, (2004), who observed higher levels of IFN- γ and TNF- α expression in an HIV-1 infected brain study. Although they associated the high levels with the pathogenesis of HIV-1 associated dementia (HAD), a broader category of neuropsychiatric impairment (NPI) and opportunistic infections (OIs). In Human T cell leukemia virus type-1(HTLV-1), Carvalho *et al.*, (2001) and Kramer *et al.*, (1989), observed that HTLV-1 virus expressed a high production of IFN- γ and TNF- α cytokines with an exaggerated T cell response. This observation could have also been as a result of intensity of urinary schistosomiasis infection in the individual as well as other genetic and inherent factors which could have led to the expression of high levels of these cytokines in their immune system.

In this study, however, the application of Praziquantel treatment in our study population demonstrated that it is an effective treatment for schistosomiasis despite the patient's HIV-1 serostatus. In the schistosomiasis infected subjects, significant decline in mean concentration levels of IFN- γ , TNF- α and IL-4 was observed at 3 and 6 months post treatment and a gradual increase at 9 and 12 months post treatment. The reason for the observation at 3 and 6 months post treatment could be that the Praziquantel treatment killed most of the worms which consequently reduced the worm burden as well as the egg load. But subsequent elevation observed from 6 months could be as a result of the emergence of some of the worms which survived the treatment therapy, continuing their destructive act. Consequently, more activated IFN- γ , TNF- α and IL-4 cells are recruited and their numbers elevated.

Similarly, among HIV/urinary schistosomiasis co-infected individuals, a comparative high level of IL-4 was obvious among infected group, compared to the sustained low plasma levels in APH control group. The decline observed we believe could be one of a struggle between the HIV virus trying to replicate further in the IL-4 cells, thus killing many of them and the IL-4 trying to induce the activation and proliferation of more cells that would contain the HIV cells. However, with the boost in their immune status (i.e. the Praziquantel treatment), the levels of IL-4 increased at the 6th, 9th and 12 months. Additionally, the intensity of disease burden might have increased due to the worms that could have survived the treatment period, thus eliciting and increasing the production of more IL-4.

Females co-infected with genital schistosomiasis/HIV responded to Praziquantel treatment in an almost similar manner to those who were HIV-1 seronegative. Moreover, levels of IFN- γ , TNF- α , and IL-4 were reduced by treatment for schistosomiasis.

The significant decrease observed at 3 months with subsequent increase in the cytokine levels at 9 and 12 months post treatment only confirms our observation that a single dose of Praziquantel treatment only reduces the level of urinary schistosomiasis pathology and not total clearance of the infection. Secondly, the increase we observed, in these individuals reflects re-activation of some of the worms which were not killed by the drugs, emerging to continue its destructive acts in the system of its hosts, even as re-infection could have also occurred along the line. Hoffman *et al.*, (2000) and Wynn *et al.*, (1997) observed similar trend in mice with exaggerated type 1 and type 2 responses caused by IL-10 deficiency which lead to immunopathological changes and not clearance of the infection. In contrast, treatment of *S. mansoni* in HIV co-infected Ugandan adults resulted in increased helminthes specific type 2 cytokine responses and HIV-1 viral load (Borkow and Bentwich, 2000) and diminished IL-10 concentration. On the other hand, *S. mansoni*/ HTLV-1 co-infection reduced the efficacy of anti-schistosomal drugs in infected subjects and at least one patient failed to eradicate *S. mansoni* infection after eight courses of treatment (Porto *et al.* 2004). Moreover, a reduction of IFN- γ and IL-4 levels among schistosomiasis infected persons treated with Praziquantel supported a report of attenuation of an accelerated increase in HIV RNA and decrease in CD4 cell count among schistosomiasis co-infected persons (Kallestrup *et al.*, 2005). At the 12 months post treatment, the cytokine levels had nearly reached the pre-treatment levels, suggesting that mass treatment should be performed on a 6 month basis if development of pathology is to be prevented or kept at a low level. Alternatively, treatment on a yearly basis will still keep the prevalence of pathology at a lower level than the pretreatment level, but will allow more cases with severe pathology to develop.

5.10.2 The Impact of CD4 T-Cell Response of the Study Population

In this study, with regards to CD4+ T cell levels, all patients with (both HIV-1 positive and negative) urogenital schistosomiasis infection demonstrated increased CD4 cell count following treatment with Praziquantel although statistically, there was no significant difference for both groups. However, at 9 and 12 months post treatment, a dip in the CD4 count levels was observed with some of the patients' CD4 cell counts only moderately higher than their initial CD4 cell levels. This is not surprising, because as we stated earlier, a re-activation of some of the worms which were not killed by the drugs, emerging to continue its destructive acts in the system of its hosts could be the reason for the dip observed in the study. Secondly, since these inflammatory cells (IFN- γ and TNF- α) express CD4+ T cell receptors, which are the primary targets for HIV, this makes it easy for its destruction by the HIV virus, thus lowering its numbers in the immune system of affected hosts. Thirdly, the participants in the study, could have continued in their daily economic and domestic activities (even after treatment), which frequently exposes them to the *Schistosoma* infected water bodies suggesting that the individuals might have been re-infected - thus, ensuring a continuous maintenance of urogenital schistosomiasis chronicity and endemicity in the study.

The CD4 cell levels of schistosomiasis/HIV positive subjects in this study, was lower than that of schistosomiasis/HIV negative individuals even with the Praziquantel treatment. The reason could be due to the fact that urogenital schistosomiasis recruits CD4+ T cell receptors that easily bind and are destroyed by the HIV virus. Consequently, subjects co-infected with Urogenital schistosomiasis/HIV experience a faster depletion of CD4 T cell which leads to a reduced CD4 cell count compared with individuals infected with only urogenital schistosomiasis or HIV alone. This result is consistent with a Western Kenyan Study, where Karanja *et al.*, (1998) described Praziquantel treatment as an effective treatment for both HIV positive schistosomiasis

patients with decreased CD4+ T cells and HIV negative patients. He ascribed the reason to the likelihood that *Schistosoma* infection might have preceded the HIV infection based on the age prevalence curve for both pathogens. Therefore, the anti-schistosome antibody responses critical for Praziquantel treatment efficacy (Brindley and Sher, 1987) could have developed prior to the depletion of the CD4+ T cell help that is necessary for antibody production.

Patients with HIV alone are characterized by the continuous depletion of CD4 T cells, both naïve and memory cells with accelerated T cell turnover (Silvestri and Feinberg, 2003; Graziosi *et al.*, 1998; Pantaleo and Fauci, 1996). Both the direct cytopathic effects of the virus on T cells and the indirect effects exerted on bystander T cells have been implicated in the eventual progression towards AIDS (Silvestri and Feinberg, 2003; Grossman *et al.*, 2002; Hazenberg *et al.*, 2000). At all stages of HIV infection, a higher frequency of HIV specific CTLs has been associated with low plasma viral load and a slower decrease in CD4 cell counts (Ogg *et al.*, 1998). Secor *et al.*, (2003) in another study observed that CD4+ T cells from schistosomiasis patients express higher levels of CXCR4 and CCR5 than do cells from patients who had previously had schistosomiasis but had been treated. They further implied that the increased density of CXCR4 and CCR5 on the cells of positive helminthiasis patients was probably due to the result of up-regulation by the Th₂ associated cytokines (IL-4 and IL-10) (Wong *et al.*, 2002). Moreover, CD4+ T cells with a type 2 phenotype are more readily infected and subsequently destroyed by HIV-1 than are Th1 cells (Maggi *et al.*, 1994). This was evident in a Kenya study, where individuals co-infected with HIV and *S. mansoni* had their Th2 type CD4+ T cells destroyed more quickly than those of HIV infected individuals alone (Mwinzi *et al.*, 2001). The reduction of HIV-1 co-receptor densities on the surface of CD4+ T cells from HIV-1 positive schistosomiasis

patients after Praziquantel treatment may provide beneficial effect in terms of reducing the susceptibility of the patient to the virus at exposure (Secor *et al.*, 2003). For example, HIV positive patients who were randomized to receive Praziquantel immediately had smaller HIV RNA level increases and increased CD4+ T cell count compared with those randomized to treatment after 3 months (Kallestrup *et al.*, 2005). The levels of these co-receptors dropped in individuals who were studied pre and post Praziquantel treatment (Secor *et al.*, 2003). This highlights the potential role that widespread anti-schistosomal treatment could play in reducing the progression and spread of HIV. In addition, patients infected with both *S. mansoni*/HIV were randomized either to Praziquantel treatment at enrollment or to Praziquantel treatment at 3months (Kallestrup *et al.*, 2005). When compared, the group with early treatment experienced significantly smaller declines in their CD4+ cell counts compared to those that had delayed treatment, that is after 3months (1.7cells/ μ l vs. 35.2cells/ μ l). The HIV RNA levels in both groups of patients also increased during the 3months but the mean increase in the early treatment (0.001log₁₀ copies/ml) was significantly lower than in the delayed treatment group (0.21Log₁₀ copies/ml) (Watson and John-Stewart, 2008).

Furthermore, several studies in humans and chimpanzees have demonstrated a significant association between a strong and sustained virus specific CD4+ and CD8+ T cell responses and spontaneous viral clearance in acute HCV infection (Shoukry *et al.*, 2004) whereas individuals who fail to mount or sustain such responses develop persistent vermeil and chronic infection (Shoukry *et al.*, 2004; Thimine *et al.*, 2002; Kamal *et al.*, 2001; Lechner *et al.*, 2000; Cooper *et al.*, 1999; Gerlach *et al.*, 1999). They attached two reasons for such responses, first due to a rebound in viremia and chronic disease course (Gerlach *et al.*, 1999) and secondly, difficulty in detecting HCV specific

CD4+ T cells in persistently infected individuals (Shoukry *et al.*, 2004; Kamal *et al.*, 2001).

5.11 CONCLUSION

This study has observed a high prevalence of urogenital schistosomiasis in Jos, Plateau State. Parasitological findings indicate that urogenital schistosomiasis and its morbidity (FUGS) is a major problem in these communities. School-age children, young adults and the middle aged females were the most infected. It also revealed the lack of basic toilet facilities and unhygienic practices in the communities, such as the indiscriminate disposal of fecal and urine wastes into and near the streams. Furthermore, the study revealed the unique contributions of the edaphic features of the communities; which provide perennial shallow fresh water environment for snail hosts to thrive and for domestic and economic needs.

Water contacts for economic and domestic purposes were leading factors that ensure risk exposure to *Schistosoma* cercariae. This is the link between the snail intermediate hosts and daily water-contact activities of community residents that combine to maintain the vicious cycle of: human - freshwater contamination - snail infection and human re-infection/infection.

In addition we observed that HIV is still endemic mostly in young women/girls. Urogenital schistosomiasis and HIV co-infection was also established in the study.

FUGS, a morbidity of *S. haematobium* schistosomiasis were confirmed by the identification of pathological indices in urine of infected hosts. The pathological consequences on urogenitalia were evident in our subjects urine through digital imaging. This study also calls for re-emphasis on the routes of HIV transmission, and

reveals possible prevention and control measures through massive campaign against schistosomiasis. The trends observed in the study revealed the epidemiological association between urogenital schistosomiasis, FUGS and HIV. It also provides a platform for further in-depth studies for comprehensive association of the two infections and disease transmission or progression. It is hoped that the exposure of FUGS morbidity will rekindle public health interest in the control of schistosomiasis and HIV in Jos, Plateau State and beyond.

This study has also confirmed the presence of high levels of urogenital schistosomiasis pathology (through the UTP indicators) and inflammatory cytokines (TNF- α , IL-4) the immunological markers of urogenital schistosomiasis), which has been associated with the pathogenesis and progression of the HIV virus in the study population. Since these cytokines and urogenital pathology indices play key role in HIV transmission; their presence may constitutes a significant risk factor in HIV acquisition in Jos and perhaps north central Nigeria where *S. haematobium* is endemic.

Schistosoma haematobium infection is highly prevalent in sub-Sahara Africa. Increasing evidence supports that it is a plausible risk factor for HIV acquisition due both to its local genital tract effects in women and to its chronic immunomodulatory effects in both men and women. It could also facilitate HIV transmission to the sexual partners of HIV positive individuals with *Schistosoma* co-infection and could enhance HIV disease progression. Thus, the immunological and epidemiological evidence is strongly suggestive of a cause-effect relationship between *S. haematobium* and HIV infection. Thus, both schistosomiasis and HIV may accelerate immune activation which may eventually increase the biological plausibility of a possible link between female urogenital schistosomiasis and HIV acquisition in women.

The control of schistosomiasis associated morbidity is a major objective in schistosomiasis control programmes (WHO, 1993). Meanwhile, in schistosomiasis – endemic areas where coverage for preventive chemotherapy with Praziquantel remains low, millions of individuals may be at higher risk for HIV infections. Urogenital schistosomiasis by itself leads to significant morbidity that can be lessened with inexpensive preventive chemotherapy. Praziquantel stands as a powerful and economical public health intervention with the potential to prevent the development of urogenital lesions, prolong survival and decrease new HIV infections on the African continent.

5.12 SUMMARY OF RESULTS

- The prevalence of Urogenital schistosomiasis and its morbidity (FUGS) were established in the study.
- School-age children/young adult females were the most affected.
- Unique edaphic features of study communities provided favorable environment for snail hosts to thrive.
- Water contact for economic and domestic needs were major risk factors for acquiring schistosomiasis infection and a link that maintains the vicious cycle of: human - freshwater contamination - snail infection and human re-infection/infection through the daily water-contact activities of community residents.
- HIV prevalence was confirmed in more young females in the study area.
- FUGS was established in the study using digital imaging and urinary tract pathological indices.
- The study also confirmed high levels of inflammatory cytokines in urogenital schistosomiasis, which are associated with accelerated HIV pathogenesis and AIDS disease.

- Furthermore, this study provides reliable population evidence of urogenital schistosomiasis as a risk factor in HIV acquisition and pathogenesis.
- It may also facilitate HIV transmission in positive individuals with *Schistosoma* co-infection.

5.13 CONTRIBUTION TO KNOWLEDGE

- This study observed the continuing endemicity of urogenital schistosomiasis in Jos, Plateau State. It further implicated the different risk (water contact) activities, of the residents in the drive to meet basic needs in the escalating poverty, lack of basic amenities like good drinking water and toilets. It revealed the unique contributions of the peculiar edaphic features of the communities which provide perennial shallow fresh water bodies for snail hosts (*Bulinus spp.*) to thrive.
- It exposed the strategies of fecal waste disposal by the communities and residents that promoted the sustenance of the vicious cycle of water- human-water/snail water contact.
- The study also demonstrated that HIV/AIDS, is still endemic and young women/girls are the most affected.
- Urinary schistosomiasis, FUGS and HIV co-infection were all established in the study.
- FUGS, a morbidity of *S. haematobium* schistosomiasis adds to the disease burden in women of all age group and the pathological consequences may lead to accelerated HIV transmission.
- The concentration of some choice drugs for the treatment of HIV in the genitalia of positive individuals will help in the control of FUGS and other STIs of the lower reproductive tracts.

- The use of CCA as an additional tool for measuring urogenital schistosomiasis morbidity will yield good result, compared to known diagnostic methods and will serve as an important invasive method for FUGS diagnosis in field studies.
- The presence of high levels of urogenital schistosomiasis pathology (through the UTP indicators and inflammatory cytokines such as TNF- α , IL-4) was associated with the pathogenesis and progression of HIV virus in the study.

5.14 RECOMMENDATIONS

- This study has provided additional information to government on the need for safe water supply to endemic communities for agriculture, drinking and other uses.
- In view of the association between urogenital schistosomiasis and HIV transmission in areas where these infections are co-endemic, a salient effect on the health of millions of individuals could presumably be achieved if anti-*Schistosoma* treatment and HIV prevention interventions were integrated. The WHO – recommended policy of early regular treatment of school age children with Praziquantel needs to be extended to adults and prioritized in national programmes as a possible means of further preventing HIV infection in sub-Saharan Africa.
- This study also calls for re-emphasis on the routes of HIV transmission, and reveals possible prevention and control measures through massive campaign against schistosomiasis.
- It also provides a platform for further in-depth studies of association of the two infections transmission and pathogenesis. It is hoped that the exposure of FUGS morbidity will rekindle public health interest in the control of schistosomiasis and HIV in Jos, Plateau State and beyond.

- It is also hoped that this improved public health knowledge on the significance of urinary schistosomiasis; its morbidity and predisposing factors, will assist in curbing the spread of FUGS, HIV and related sexually transmitted pathogens, as well as reduce the high females burden of HIV in Nigeria and perhaps the tropics.

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Appendix A: Two – Way Analysis of variance (ANOVA, with blocks) on the significance of variations in the water contact scores of individuals/activities in different study sites.

Treatment: Major activities Block:	Children	Adult	B	B ² /k
Bathing	22	8	30	450
Playing	70	0	70	2450
Washing utensils	75	217	292	42632
Fetching household water	39	11	50	1250
Fetching for block industry	33	22	55	1512.5
Soil preparation planting	99	83	182	16562
Irrigation	69	97	166	13778
Weeding	11	31	42	882
Crop watering	223	223	446	99458
Defecation	30	13	43	924.5
Total	671	705	1376	179899
T ² /b	45024.1	49702.5	94726.6	

$$\Sigma (T^2/b) = 94726.6, \quad \Sigma X^2 = 193846, \quad b = 10, \quad k = 2$$

$$\Sigma X/GT = 1376, \quad \Sigma (B^2/k) = 179899$$

$$C = (GT)^2/kb = (1376)^2/2 \times 10 = (1376)^2/20 = 94668.8$$

$$SS = \Sigma X^2 - C = 193846 - 94668.8 = 99177.2$$

$$SST = \Sigma (T^2/b) - C = 94726.6 - 94668.8 = 57.8$$

$$SSB = \Sigma (B^2/k) - C = 179899 - 94668.8 = 85230.2$$

$$SSE = SS - (SST + SSB) = 99177.2 - (57.8 + 85230.2)$$

$$= 99177.2 - 85288 = 13889.2$$

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F- Ratio
Treatment	57.8	(k-1) = 1	57.8/1 = 57.8	57.8/1543.24 = 0.0375
Block	85230.2	(b-1) = 9	85230.2/9 = 9470.02	9470.02/1543.24 = 6.1365
Error	13889.2	(k-1)(b-1) = 9	13889.2/9 = 1543.24	
Total	99177.2	(kb-1) = 8		

Treatment (Children and Adults): Tab. $F_{0.05, df1, 9} = 5.12 > \text{Cal. } F = 0.0375$; i.e. $P < 0.05$ (significant); Block (Different WCAs): Cal. $F = 6.13 > \text{Tab. } F_{0.05, df_9, 9} = 3.23$; $P < 0.05$ (significant).

Appendix B: Chi-Squared (χ^2) Analysis of Age related prevalence of urinary schistosomiasis in apparently healthy individuals in study population

	Age group	No Sample d	O	E	O - E	(O - E) ²	(O - E) ² /E
Jos Urban	0- 10	7	1	0.477	0.523	0.2735	0.5734
	11- 20	27	2	1.836	0.164	0.0269	0.0146
	21 - 30	91	9	6.188	2.812	7.9073	1.2779
	31 - 40	44	3	2.992	0.008	0.0001	0.00002
	41 - 50	46	3	3.128	-0.128	0.0164	0.0052
	≥51	27	1	1.836	-0.836	1.672	0.9107
Abattoir	0-10	4	0	0.272	-0.272	0.074	0.272
	11-20	20	1	1.36	-0.36	0.1296	0.0953
	21-30	28	2	1.904	0.096	0.0092	0.0048
	31-40	33	1	2.244	-1.244	1.5475	0.6896
	41-50	13	0	0.884	-0.884	0.7815	0.884
	≥51	0	0	0.000	0.000	0.000	0.000
Tudun Wada	0-10	3	0	0.204	-0.204	0.0416	0.204
	11-20	15	1	1.02	-0.02	0.0004	0.0004
	21-30	20	2	1.36	0.64	0.4096	0.3012
	31-40	14	1	0.952	0.048	0.0023	0.0024
	41-50	4	0	0.272	-0.272	0.074	0.272
	≥51	0	0	0.000	0.000	0.000	0.000
						Cal. $\chi^2 = 5.5075$	

Df. = (r-1) (3-1) = (5) (2) = 10

Average incidence = $27/369 = 0.068$ \therefore Expected values = $0.068 \times 7 = 0.476$

Cal $\chi^2 = 5.5075 < \text{Tab } \chi^2 = 18.31$

\therefore Since $\text{Tab } \chi^2 = 18.31 > \text{Cal } \chi^2 = 5.51$ at $df_{10} P > 0.05$. This means, that there is no significant difference in terms of age. Thus, the disease has no specified age group, it could attack. Even age group could get infected at any time.

Appendix C: Chi- squared (χ^2) Analysis of Age related prevalence of urinary schistosomiasis in symptomatic individuals in study population

Symptomatic (Hospital) population

Age group	Urine Analysis		High Vaginal Swab	
	No Scrn.	No positive	No Scrn.	No positive
0-10	12	2	32	1
11-20	27	8	17	3
21-30	73	15	4	0
31-40	63	13	14	0
41-50	48	0	4	1
≥ 51	15	4	3	0
T	238	51	74	5

Average incidence $51/238 = 0.214$

$5/74 = 0.068$

Expected value = 0.214×12

Expected value = $0.068 \times 32 = 2.162$

	O	E	O-E	$(O-E)^2$	$(O-E)^2/E$
0-10	2	2.568	-0.568	0.3226	0.1256
11-20	8	5.778	2.222	4.9373	0.8545
21-30	15	15.622	-0.622	0.3869	0.0248
31-40	13	13.482	0.482	0.2323	0.0172
41-50	9	10.272	-1.272	1.6180	0.1575
≥ 51	4	3.21	0.79	0.6241	0.1944
0-10	1	2.162	-1.3502	1.3502	0.6245
11-20	3	1.156	1.844	3.4003	0.9415
21-30	0	0.272	-0.272	0.0740	0.272
31-40	0	0.952	-0.952	0.9063	0.952
41-50	0	0.272	0.728	0.5200	1.9485
≥ 51		0.204	-0.204	0.0416	0.204
				Cal. P =	8.3165

Tab. $\chi^2 = 11.07 > \text{Cal. } \chi^2 = 8.32$ at $P > 0.05$.

This means that the contraction of the schistosomiasis disease is not significantly dependent on age.

Appendix D: Two – Way Analysis of variance (ANOVA) on the significance of variation in the seasonal distribution of *Bulinus species* in different study communities.

Treatment	Tudun-Wada	Abattoir	Nabong	B	B ² /K
Block:	561	724	350	1635	891075
Dry season	167	126	69	362	43681.33
Rainy season	728	850	419	1997	934756.33
Total	264992			361250	87780.5

T²/b

$$\Sigma (T^2/b) = 714022.5, \quad \Sigma X^2 = 1009923, \quad b = 2, \quad k = 3$$

$$\Sigma X/GT = 1997, \quad \Sigma (B^2/k) = 934756.33$$

$$C = (GT)^2/kb = (1997)^2/2 \times 3 = (1997)^2/6 = 664668.167$$

$$SS = \Sigma X^2 - C = 1009923 - 664668.167 = 345254.83$$

$$SST = \Sigma (T^2/b) - C = 714022.5 - 664668.167 = 49354.33$$

$$SSB = \Sigma (B^2/k) - C = 934756.33 - 664668.167 = 270088.163$$

$$SSE = SS - (SST - SSB) = 345254.83 - (49354.33 + 270088.163)$$

$$= 345254.83 - 319442.493 = 25812.337.$$

Source of Variation	Sum of Squares	Degrees of freedom	Mean Squares	F- Ratio
Treatment	49354.33	(k-1) = 2	49354.33/2 = 24677.165	24677.165/12906.1685 = 1.9120
Block	270088.163	(b-1) = 1	270088.163/1 = 270088.163	270088.163/12906.1685 = 20.9271
Error	25812.337	(k-1)(b-1) = 2	25812.337/2 = 12906.1685	
Total	345254.830	(kb-1) = 5		

Treatment (Effect of different communities): Tab. $F_{0.05, df_{2,2}} = 19.0 > \text{Cal. } F = 1.9120$; ie, $P > 0.05$ (not significant); Block (Effects of dry and rainy seasons): Tab. $F_{0.05, df_{1,2}} = 18.5 < \text{Cal. } F = 20.9271$; ie, $P < 0.05$ (significant).

**Appendix E: Correlation (r) analysis to establish any linear relationship
between urinalysis diagnostic outcome and HVS sourced sampling**

Age group	X	Y	X ²	Y ²	XY
0-10	2	1	4	1	2
11-20	8	3	64	9	24
21-30	15	0	225	0	0
31-40	13	0	169	0	0
41-50	9	1	81	1	9
≥ 51	4	0	16	0	0

$$\Sigma X = 51 \quad \Sigma Y = 5 \quad \Sigma X^2 = 559 \quad \Sigma Y^2 = 11 \quad \Sigma XY = 35$$

$$n = 6, (\Sigma x)^2 = 2601 \quad (\Sigma y)^2 = 25 \quad df = n-1 \text{ ie, } 6-1 = 5$$

$$df_5$$

$$SS_{xy} = \Sigma (xy - \Sigma x \Sigma y / n) = (35 - (51)(5)/6)$$

$$= 35 - (255/6) = 35 - 42.5$$

$$= -7.5$$

$$SS_x = \Sigma x^2 - (\Sigma x)^2 / n = 559 - (2601/6)$$

$$= 559 - 433.5 = 125.5$$

$$SS_y = \Sigma y^2 - (\Sigma y)^2 / n = 11 - (25/6)$$

$$= 11 - (4.1667) = 6.83$$

$$\therefore r = \frac{SS_{xy}}{\sqrt{SS_x SS_y}} = \frac{-7.5}{\sqrt{(125.5 \times 6.83)}} = \frac{-7.5}{\sqrt{857.165}}$$

$$= -0.0087$$

$$\therefore \text{Tab } r \text{ at } df_5 = 0.754$$

$$\text{Cal } r = -0.0087$$

$$\text{Cal. } r = -0.0087 < \text{Tab. } r \text{ at } 0.05, df_5 = 0.754$$

This means that, there is no linear relationship between urine analysis and HVS.

Appendix F: Two – Way Analysis of variance (ANOVA, with replication) on the significance of variations in the urinary tract pathological indices in individuals in the study population.

Treatment	Positive Subjects	Negative Subjects	Block Total	B ² /Kn
Glucose	41	141	182	1656.2
Bilirubim	188	22	210	2205
Nitrite	379	26	405	8201.25
Uroglobulin	128	12	140	980
Protein	324	21	345	5951.25
pH	292	111	403	8120.45
Blood	381	26	407	8282.45
Specific gravity	232	80	312	4867.2
Ketone	210	74	284	4032.8
Leukocyte	378	156	534	14257.8
Treatment Total	2553	669	3222	58554.4
T ² /bn	1629452.25	111890.25	1741342.5	

$$GT=3222; \quad x=1611; \quad \Sigma X^2=428412;$$

$$C=(GT)^2/Kbn=(3222)^2/40=259532.1$$

$$SS=\Sigma X^2-C=428412-259532.1=168879.9$$

$$SST=\Sigma T^2/bn - C = 1629452.25/4 - 259532.1 = 147830.96$$

$$SSB=\Sigma B^2/kn - C = 58554.4/20 - 259532.1 = -259532.1$$

Q2/n for each subtreatment was calculated as follows:

	Positive	Negative	Total
Glucose	840.5	9940.5	10781
Bilirubim	17672	242	17914
Nitrite	71820.5	338	72158.5
Uroglobulin	8192	72	8264
Protein	52488	220.5	52708.5
pH	42632	6160.5	48792.5
Blood	72580.5	338	72918.5
Specific gravity	26912	3200	30112
Ketone	22050	2738	24788
Leukocyte	71442	12168	83610
Total			422047

$$SSQ=\Sigma Q/n - C = 422047 - 259532.1 = 162514.9$$

$$\begin{aligned} \text{SSTB} &= \text{SSQ} - (\text{SST} + \text{SSB}) = 162514.9 - (147830.96 + (-259532.1)) \\ &= 162514.9 + 111701.14 = 274216.04 \\ \text{SSE} &= \text{SS} - \text{SSQ} = 168879.9 - 274216.04 = -105336.14 \end{aligned}$$

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F-Ratio
Treatment	147830.96	9	147830.96/9 = 16425.66	16425.66/- 5266.81 = -3.12
Block	-256604.38	1	-256604.38/1 = -256604.38	-256604.38/- 5266.81 = 48.7
Interaction	274216.04	9	274216.04/9 = 30468.45	30468.45/- 5266.81 = -5.78
Error	-105336.14	20	-105336.14/20 = -5266.81	
Total	168879.9			

Treatment (Indices): Tab. F at $df_{9, 20} = 2.45 < \text{Cal. F} = 3.12$ (Significant)

Block (Age): Tab. F at $df_{1, 20} = 5.12 < \text{Cal. F} = 48.7$ (Significant)

Interaction (Infected vs Uninfected): Tab. F at $df_{9, 20} = 2.45 < \text{Cal. F} = 5.78$ ($p < 0.05$.)

Appendix G: Two – Way Analysis of variance (2-WAY ANOVA, without replication) on the significance of IFN- γ production by urinary schistosomiasis positive and urinary schistosomiasis/HIV coinfecting females.

Block	A	B	C	D	E	Block Total	B2/K
	58.26	16.26	53.74	64.58	79.48	272.32	14831.64
	196.45	138.19	216.32	159.87	241.61	952.44	181428.39
	1295.64	421.34	434.89	766.37	619.14	3537.38	2502611.45
	1387.32	484.57	489.08	491.79	0	2852.76	1627647.92
	1364.28	1002.55	1007.07	1010.68	1315.96	5700.54	6499231.26
	299.41	142.25	219.93	234.38	292.19	1188.16	282344.84
	153.10	235.74	247.93	459.28	471.02	567.07	491141.68
	948.81	425.41	437.60	938.88	563.15	3313.85	2196320.37
	1121.32	792.56	804.75	1387.77	1397.25	5503.65	6058032.67
	961.91	486.37	1021.97	1029.10	0	3499.35	2449090.09
	387.47	146.32	223.54	169.35	323.80	1250.48	312740.05
	419.92	239.80	349.09	59.61	479.60	1548.02	1198182.96
	1133.97	841.78	807.01	548.69	1123.13	4454.58	3968656.60
	953.33	532.89	1024.68	1031.91	1220.67	4763.48	4538148.34
	1420.74	1049.52	1008.42	1012.94	1413.06	5904.68	6973049.18
	272.31	194.19	161.22	172.06	330.57	1130.35	255538.22
	649.85	432.18	811.53	123.73	576.70	2593.99	1345756.82
	1115.00	799.33	1026.49	246.12	1414.41	4601.35	4234484.37
	1146.61	488.18	1009.33	1033.71	1221.58	4899.41	4800843.67
	1249.58	1006.16	95.74	0	1423.44	3774.92	2850004.20
	436.25	155.80	454.76	30.71	410.50	1488.02	442840.70
	621.85	294.44	773.14	269.61	597.47	2556.51	1307148.68
	1097.84	851.72	1027.84	826.43	1126.74	4930.57	4862104.11
	1271.25	538.31	1011.13	1036.42	1403.12	5260.23	5534003.93
	1362.03	1053.58	102.51	1466.80	1450.54	5435.46	5908845.08
	443.02	203.67	529.28	178.83	418.63	1773.43	629010.79
	629.98	297.15	941.59	317.47	605.60	2791.79	1558818.28
	850.81	808.82	494.05	0	1425.70	3579.38	2562392.24
Treatment Totals	23248.31	14079.08	16784.63	15067.09	21945.06	91124.17	
Treatment Means	830.3	502.82	599.45	538.11	783.75		
T²/b	19302997.07	7079303.35	10061564.44	8107757.18	17199487.8		

GT=91124.17; \bar{x} =3254.427/5= 650.89;

$$\Sigma x^2 = 24428569.35 + 6235761.01 + 13446439.02 + 13725983.16 + 24242828.08 = 82079580.62$$

$$C = (GT)^2/kb = (91124.17)^2/28 \times 5 = 8303614358/140 = 59311531.13$$

$$SS = \Sigma x^2 - C = 82079580.62 - 59311531.13 = 22768049.49$$

$$SST = \Sigma T^2/b - C = 61751109.84 - 59311531.13 = 2439578.71$$

$$SSB = \Sigma B^2/k - C = 75585248.48 - 59311531.13 = 16273717.35$$

$$SSE = SS - (SST + SSB) = 22768049.49 - (2439578.71 + 16273717.35)$$

$$22768049.49 - 18713296.06 = 4054753.43$$

Source of Variation	Sum of Squares	Degrees of freedom	Mean Squares	F-ratio
Treatment	2439578.71	27	$2439578.71/27 = 90354.77$	$90354.77/37544.01 = 2.41$
Block	16273717.35	4	$16273717.35/4 = 4068429.34$	$4068429.34/37544.01 = 108.36$
Error	4054753.43	108	$4054753.43/108 = 37544.01$	
Total	22768049.49	139		

Therefore, Tab. F at $df_{27,108} = 1.39 < \text{Cal.F} = 2.41$ (Significant)

Tab.F at $df_{4,108} = 2.52 < \text{Cal.F} = 108.36$ (Significant)

Appendix H: Two-Way Analysis of variance (2-WAY ANOVA, without replication) on the significance of TNF- α production by urinary schistosomiasis positive and urinary schistosomiasis/HIV coinfecting females.

Block	A	B	C	D	E	Block Total	B ² /k
	6.99	4.51	2.70	6.77	6.55	18.5	12.22
	10.77	1.10	12.38	9.54	12.30	46.09	75.87
	36.04	14.85	15.80	27.74	26.94	121.37	526.10
	97.55	61.15	61.95	62.54	66.98	350.17	4379.25
	172.61	110.07	120.48	131.26	126.60	661.02	15605.27
	5.17	4.66	3.79	7.28	6.04	17.62	11.09
	12.89	1.38	9.68	10.41	12.74	47.1	79.23
	23.08	6.26	7.79	8.74	25.55	71.42	182.17
	36.48	14.41	12.23	16.60	34.43	114.15	465.37
	49.58	23.59	20.17	32.69	0	126.03	567.27
	222.11	134.53	155.43	213.45	215.12	940.64	31600.13
	7.79	3.64	4.95	10.92	0	20.02	14.31
	22.93	7.72	8.81	16.67	72.44	128.57	590.37
	37.13	16.60	24.17	29.63	55.55	163.08	949.82
	68.94	31.74	32.47	56.78	61.95	251.88	2265.84
	178.80	114.59	127.18	182.44	127.47	730.48	19057.18
	7.79	3.06	8.74	3.86	1.02	18.35	12.03
	24.68	6.77	15.36	12.89	6.84	66.54	158.12
	26.21	8.74	26.86	24.10	12.45	98.36	345.52
	37.78	16.80	34.80	0	35.89	125.27	560.45
	121.87	62.97	97.41	129.00	86.92	498.17	8863.33
	199.04	114.44	128.42	203.33	131.99	777.22	21573.96
	9.25	2.40	2.62	2.84	2.18	14.49	7.50
	28.40	3.42	15.36	20.82	8.01	76.01	206.34
	39.46	9.03	27.23	32.18	43.90	151.8	822.97
	53.22	34.29	61.01	72.07	63.55	284.14	2883.41
	135.19	70.98	97.84	130.53	60.44	494.98	8750.19
	267.32	201.66	207.70	0	227.28	903.96	29183.70
T. Totals	1939.07	1048.82	1343.33	1455.08	1531.13	7317.43	
T Means	69.25	37.46	47.98	51.97	54.68	52.27	
T2/b	751998.49	220004.68	360907.10	423451.56	468871.82	2225233.65	

$$GT=7317.43 \quad x=52.27$$

$$\Sigma x^2=285579.80+113964.04+147206.09+189428.25+185978.51=922156.69$$

$$C = (GT)^2/kb = (7317.43)^2/28 \times 5 = 382462.73$$

$$SS = \Sigma x^2 - C = 922156.69 - 382462.73 = 539693.96$$

$$SST = \Sigma T^2/b - C = 2225233.65 - 382462.73 = 1842770.92$$

$$SSB = \Sigma B^2/k - C = 149749.02 - 382462.73 = -232713.71$$

$$SSE = SS - (SST + SSB) = 539693.96 - (1842770.92 - 232713.71) = -1070363.25$$

Source of Variation	Sum of Squares	Degree of Freedom	Mean Squares	F-ratio
Treatment	1842770.92	27	$1842770.92/27=68250.77$	$68250.77/-9910.77 = 6.89$
Block	-232713.71	4	$-232713.71/4= -58178.43$	$-58178.43/-9910.77 = 5.87$
Error	-1070363.25	108	$-1070363.25/108= -9910.77$	
Total	539693.96	139		

Tab. F at $df_{27,108} = 1.39 < \text{Cal. F at } df_{27,108} = 6.89$ (P < 0.05)

Tab. F at $df_{4, 108} = 2.53 < \text{Cal. F at } df_{4, 108} = 5.87$ (P < 0.05, Significant)

Appendix I: Two – Way Analysis of variance (2-WAY ANOVA, without replication) on the significance of IL-4 production by urinary schistosomiasis positive and urinary schistosomiasis/HIV coinfecting females.

Block	A	B	C	D	E	Block Total	B²/k
	52.668	30.335	37.391	44.006	50.180	214.58	1644.45
	50.369	32.067	38.336	47.754	51.062	219.59	1722.13
	5.324	0.693	0.284	0.126	0.0788	4.39	0.69
	12.222	1.292	2.867	5.954	5.954	28.289	28.58
	26.46	21.137	27.374	28.665	29.232	132.87	630.52
	81.822	65.300	78.278	87.602	87.696	400.698	5734.25
	2.111	15.278	26.334	2.111	2.426	48.26	83.18
	0.441	0.662	0.063	1.040	0.756	1.638	0.096
	2.016	0.315	1.197	2.142	1.89	6.93	1.72
	11.781	36.871	48.51	59.535	55.976	212.67	1615.30
	53.046	63.977	83.507	0	54.338	254.87	2319.95
	55.913	0.882	0.662	89.870	90.563	236.126	1991.27
	90.909	0.504	0.158	0.882	0.0945	92.36	304.65
	0.032	0.189	0.095	1.481	1.418	2.583	0.24
	0.189	33.768	33.989	0.819	1.103	69.49	172.46
	0.536	38.745	42.557	37.107	43.722	162.667	945.02
	49.266	5.009	24.318	52.196	52.448	183.24	1199.14
	26.744	4.851	5.355	25.389	28.571	90.91	295.17
	5.261	50.873	5.387	5.607	6.521	73.649	193.72
	5.513	40.761	55.850	5.450	8.726	116.3	483.06
	49.896	0.63	0.126	52.763	55.944	159.107	904.11
	50.621	2.268	0.882	42.400	48.699	144.87	749.55
	0.205	2.741	2.52	0.347	0.315	5.434	1.05
	0.473	1.512	2.709	0.252	0.945	5.89	1.24
	2.300	5.418	5.292	2.678	3.119	18.807	12.63
	12.175	37.217	75.222	9.261	15.372	149.247	795.52
	26.177	43.502	43.565	29.641	30.366	173.251	1071.997
	91.539	61.929	46.211	84.578	91.35	375.607	5038.59
T.	765.567	593.244	688.029	718.962	818.52	3584.32	27940.28
Totals							
T. means	27.34	21.19	24.57	25.68	29.23	25.602	
T²/b	117218.57	70387.69	94676.78	103381.27	133995.00	519659.31	

$$GT = 3584.32, \quad x = 25.602$$

$$\Sigma x^2 = 44707.03 + 26446.09 + 34154.44 + 41896.97 + 48619.40 = 195823.93.$$

$$C = (GT)^2/kb = (3584.32)^2/(28 \times 5) = 1284734.99/140 = 91766.78$$

$$SS = \Sigma x^2 - C = 195823.93 - 91766.78 = 104057.15$$

$$SST = \Sigma T^2/b - C = 519659.31 - 91766.78 = 427892.53$$

$$SSB = \Sigma B^2/k - C = 27940.28 - 91766.78 = -63826.5$$

$$SSE = SS - (SST + SSB) = 104057.15 - (427892.53 - 63826.5) = -260008.88$$

Source of variation	Sum of squares	Degrees of freedom	Mean of squares	F-ratio
Treatment	427892.53	27	$427892.53/27 = 15847.87$	$15847.87/-2407.49 = -6.58$
Block	-63826.5	4	$-63826.5/4 = -15956.63$	$-15956.63/-2407.49 = 6.63$
Error	-260008.88	108	$-260008.88/108 = -2407.49$	
Total	104057.15	130		

Tab. F at $df_{27,108} = 1.39 < \text{Cal. F at } df_{27,108} = 6.58$ (P < 0.05 Significant)

Tab. F at $df_{4,108} = 2.53 < \text{Cal. F at } df_{4,108} = 6.63$ (P < 0.05 Significant)

**Appendix J: Two-Way Analysis of Variance for the CD4 Count of HIV/
Schistosomiasis coinfected Individuals**

Block	0	3	6	9	12	TOTAL	B²/k
	261	303	314	298	291	1467	307441.29
	266	317	334	301	0	1218	211932.00
	107	258	265	251	229	1110	176014.29
	161	264	273	0	233	931	123823.00
	359	415	431	409	387	2001	572000.14
	82	139	170	155	127	673	64704.14
	497	532	539	0	483	2051	600943.00
TOTA	1733	2228	2326	1414	1750	9451	2056857.86

L

T²/b	60065	99279	10820	39987	61250
	7.8	6.8	55.2	9.2	0.

$$K = 7, b = 5, GT = 9451$$

$$\Sigma X^2 = 553181 + 803128 + 860088 + 433712 + 590598 = 3240707$$

$$C = (GT)^2/kb = 2552040.029$$

$$SS = \Sigma X^2 - C = 3240707 - 2552040.029 = 688666.9710$$

$$SST = \Sigma T^2/b - C = 3687889.00 - 2552040.029 = 1135848.971$$

$$SSB = \Sigma B^2/k - C = 2056857.857 - 2552040.029 = -495182.1720$$

$$SSE = SS - (SST + SSB) = 688666.9710 - 640666.7990 = 48000.172$$

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F
Treatment	1135848.971	6	1135848.971/6 = 189308.1618	189308.1618/2000.00716 7 = 94.7
Block	-495182.172	4	-495182.172/4 = -123795.543	- 123795.5430/2000.00716 7 = 61.9
Error	48000.172	24	48000.172/24 = 2000.007167	
Total	688666.971	34		

Treatment: Tab. F at $d_{6,24} = 2.51 < \text{Cal. F} = 94.7$ (significant)

Block: Tab. F at $df_{4,24} = 2.78 < \text{Cal. F} = 61.9$ (significant).

**Appendix K: Two (2) - Way Analysis of Variance without Replication of CD4
Count of Apparently Healthy Individuals in the Study**

BLOCK	0	3	6	9	12	Block Total	B2/k
	300	427	488	453	381	2049	182539.17
	240	384	449	421	373	1867	151551.70
	388	551	601	584	542	2666	309024.17
	532	702	911	896	674	3715	600053.26
	899	989	1041	894	833	4656	942536.35
	441	750	783	621	0	2595	292783.70
	622	827	859	801	799	3908	664020.17
	581	708	777	738	698	3502	533217.57
	664	805	847	823	763	3902	661982.78
	674	757	813	707	685	3636	574804.17
	779	849	865	839	768	4100	730869.57
	725	877	882	854	822	4160	752417.39
	651	741	766	753	719	3630	572908.70
	402	513	537	526	481	2459	262899.17
	646	822	836	808	797	3909	664360.04
	448	581	588	571	533	2721	321906.13
	905	1043	1057	1015	1008	5028	1099164.52
	576	683	691	678	665	3293	471471.7
	201	355	362	351	342	1611	112840.04
	928	981	1005	990	0	3904	662661.57
	412	608	619	593	0	2232	216601.04
	370	509	522	517	497	2415	253575.0
	818	1004	1019	995	887	4723	969857.78
T Total	13202	16466	17318	16428	13267	76681	
T mean	574	715.91	752.96	714.26	663.35		
T2/b	54225	34858560.8	59982624.8	53975836.8	35202657.8		831.2

$$GT = 76681$$

$$\Sigma X^2 = 9445377 + 13923344 + 12532622 + 8567512 + 12667988 = 57136843$$

$$C = (GT)^2/kb = 51130224.01$$

$$SS = \Sigma X^2 - C = 57136843 - 51130224 = 6006618.99$$

$$SST = \Sigma T^2/b - C = 238245511.4 - 51130224.01 = 187115287.4$$

$$SSB = \Sigma B^2/k - C = 12004045.69 - 51130224.01 = -39126178.32$$

$$SSE = SS - (SST + SSB) = 6006618.990 - 147989109.1 = -141982490.1$$


Source of Variation	Sum of Squares	Degrees of Freedom	Mean Squares	F-ratio
Treatment	187115287.4	22	187115287.4/22 =8505240.336	8505240.336/- 1613437.388 = -5.27
Blocks	-39126178.32	4	-39126178.32/4 = -9781544.580	-9781544.580/- 1613437.388 = 6.06
Error	-141982490.1	88	-141982490.1/88 = -1613437.388	
Total	6006618.990	114		

Treatment: Tab. F at $df_{22, 88} = 1.92 < \text{Cal. F} = 5.27$ (significant)

Block: Tab. F at $df_{4, 88} = 2.53 < \text{Cal. F} = 6.06$ (significant)

Appendix L: Ethical Clearance Granted by Plateau State Specialist Hospital, Jos.

**PLATEAU STATE SPECIALIST HOSPITAL,
JOS**



Email: psshjos@gmail.com

Ref. No. PSSH/ADM/455/1/X
Old Bukuru Road,
P.M.B. 2113,
Jos, Nigeria
Tel. 073-462180
Fax: 073-464031

Date: September 18, 2006

Njoku Chizoma Jane,
Department of zoology,
University of Jos,
Plateau State.

RE: RESEARCH PROPOSAL

Refer to your request to carryout a research titled "The Seroprevalence and Epidemiological Survey of Female Genital Schistosomiasis in Apparently Healthy and HIV – Infected Individuals" in Jos Plateau State Nigeria followed the correction you made thereof.

Clearance is hereby granted for you to carryout your research and we wish you success in your undertaking, please.


R. N. Mutfwang (Mrs.)
Secretary Ethical Committee