Rationale

In many areas of the world, including Sudan, malaria and helminthes are co-endemic, so, co-infections are common. However, little is known how concurrent infections affect the epidemiology and/or pathogenesis of each other. The question of whether and how these two types of parasite might interact within co-infected hosts has attracted much interest and controversy (Andargachew *et al*., 2013). Therefore, this study was conducted to assess the effects of intestinal helminth infections on the epidemiology and clinical patterns of malaria in Abu-Naama in Sinnar State where both infections are prevalent.

Chapter One

Introduction and Literature Review

1.1 Introduction:

Malaria is the most important tropical disease known to man. It remains a significant problem in many tropical areas, especially in sub-Saharan Africa. Malaria is spreading as a result of environmental changes, including global warming, civil disturbances, increasing travel and drug resistance (Greenwood, 1997). Around 50,000 species of multicellular helminthes (worms) have been described from a wide range of hosts. Round worms (nematodes) cause much morbidity and mortality in humans and animals throughout the world. Serious infections include diseases of filariasis, hook worms and thread worms. Larval and adult tape worms (cestodes) may be found in many vertebrate hosts. Flukes (trematodes) include many important species such as sheep liver fluke and human *Schistosomes* (www.parasite.org.au). Throughout evolutionary history humans have been infected with parasites. Today, it is estimated that over a third of the world's population, mainly those individuals living in the tropics and sub-tropics, are infected by parasitic helminths (worms) or one or more of the species of *Plasmodium* (de Silva *et al*., 2003; Snow *et al*., 2005). The ubiquity of these parasites results in high rates of co-infection (Petney and Andrews, 1998). It has increasingly been speculated that helminth infections may alter susceptibility to clinical malaria (Tabitha *et al.*, 2006; Nacher, 2001; Druilhe *et al*., 2005) and there is now increasing interest in investigating the consequences of co-infection (Nacher *et al*., 2000; Sokhna *et al*. 2004; Le Hesran *et al*., 2004; Braind *et al*., 2005; Lyke *et al*., 2005; Shapiro *et al*., 2005). This is however not a new research topic. Nearly thirty years ago it was suggested that infection with the intestinal nematode *Ascaris lumbricoides* was associated with the suppression of malaria symptoms and that anti- helmintic treatment led to a recrudescence of malaria (Murray *et al*., 1977,

1978). The mechanisms underlying this finding, and those of more recent studies, are based on the assumption that helminth infections induce a potent and highly polarized immune response (Maizels *et al*., 2004) which has been proposed to modify the acquisition of immunity to malaria (Mwangi *et al*., 2006).

1.2 Literature review:

1.2.1 Helminthes:

1.2.1.1 Introduction:

Helminth (from the Greek helminthos, meaning worm) refers to all parasitic worms of humans (Samuel, 1996). Although the word "helminth" does mean "worm," in zoological terms it is more restricted to members of the phyla Platyhelminths, Nematoda, and Acanthocephala (Micheal *et al.*, 2009). They are complex, multicellular organism, ranging in size from the microscopic filarial parasites to the giant tape worms, several meters in length. Sexual reproduction occurs in all cases, usually by mating between male and female larvae. However, some helminthes are hermaphroditic, possessing both male and female reproductive organs, and can reproduce by self-fertilization, termed parthenogenesis (Samuel, 1996). There are three groups of medically important helminthes; cestodes (tape worms), nematodes (round worms) and trematodes (flukes) (Micheal *et al.*, 2009).

1.2.1.2 Classification of intestinal helminthes:

1.2.1.2.1 Phylum: Platyhelminthes

Class 1: Monogenea.

Class 2: Cestoda.

Subclass: Eucestoda.

Order 1: Pseudophyllidea (*Diphyllobothrium*).

Order 2: Cyclophyllidea (*Taenia, Echinococcus*).

Class 3: Aspidogastrea.

Class 4 Digenea (*Schistosoma, Fasciolopsis, Fasciola,Paragonimus*) .

1.2.1.2.2 Phylum: Nematoda

Order 1: Rhabditida (*Strongyloides*).

Order 2: Strongylida (Necator, *Ancylostoma* ….*etc.*).

Order 3: Ascaridida (*Ascaris, Toxocara, etc.*).

Order 4: Oxyurida (*Enterobius*).

Order 5: Spirurida (*Dracunculus, Wuchereria, Brugia, Loa, Onchocerca*).

Order 6: Enoplida (*Trichinella, Trichuris*) (Cox, 1993).

1.2.1.3 General characteristics of intestinal helminthes:

1.2.1.3.1 General characteristics of cestodes:

Cestodes characterized by tape-like body made up of head (scolex) and many proglottides (segments). Proglottides formed from the neck behind the head. Those that are newly formed are small and immature. Mature proglottides contain fully developed reproductive organs. The proglottides which contain eggs are known as gravid segments (Cheesbrough, 1987). Tape worms are hermaphroditic. The male and female organs meet in a common genital pore which may be ventrally in the midline of each segment or inside of the segment (Cheesbrough, 1987). There is no mouth or digestive system; they absorb nutrients through their body surface (Cheesbrough, 1987).

Table 1.1: Morphological differences between Cyclophylidae and Pseudophylidae (Cheesbrough, 1987).

Characteristics	Cyclophylidae	Pseudophylidae
Head	Globular with four suckers	Elongated with two slit-like sucking grooves
Common genital pore	Laterally	In the mid-line
Uterine pore	Absent	Present
Vitelline glands	Massed together	Scattered
Egg	Non-operculated, embryonated	Operculated, non-embryonated
Intermediate host	Only one	Tow hosts

- **Cyclo- phylidae:**

Include: *Taenia saginata, Taenia solium, Hymenolepis nana, Hymenolepis diminuta, Echinococcus granulosus* and *Dipylidium caninum* (Cheesbrough, 1987).

-**Pseudo- phylidae:** Include: *D.latum* (Cheesbrough, 1987).

Table 1.2: Differential morphology of the diagnostic stages of medically important cestodes found in human (CDC).

Species	Size	Unique characters	Color	Stage of development when passed
-*Taenia saginata* -*Taenia solium*	31-43μm.	Spherical with thick striated shell.	Walnut brown.	Embryonated. 6-hooked oncosphere present inside a thick shell.
Hymenolepis nana	47μmx 37μm.	Oval shell consists of 2 distinct membranes. With “knobs" and polar filaments.	Colorless, almost transparent.	Embryonated. 6-hooked oncosphere inside shell.
Hymenolepis diminuta	70-86μm x60-80μm.	Round or slightly oval. Striated outer membrane and thin inner membrane.	Yellow	Embryonated. 6-hooked oncosphere inside shell.
Dipylidium caninum	31-50μm x 27-48μm.	Spherical or oval. 5-15 eggs (or more) are enclosed in a sac or capsule.	Colorless	Embryonated. 6-hooked oncosphere inside shell.

D.latum	58-76μm x40-51μm.	Oval with and operculum at one end and a small "knob" at the other end.	Yellow to brown.	Unembryonated. Germinal cell is surrounded by a mass of yolk cells.

1.2.1.3.2 General Characteristics of Trematodes:

Flukes of medical importance belong to the subclass Digenea. Trematodes are unsegmented leaf-like worms (except schistosomes). Attach to the host by mean of suckers (ventral and oral suckers) (Cheesbrough, 1987). There is no body cavity, and the digestive system consists of mouth and an esophagus which divide to form intestinal caeca. There is no anus. Trematodes are hermaphroditic and produce operculated eggs (with exception of schistosomes where sex is separated and eggs are non-operculated) (Cheesbrough, 1987). Trematodes include: *Fasciolopsis buski, Fasciola hepatica, Clonorchis sinensis, Dicrocoelium dentriticum, Paragonimus westernami, Schistosoma mansoni* and *Schistosoma japonicum* (Cheesbrough, 1987).

Table 1.3: Differential morphology of the diagnostic stages of medically important tematodes found in humans (CDC).

Species	Size	Unique characters	Color	Stage of development when passed
Schistosoma mansoni	114-180μm.	Elongated with prominent lateral spine near posterior end.	Yellow or yellow brown.	Embryonated. Contains mature miracidium.

Schistosoma japonicum	68-100 μm.	Oval.Small lateral spine is often seen or may appear as a small hook or "knob.	Yellow or yellow brown.	Embryonated. Contains mature miracidium.
Clonorchis sinensis	27-35 μm.	Small, ovoidal, or elongated with broad rounded posterior end and a convex operculum resting on"shoulders". A small "knob" on the posterior end.	Yellow brown.	Embryonated. Contains mature miracidium.
Heterophyes heterophyes	28-30 μm.	Small, elongated or slightly ovoidal. Operculum.Slight "knob" at posterior end.	Yellow brown.	Embryonated. Contains mature miracidium.
Metagonimus yokogawai	26-30 μm.	Small, elongated or ovoidal. Operculum. No "shoulders" at anterior end. Small "knob" often seen on posterior end.	Yellow brown.	Embryonated. Contains mature miracidium.

Paragonimus westermani	68-118 μm.	Ovoidal or elongate with thick shell. Operculum is slightly flattened. Posterior end is thickened. Egg often asymmetrical.	Yellow brown.	Unembryonated. Filled with yolk material in which a germinal cell is imbedded. Cells are irregular in size.
Fasciola hepatica	120-150 μm.	Ellipsoidal, thin shell. Small, indistinct operculum.	Yellow to light brown.	Unembryonated. Filled with yolk cells in which an indistinct germinal cell is imbedded.
Fasciolopsis buski	130-159 μm.	Ellipsoidal, thin shell. Small, indistinct operculum.	Yellow to brown.	Unembryonated. Filled with yolk cells in which an indistinct germinal cell is imbedded.

1.2.1.3.3 General Characteristics of Nematodes:

Nematodes are non-segmented cylindrical worms. They possess a shiny cuticle which may be smooth, ridged or spined. Mouth is surrounded by lips or papillae. Digestive system is simple tube ends in an anus (Cheesbrough, 1987). Sexes are separated, with the male being smaller than the female and usually curved ventrally. In the male the testes at the distal end of a long tube terminates in a cobulatory organs (one or two projections) called spicules, copulatory bursa, caudal alae, or genital papillae. While the female posses one or two tubular ovaries lead to a uterus

or uteri. The uterus or united uteri open to the exterior through the vuvla. Females are either viviparous or oviparous, the discharged eggs may hatch directly into infective larvae, or may require special conditions in which to hatch and develop through three stages before becoming infective larvae (moulting) (Cheesbrough,1987). Nematodes include: *Enterobius vermicularis, Ascaris lumbricoides, Trichuris trichiura, Ancylostoma duodenale, Necator americanus* and *Strogyloides stercoralis*, (Cheesbrough, 1987).

Table 1.4: Differential Morphology of the Diagnostic Stages of Nematodes found in humans (CDC).

Species	Size	Unique characters	Color	Stage of development when passed
Ascaris lumbricoides: -Fertile egg	45-70 μm.	Round or ovoidal.With thick shell.	Brown or yellow brown	1 cell, separated from the shell at both ends.
-Infertile egg	85-95 μm.	Elongated, occasionally triangular, or other bizarre forms. Shell often very thin.	Brown.	Internal material is a mass of irregular globules and granules that fills shell.

Enterobius vermicularis	50-60 μm.	Elongated, asymmetrical with one side flattened, other side convex.	Colorless.	Embryonated. Contains tadpole-like embryo.
-Ancylostoma duodenale -Necator americanus	57-76 μm.	Oval or ellipsoidal with a thin shell.	Colorless with grayish cells.	4- to 8-cell stage.
Trichuris trichiura	49-65 μm	Elongated, barrel-shaped with a polar "plug" at each end.	Yellow to brown. "Plugs" are colorless.	1 cell or unsegmented.

1.2.1.4 Transmission:

Transmission of helminth infections from host to host is achieved by eggs or larvae. Eggs are usually directly ingested by a host. Larvae may be similarly ingested, consumed while attached to a plant or eaten while located in an intermediate host which acts as a prey item for the next host in the life cycle. Free-living mobile larval forms such as digenean miracidia and cercariae and third larval stage of nematodes (L3) larval nematodes are often able to find, recognize and invade new hosts. They possess impressive sensory and locomotory abilities which enable them to carry out these functions (Cox, 1993).

Helminth transmission can be through:

- Contaminated water and food: *A. lumbricoides*, *T.trichiuria* and *E. vermicularis.*
- Congenital infection: larva of hook worms.
- Skin penetration: Cercaria of *Schistosoma* species.

- Soil transmitted: larva of hook worms and *S.stercoralis.*
- Airborne: *E. vermicularis* and *A. lumbricoides* (Cox, 1993).

1.2.1.5 Life cycle:

Parasitic helminthes may have either simple or complicated life cycles. Firstly the adult parasites are found in the definitive host. This is where the parasite's sexual cycle usually takes place, with either cross or self fertilization with hermaphroditic parasites, or sexual reproduction if the parasites have separate sexes, followed by production of eggs, or more rarely with viviparous helminthes larvae (www.path.com.ac.uk). Secondly, in many cases the parasite larvae are found in different hosts, these are called the intermediate hosts. Parasitic helminth larvae may have one, two or more intermediate hosts in their life cycles, or they may have no intermediate hosts. Often asexual stages of reproduction occur in these intermediate hosts, (for example with platyhelminth parasites). Some parasitic nematodes (e.g. *S.stercoralis*) are facultative parasites, having completely free living life cycles in addition to parasitic ones (www.path.com.ac.uk).

1.2.1.5.1 Life cycle of cestodes:

Nearly every life cycle known for tape worms requires two hosts for its completion. One notable exception is *Hymenolepis nana.* Sexually mature tape worms live in the intestine and may live for a few days or up to many years, depending on species. During its reproductive life a single worm produces from a few to millions of eggs, each with the potential of developing into an adult, (Larry and John, 2009) see figure (1.1).

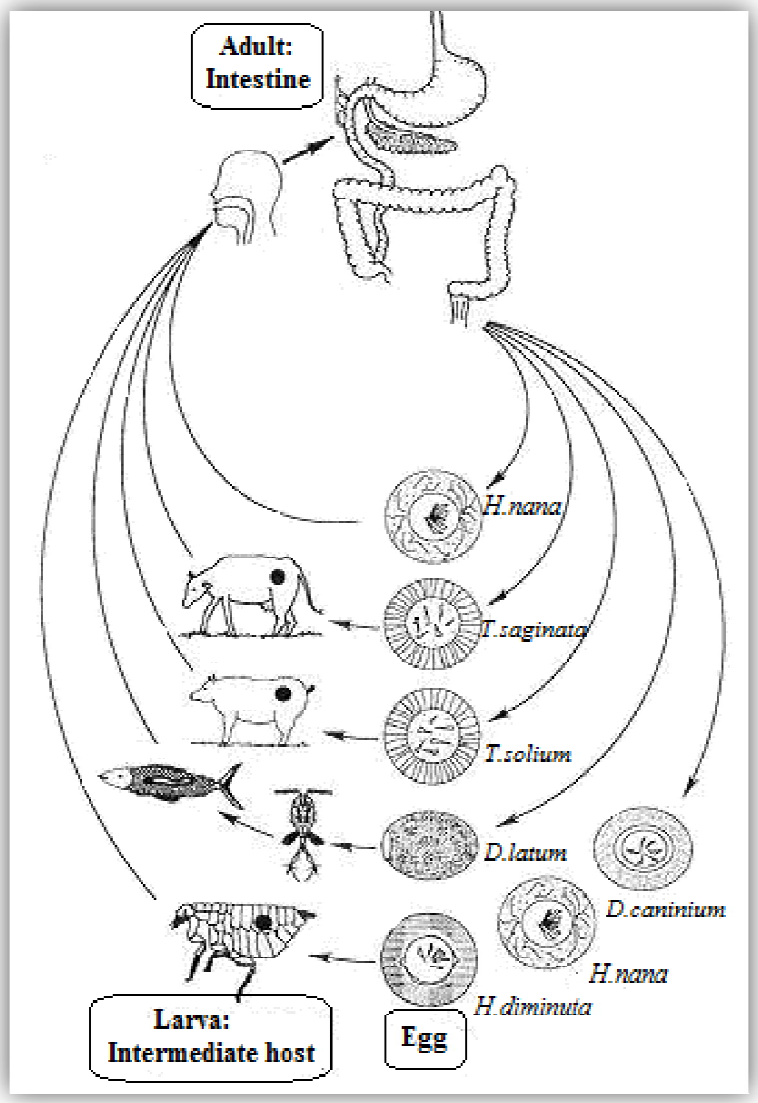

Figure 1.1: Typical life cycle of cestodes, source: www.pathobio.sdu.edu.cn.

1.2.1.5.2 Life cycle of trematodes:

A “typical” life cycle of trematode is as follows: a ciliated, free swimming larva, a miracidium, hatches from its shell and penetrates a first intermediate host, usually a snail. At the time of penetration or soon after, the larva discards its ciliated epithelium and metamorphoses into a rather simple, sac-like form, a sporocyst. Within the sporocyst a number of embryos develop asexually to become rediae. Rediae are somewhat more differentiated than sporocysts. Additional embryos develop within the redia, and these become cercariae. Cercariae emerge from the snail. They usually have a tail to aid in swimming. Although many species require further development as metacercariae before they are infective to a definitive host, cercariae are properly considered juveniles; they have organs that will develop into an adult digestive tract and suckers (Larry and John, 2009). A fully developed, encysted metacercaria is infective to a definitive host and develops there into an adult trematode. Many trematodes have a second intermediate host which bears their encysted metacercariae. Their vertebrate definitive hosts are then infected when they consume the second intermediate host (Larry and John, 2009). See figure (1.2) below.

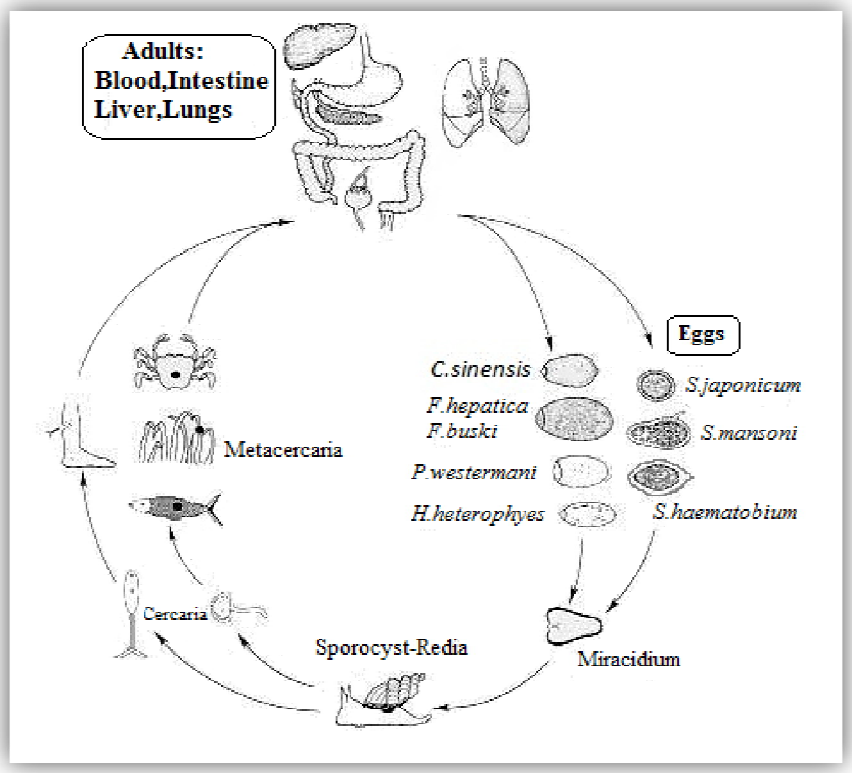

Figure 1.2: Typical life cycle of trematodes, www.pathobio.sdu.edu.cn.

1.2.1.5.3 Life cycle of nematodes:

Despite the diversity and complexity of many nematode life cycles, all of them can be related to the same basic pattern. This consists of two phases, parasitic and pre-parasitic. The parasitic phase takes place inside the definitive host while the pre-parasitic phase occurs either as a freeliving phase in the external environment or inside a second host, called an intermediate host. This basic life cycle also consists of seven stages, an egg, four larval stages (L1, L2, L3 and L4) and two adult stages comprising separate males and females. Sometimes the sexually immature adult stages are called L5 (Johnstone, 2000). See figure (1.3).

In most species sexual reproduction by adult nematodes is the norm and occurs within an infected definitive host. Eggs are laid by the female and pass from this host into the external environment (Johnstone, 2000).

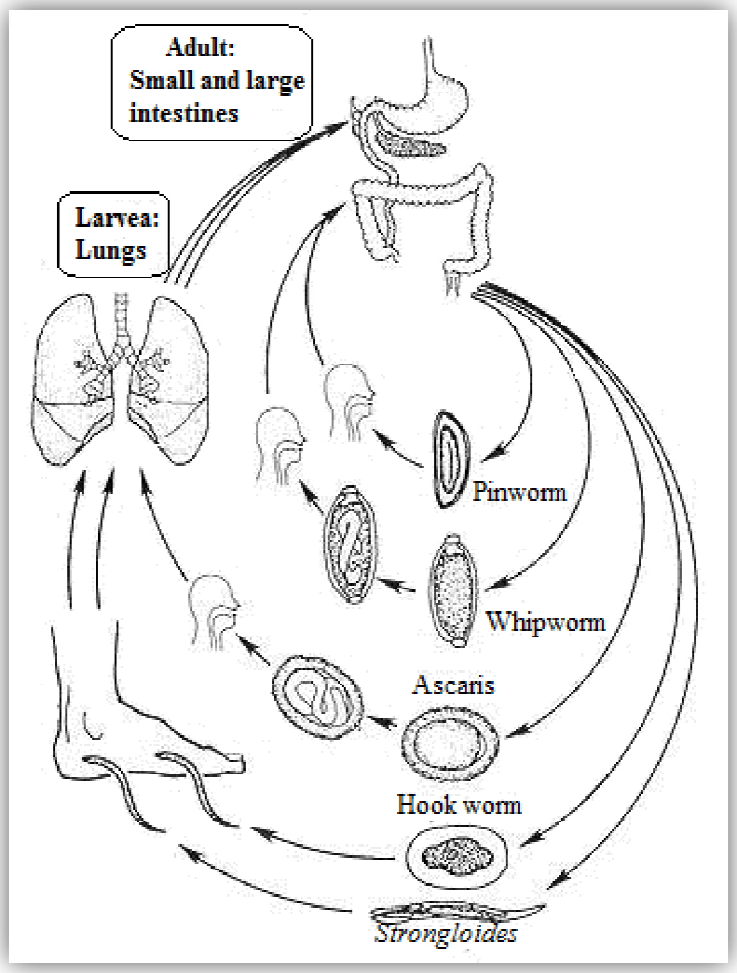

Figure 1.3: Typical life cycle of nematodes, source: www.pathobio.sdu.edu.cn.

1.2.1.6 Pathogenesis:

The majority of individuals infected with parasitic worms experience relatively minor symptoms and small percentage suffer severe life-threatening consequences.

Many of them have multistage within their mammalian hosts they often undergo extensive growth and differentiation that resulted a different pathological damages (Cox, 1993).

In terms of human pathology both adult and larval helminthes may cause pathology and disease. An important difference between infection with parasitic helminthes and infection with bacterial, viral or protozoan parasites is that, in most cases, the parasites do not increase in numbers within their hosts, (exceptions to this general rule may however be found with larval helminthes, or some nematodes such as *Strongyloides* spp). That is, each larval helminth that infects the definitive host will give rise to only one adult parasite. Therefore, pathology due to helminth infection is usually density dependent (www.path.cam.ac.uk). The damage may be from:

1. Direct damage from worm activity:

- The blockage of internal organs (migration of *Ascaris* may block the bile duct).
- The pressure effects exerted by growing parasites (cyst of *E.granulosus*).
- Physical and chemical damage (septicemia in disseminated Strongyloidiasis).
- Immunopathologic response (Allergic nasopharyngitis and itching in the throat (Halzoun -Marrara Syndrome - due to *F.hepatica*)).
- Extensive migrations through body tissues (intestinal nematodes with heart-lung migration may produce mild to severe symptoms during migration especially in lung).

2. Indirect damage from host response:

As with all infectious organisms, it is impossible to separate the pathogenic effects that result strictly from mechanical or chemical tissue damage from those caused by the immune response to the parasite. All helminthes are “foreign bodies” not only in the large and invasive but also in the immunologic sense: they are antigenic and therefore stimulate immunity. An excellent illustration of this interrelation between direct and indirect damage is seen in the pathology associated with *Schistosoma* infections. The severity of these indirect changes is a result of the chronic nature of the infection (Cox, 1993).

1.2.1.7 Diagnosis:

Most helminthes are acquired oversees, and the importance of a carefully taken travel history cannot be over emphasized. The majority of patients with gut helminthes will be either relatively asymptomatic or will present having passed a whole worm or worm segment. Identification of worms can be undertaken by local microbiology laboratories or by reference centers. Individuals with gastrointestinal symptoms or concerns following travel can be initially screened by stool examination for ova, larvae, segment, and sometimes adult. Full blood count for eosinophilia also helps (Allifia and William**,** 2011). Stool microscopy and eosinophil count may not be sufficiently sensitive for screening for infections such as *Strongyloides*, and serological tests may be needed. Interpretation can be complex and their use is probably best discussed with local infectious diseases or microbiology departments (Allifia and William, 2011).

1.2.1.8 Treatment:

Drugs used in the treatment of helminthes may be difficult to obtain and several are available on a named-patient basis only. For exotic helminth infections, referral to an infectious diseases service will probably be necessary from the point of view of accurate diagnosis, obtaining medication and for appropriate follow-up. Data on the

use of all anti-helmintic drugs during pregnancy and breast-feeding are limited, so their use in these situations should be discussed with specialists. Most anti-helmintic drugs are not licensed for use in children less than two year, or have age- or weight-adjusted dosing regimens; see (Table 1.5) below (Allifia and William, 2011).

Table 1.5: Drugs used in the treatment of helminth infections (Allifia and William, 2011).

Drug	Organism	Dosage	
		Adult	**Children**
Mebendazole *	*Ascaris, Trichuris,* hook worm	100 mg twice daily for 3 days.	1-18 years as adults.
	Enterobius	100 mg single dose, repeat at 2 weeks.	6 months -18 years as adults.
Albendazole†	*Ascaris, Enterobius Trichuris,* hook warms.	400 mg single dose.	Over 2 years 400 mg single dose.
	Cutaneous larva migrans,	400 mg once daily for 3 days (unlicensed use).	Over 2 years 400 mg twice daily for 3 days
	Strongyloides	400 mg twice daily for 3 days.	
	Trichinella	400 mg twice daily for 1 week.	

Piperazine*	*Ascaris,* hookworm	4g single dose, repeat monthly up to 3m if reinfection risk	3 months-1 year 2.5 ml level spoon single dose 1-6 years 5ml level spoon single dose, repeat monthly for up to 3 months if re-infection risk.
	Enterobius	4 g single dose, repeated at 2 weeks	First dose as for *Ascaris*; repeat at 2 weeks.
Ivermectin†	*Strongyloides,* Cutaneous larva migrans	200 μg/kg once daily for 2 days.	*Strongyloides*:over 5 years 200 μg/kg once daily for 2 days.
Niclosamide†	Tape worm	2 g single dose.	under 2 years 500mg single dose 2-6 years 1g single dose.
Praziquantel †	Tape worm	5-10 mg/kg single dose.	over 4 years 5-10 mg/kg single dose after food.
	Hymenolepis nana	25mg/kg single dose.	over 4 years 25mg/kg single dose.
	Schistosomiasis	20 mg/kg 2 doses, 4-6 hours apart.	over 4 years 40mg/kg in 2 divided doses 4-6 hours.

Levamisole†	*Ascaris* Hook worms	120-150 mg single dose. 120-150 mg single dose, repeat at 1 week if heavy infestation.	1 month-18 years: 2.5-3mg/kg(max. 150mg) single dose. 1 month-18 years: 2.5mg/kg (max. 150 mg) single dose, repeat at heavy 1 week if infestation.
* Available over the counter. † Available on named- patient basis only.			

1.2.1.9 Prevention and control:

The strategy for control of soil-transmitted helminth infections is to control morbidity through the periodic treatment of at-risk people living in endemic areas. People at risk are:

- Preschool children.
- School-age children.
- Women of childbearing age (including pregnant women in the second and third trimesters and breastfeeding women).
- Adults in certain high-risk occupations, such as tea-pickers or miners (WHO, 2014)

WHO recommends periodic drug treatment (deworming) without previous individual diagnosis to all at-risk people living in endemic areas. Treatment should be given once a year when the prevalence of helminthes infections in the community is over 20%, and twice a year when the prevalence of helminthes infections in the community is over 50%. This intervention reduces morbidity by reducing the worm burden (WHO, 2014).

In addition, health and hygiene education reduces transmission and re-infection by encouraging healthy behaviors and provision of adequate sanitation is also important but not always possible in resource-poor settings (WHO, 2014).

The aim of control activities is morbidity control. Periodic deworming can be easily integrated with child health days or supplementation programmes for preschool children, or integrated with school health programmes. In 2011, over 300 million preschool-aged and school-aged children were treated with anti-helminthic medicines in endemic countries, corresponding to 30% of the children at risk. Schools provide a particularly good entry point for deworming activities, as they allow easy provision of the health and hygiene education component such as the promotion of hand washing and improved sanitation (WHO, 2014).

1.2.1.10 Epidemiology:

Intestinal helminth infections are caused by different species of parasitic worms; most are soil-transmitted helminth which is transmitted by eggs present in human feces, which contaminate the soil in areas where sanitation is poor. Approximately two billion people are infected with soil-transmitted helminthes worldwide. Infected children are physically, nutritionally and cognitively impaired. Control is based on:

- Periodical deworming to eliminate infecting worms.
- Health education to prevent re-infection.
- Improved sanitation to reduce soil contamination with infective eggs.

Helminth infections are among the most common infections worldwide and affect the poorest and most deprived communities. The main species that infect people are the roundworm (*A.lumbricoides*), the whipworm (*T.trichiura*) and the dwarf tape warm (*H.nana*) (WHO, 2014).

More than 1.5 billion people or 24% of the world's population are infected with helminth worldwide. Infections are widely distributed in tropical and subtropical areas, with the greatest numbers occurring in sub-Saharan Africa, the Americas, China and East Asia. Over 270 million preschool-age children and over 600 million school-age children live in areas where these parasites are intensively transmitted, and are in need of treatment and preventive interventions (WHO, 2014).

1.2.2 Malaria:

1.2.2.1 Introduction:

Human malaria is still a serious problem in sub-Saharan Africa and the risk exists throughout the region. It is a real fact that most malaria cases and deaths occur in sub-Saharan Africa. This region has some of the poorest countries of the world with 90% of deaths occurring (approximately 3,000 deaths each day) (www.rbm.who.int, 2003**).** The disease remains one of the leading causes of morbidity and mortality in the tropics. It is the most important and widespread of the tropical deadly diseases. It exacts a heavy toll of illness and death on children and pregnant women (Okwa, 2003). Malaria is spreading as a result of environmental changes, including global warming, civil disturbances, increasing travel and drug resistance (Michael *et al.*, 2009). Human Malaria is a parasitic disease caused by apicomplexan protozoan coccidian. A contributing factor to the malaria problem in sub-Saharan Africa is the diversity of the parasite that infects humans. Four species infect man of which *P. falciparium* is the most virulent. The other species are *P.vivax, P.malariae* and *P.ovale* (WHO, 20l0). *P.falciparium* posses the greatest threat in sub-Saharan Africa, because of its high level of mortality and the complications arising. *P.vivax* is worldwide in tropical and some temperate regions. *P.vivax* accounts for more than half of all malaria cases outside sub- Saharan Africa (WHO, 20l0).

1.2.2.2 Transmission:

Malaria transmission most often occurs through the bite of an *Anopheles* mosquito. No other types of mosquitoes are known to transmit this disease. This type of mosquito becomes infected with one of the four *Plasmodium* parasites that cause malaria in humans, through a previous blood meal from an infected person. Because the malaria parasite is found in red blood cells, transmission may also occur through contact with infected blood. This can occur through a blood transfusion, an organ transplant, the shared use of needles or syringes that are contaminated with blood, or may also transmitted from a mother to her fetus, before or during delivery (congenital malaria) (Cheesbrough, 1987).

1.2.2.3 Life cycle:

The life cycle of malaria is passed in two hosts (alternation of hosts) and has sexual and asexual stages (alternation of generations). One is vertebrate host-man (intermediate host), where the asexual cycle takes place. The parasite multiplies by schizogony and there is formation of male and female gametocytes (gametogony) (Dawit *et al*., 2004). Other is the invertebrate host-mosquito (definitive host) where the sexual cycle takes place. Union of male and female gametes ends in the formation of zygot sporozoites (sporogony). The life cycle passes in four stages which three in man: Pre-erythrocytic schizogony, Erythrocytic schizogony and Exo-erythrocytic schizogony. And the fourth stage in mosquito (Sporogony). Introduction into humans, when an infective female Anopheles mosquito bites man, it inoculates saliva containing sporozoites (infective stage) (Dawit *et al*., 2004).

Pre-erythrocytic schizogony:

Sporozoites reach the blood stream and within 30 minutes enter the parenchymal cells of the liver, initiating a cycle of schizogony. Multiplication occurs in tissue schizonts, to form thousands of tiny merozoites. Merozoites are then liberated on rupture of schizonts about 7^{th} - 9^{th} day of the bites and enter into the blood stream. These merozoites either invade the RBC's or other parenchymal liver cells. In case

of *P.falciparum* and possibly *P.malariae,* all merozoites invade RBC's without re-invading liver cells. However, for *P.vivax* and *P.ovale,* some merozoites invade RBC's and some re-invade liver cells initiating further exo-erythrocytic schizogony, which is responsible for relapses. Some of the merozoites remain dormant (hypnozoites) becoming active later on (Dawit *et al*., 2004).

Erythrocytic schizogony (blood phase):

Erythrocytic schizogony is completed in 48 hrs in *P.vivax*, *P.ovale,* and *P.falciparum,* and 72 hrs in *P.malariae*. The merozoites reinvade fresh RBC's repeating the schizogonic cycles. Erythrocytic merozoites do not reinvade the liver cells. So malaria transmitted by blood transfusion reproduces only erythrocytic cycle (Dawit *et al*., 2004).

Gametogony:

Some merozoites that invade RBC's develop into sexual stages (male and female gametocytes). These undergo no further development until taken by the mosquito (Dawit *et al*., 2004).

Sporogony (extrinsic cycle in mosquito):

When a female *Anopheles* mosquito vector bites an infected person, it sucks blood containing the different stages of malaria parasite. All stages other than gametocytes are digested in the stomach. The microgametocyte undergoes ex-flagellation. The nucleus divides by reduction division into 6-8 pieces, which migrate to the periphery. At the same time, 6-8 thin filaments of cytoplasm are thrust out, in each passes a piece of chromatin. These filaments, the microgametes, are actively motile and separate from the gametocyte. The macrogametocyte by reduction division becomes a macrogamete (Dawit *et al*., 2004). Fertilization occurs by entry of a microgamete into the macrogamete forming a zygote. The zygote changes into a worm like form, the ookinete, which penetrates the wall of the stomach to develop into a spherical oocyst between the epithelium and basement membrane. The oocystes increase in size. Thousands of sporozoites

develop inside the oocysts. Oocysts rupture and sporozoites are liberated in the body cavity and migrate everywhere particularly to the salivary glands. Now the mosquito is infective. The sporogonous cycle in the mosquito takes 8-12 days depending on temperature. See figure (1.2) (Dawit *et al*., 2004).

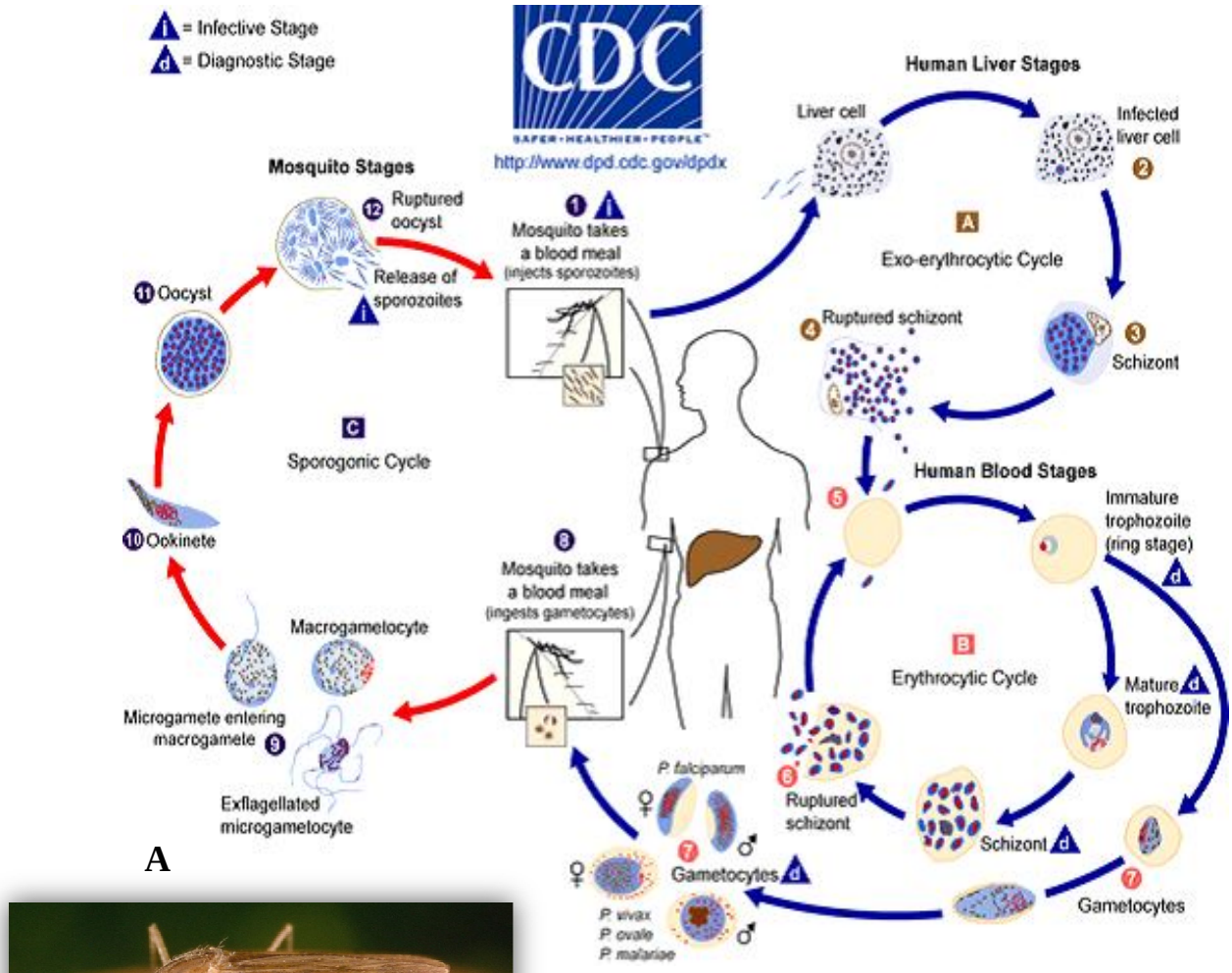

Figure 1.2: (A) Malaria life cycle and (B) *Anopheles* mosquito pumping blood, Source: CDC.

1.2.2.4 Pathogenesis:

The pathogenesis affect of malarial infection have been considered to be directly related to hemolysis of infected red blood cells, liberation of the metabolites of parasites, and the immunogenic pigments (Edward *et al*., 1999). *P.vivax*, *P.ovale*, *P.malarie* and uncomplicated *P.falciparum* have similar features with fever rigors, headache, muscle aches, malaise and anorexia. Anemia may develop and liver and spleen may become enlarged. Because clinical appearance is non-specific, malaria may be misdiagnosed (Edward *et al*., 1999).

The hallmark of *P.falciparum* malaria is the sequestration of infected erythrocytes within the capillaries and post capillary venules in the brain, lung, heart, bone marrow, kidney, liver, pancreas, intestine and other organs, and the intravillous spaces of the placenta (Stephen *et al.*, 2001, Luse and Miller, 1971; White and Ho, 1992). The slower blood flow and low oxygen tension provides a favorable environment for further parasite development. Sequestration also allows mature parasites to avoid passage through the spleen and likely clearance. *P.vivax* and *P.malariae* do not sequester, do not cause microcirculatory obstruction and are rarely fatal (Stephen *et al.*, 2001, Luse and Miller, 1971). In addition to adhesion to vascular endothelium, infected erythrocytes can adhere to uninfected erythrocytes and clumps or layers of erythrocytes are sometimes observed extending into the vessel lumen in cerebral malaria. Adherence of trophozoite and schizont-infected erythrocytes in target organs appears to be a major feature of the pathophysiology of *P.falciparum* malaria. As parasites mature, the infected erythrocytes become more rigid, less deformable, and changes occur in parasite and host surface proteins (Stephen *et al.*, 2001, Luse and Miller, 1971; White and Ho, 1992).

1.2.2.5 Clinical features:

In people with malaria, symptoms typically begin to develop 10 to 30 days after infection. They can range from mild symptoms to severe disease, and even death **(**www.malariasymptoms.org).

Clinical features appear as paroxysm which includes three stages:

1- Cold stage: characterized by rigor and headache. The patient feels cold and shivers even though his or her temperature is rising.
2- Fever stage: in which the temperature rises to its maximum and the back and joints and often vomiting and diarrhea.
3- Sweating stage: in which the patient perspires, the temperature falls and the headache and other pains are relived until the next rigor (Cheesbrough, 1987).

Early symptoms of malaria are generally non-specific such as fever**,** sweats**,** shaking chills**,** headaches**,** tiredness**,** muscles aches, nausea, vomiting, and diarrhea **(**CDC). More complicated diseases lead to severe malaria symptoms which include: kidney failure, seizures**,** mental confusion**,** coma**,** severe anemia**,** fluid in the lungs (pulmonary edema)**,** acute respiratory distress syndrome (ARDS)**,** and bleeding due to blood clotting problems and death (www.emedtv.com).

1.2.2.6 Complications:

P.vivax, P.ovale and quartan malaria are relatively benign, and complications that arise during the course of infection with one of these parasites are usually due to debility or undercurrent disease (Edward *et al*., 1999).

Infection with *P.falciparum* can rapidly build up to level not obtained with other 3 species and because of physiologic characteristic of red blood cells infected with *P.falciparum,* may lead to localized capillary obstruction, decreased blood flow, tissue hypoxia, infraction , and death (Edward *et al*., 1999).

Chronic *P.malariae* infection may result in immune complex deposition on glomerular walls, leading to nephrotic syndrome (Edward *et al*., 1999).

Malaria can be fatal, particularly the variety that's common in tropical parts of Africa. The Centers for Disease Control and Prevention estimate that ninety percent of all malaria deaths occur in Africa, most commonly in children under the age of 5 (www.mayoclinic.org).

In most cases, malaria deaths are related to one or more of these serious complications:

Cerebral malaria:

If infected red blood cells block small blood vessels of brain, swelling of brain or brain damage may occur. Cerebral malaria may cause coma (www.mayoclinic.org), and it may be produced by hypoglycemia, hypoxia, and lactic acidosis (Guerrant *et al*., 2001)

Severe anemia:

Malaria damages red blood cells due to lysis by mature asexual intra erythrocytic parasites (schizont), which can result in severe anemia (www.mayoclinic.org), also due to suppression of erythropoiesis by cytokines such as TNF-α, IL-1 and others, and peripheral destruction of red blood cells by spleen (Gurrant *et al*., 2001).

Renal failure:

Comes with urine output < 400 ml/ 24 hours in adults (< 12 ml/ kg/ 24 hours in children), and a serum creatinine > 265 µmol/ l (> 3.0 mg/ dl) despite adequate volume repletion (www.malariasite.com).

Pulmonary oedema or adult respiratory distress syndrome:

Pulmonary edema may develop rapidly in an oliguric or anuric patient, secondary to overzealous parenteral fluid administration or it may develop without evidence of fluid retention or cardiac decompensation, possibly as the result of disseminated intravascular coagulation (DIC) or anoxia affecting the pulmonary microcirculation (Edward *et al*., 1999). Accumulated fluid in lungs (pulmonary edema) can make it difficult to breathe (www.mayoclinic.org).

Tropical splenomegally syndrome:

Splenomegally of unknown reason appear most commonly occur in children with chronic malaria, it is characterized by elevated IgM level and infiltration of the liver sinusoids with B cells (www.mayoclinic.org).

Black water fever "Haemoglobinuria":

Macroscopic black, brown or red urine; not associated with effects of oxidant drugs or enzyme defects (like G6PD deficiency) (www.malariasite.com).

Hypoglycemia:

Severe forms of malaria itself can cause low blood sugar, as can quinine, one of the most common medications used to combat malaria. Hypoglycemia due to decreased oral intake, decreased appetite from the acute malaria illness, depletion of liver glycogen and parasite consumption of glucose (Gurranet *et al.*, 2001). Very low blood sugar can result in coma or death (www.mayoclinic.org).

Hyperparasitemia:

Five per cent parasitized erythrocytes or > 250, 000 parasites/ µl (in non immune individuals) (www.malariasite.com).

Metabolic (Lactic) acidosis:

Metabolic acidosis is defined by an arterial blood pH of < 7.35 with a plasma bicarbonate concentration of < 22 mmol/ L; and lactic acidosis is characterized by a pH < 7.25 and a plasma lactate > 5 mmol/ L (www.malariasite.com).

Repeated generalized convulsions:

Three or more generalized seizures within 24 hours (www.malariasite.com).

Hypotension and shock (algid malaria):

Systolic blood pressure being < 50 mmHg in children between 1-5 years, or < 70 mmHg in patients ≥ 5 years; cold and clammy skin or a core-skin temperature difference > 100 C^{o} (www.malariasite.com).

1.2.2.7 Epidemiology and distribution:

The prevalence of malaria has increased at an alarming rate during the last decades. There are now an estimated 300-500 million cases annually, which occur in some 101 countries and territories, of which almost half are situated in Africa south of the sahara (WHO, 1998b). Recent epidemics have caused a high number of deaths, many in areas previously free of the disease (Nchinda, 1998).

It is estimated that 3000 children under the age of 5 years die from malaria every day (WHO, 1998b). Frequent international air travel has also resulted in increasing numbers of imported cases and deaths in returned travelers and visitors to developed countries previously declared free of the disease (WHO, 1998b). A number of factors appear to have contributed to the resurgence of malaria (Nchinda, 1998). These include breakdown of control programmes, rapid spread of resistance of malaria parasites to chloroquine and other quinolines, and the migration of non-immune populations (for the purposes of agriculture, commerce or trade) from areas that are free from malaria to areas where transmission is high. In addition, armed conflicts have caused displacement of large populations of refugees to areas where living conditions are difficult and the prevalence of malaria is often high. Changing rainfall patterns and land use, leading to new mosquito breeding sites, and changes in vector behavior have further compounded the problem. In general, governments have responded slowly to the changing malaria situation because of adverse socioeconomic conditions and limited resources for health (Murphy and Oldfield, 1996). Malaria occurs most commonly in the tropics as high humidity and ambient temperatures of 20-30C^{o} provide optimal conditions for the developing of vectors and malaria parasites. Transmission does not occur below 16C^{o} (Murphy and Oldfield, 1996). Ninety per cent of malaria cases occur in sub-Saharan Africa. *P.falciparum* is predominant in Africa responsible of the death of one in 20 rural African children under 5 years (Murphy and Oldfield, 1996). Seventy four per cent (74%) of the populations in Africa live in areas where malaria transmission is intense and per annual (WHO, 1996). The transmission and pattern of clinical malaria may vary considerably, even within small geographic areas, depending on the characteristic of vector and susceptibility and accessibility of human hosts. Transmission may be related to the vector density, the number of times the mosquito bites man each day, and the longevity of the mosquito. Different species of anopheline mosquito vary in their ability to transmit malaria, and of nearly 400

known species only about 60 are considered important vectors. Also each anopheline has its own behavior pattern, which influences its rule in transmission. For example, *A.gambiae* complex are the most successful malaria vectors because they are resilient, long lived and bite humans frequently (Stephen *et al.*, 2001, Bruce-Chwatt, 1985 and Zheng and Kafatos, 1999).

1.2.2.8 Laboratory diagnosis:

The definitive diagnosis of malaria infection is still based on finding malaria parasites in blood films. In thin films the red blood cells are fixed so the morphology of the parasitized cells can be seen and species identification can be made. However, malaria parasites may be missed on a thin blood film when there is a low parasitemia (Michael *et al.*, 2009). Therefore, examination of a thick blood film is recommended. With a thick blood film, the red cells are approximately 6-20 layers thick which results in a larger volume of blood being examined (Michael *et al.*, 2009). Films should be made without delay since morphological alteration of parasites occurs with storage of EDTA-anticoagulated blood (Bailey, 1997). After malaria parasites are detected on a blood smear, the parasite density should then be estimated. The parasite density can then be estimated from the percentage of infected RBCs, after counting 500 to 2000 RBCs (CDC, 2013). In addition to microscopy, other laboratory diagnostic tests are available. Several antigen detection tests (rapid diagnostic tests or RDTs) using a “dipstick” or cassette format exist. RDTs can more rapidly determine that the patient is infected with malaria, but they cannot confirm the species or the parasitemia (CDC, 2013). Parasite nucleic acid detection using polymerase chain reaction (PCR) it is at least 10-fold more sensitive than microscopy (Padley, 2003) and specific than microscopy but can be performed only in reference laboratories and so results are not often available quickly enough for routine diagnosis. However, PCR is a very useful tool

for confirmation of species and detecting of drug resistance mutations (CDC, 2013).

1.2.2.9 Treatment:

Most drugs used in treatment are active against the parasite forms in the blood (the form that causes disease) and include: Chloroquine, Atovaquone-Proguanil (Malarone ®), Artemether-Lumefantrine (Coartem ®), Mefloquine (Lariam ®), Quinine, Quinidine, Doxycycline (used in combination with Quinine), Clindamycin (Used In Combination With Quinine), Artesunate and primaquine which is active against the dormant parasite liver forms (hypnozoites) and prevents relapses (www.cdc.gov).

It is preferable that treatment for malaria should not be initiated until the diagnosis has been established by laboratory investigations.

Treatment should be guided by three main factors:

• The infecting *Plasmodium* species.

• The clinical status of the patient.

• The drug susceptibility of the infecting parasites as determined by the geographic area where the infection was acquired and the previous use of antimalarial medicines (www.cdc.gov, 2013).

The infecting *Plasmodium* species: Determination of the infecting *Plasmodium* species for treatment purposes is important for three main reasons:-

Firstly, *P.falciparum* and *P.knowlesi* infections can cause rapidly progressive severe illness or death while the other species are less likely to cause severe manifestations. Secondly, *P.vivax* and *P.ovale* infections also require treatment for the hypnozoite forms that remain dormant in the liver and can cause a relapsing infection. Finally, *P.falciparum* and *P.vivax* species have different drug resistance patterns in differing geographic regions. For *P.falciparum* and *P.knowlesi*

infections, the urgent initiation of appropriate therapy is especially critical (www.cdc.gov, 2013).

The clinical status of the patient: Patients diagnosed with malaria are generally categorized as having either uncomplicated or severe malaria. Patients diagnosed with uncomplicated malaria can be effectively treated with oral antimalarials. However, patients who have one or more of complications (as in hypoglycemia) are considered to have manifestations of more severe disease and should be treated aggressively with parenteral antimalarial therapy (www.cdc.gov, 2013).

The drug susceptibility of the infecting parasites: Finally, knowledge of the geographic area where the infection was acquired provides information on the likelihood of drug resistance of the infecting parasite and enables the treating clinician to choose an appropriate drug or drug combination and treatment course. If the diagnosis of malaria is suspected and cannot be confirmed, or confirmed but species determination is not possible; antimalarial treatment effective against chloroquine-resistant *P.falciparum* must be initiated immediately (www.cdc.gov, 2013).

Uncomplicated malaria

For infections acquired in areas with chloroquine resistance, four treatment options are available. The first two treatment options are Atovaquone-Proguanil (Malarone) or Artemether-Lumefantrine (Coartem). These are fixed dose combination medicines that can be used for non-pregnant adult and pediatric patients. Both of these options are very efficacious. Quinine sulfate plus doxycycline, tetracycline, or clindamycin is the next treatment option. Quinine treatment should continue for 7 days for infections acquired in South East Asia and for 3 days for infections acquired in Africa or South America. The fourth option, Mefloquine, is associated with rare but potentially severe neuropsychiatric reactions when used at treatment doses. We recommend this fourth option only when the other options cannot be used. (www.cdc.gov, 2013). For pediatric patients, the treatment options are the

same as for adults except the drug dose is adjusted by patient weight (www.cdc.gov, 2013).

For infections acquired in areas without chloroquine-resistant strains, which include Central America west of the Panama Canal, Haiti, the Dominican Republic, and most of the Middle East, patients can be treated with oral chloroquine (www.cdc.gov, 2013). In addition, any of the regimens listed for the treatment of chloroquine-resistant malaria may be used for the treatment of chloroquine-sensitive malaria. Prompt initiation of an effective regimen is vitally important and so using any one of the effective regimens that readily at hand would be the preferred strategy (www.cdc.gov, 2013).

Severe Malaria:

Patients who are considered to have manifestations of more severe disease should be treated aggressively with parenteral antimalarial therapy regardless of the species of malaria seen on the blood smear. Oral antimalarial drugs are not recommended for the initial treatment of severe malaria. If severe malaria is strongly suspected but a laboratory diagnosis cannot be made at that time, blood should be collected for diagnostic testing as soon as it is available and parenteral antimalarial drugs may be started (www.cdc.gov, 2013).

1.2.2.10 Malaria control:

- Diagnosis and treatment.
- Prevention of infection through vector control (use of insecticide-treated mosquito nets, shown to reduce all-cause child mortality by 20 %- 25%).
- Prevention of disease by administration of antimalarial drugs to particularly vulnerable population groups such as pregnant women.

1.2.2.11 Malaria in Sudan:

There is a high burden of malaria-related morbidity and mortality in Sudan. However, the national malaria control programme, with the WHO's support, has reduced the number of malaria cases from more than four million in 2000 to less than one million in 2010 (WHO, 2011), the incidence was estimated to be about 9 million episodes in 2002 and the number of deaths due to malaria was about 44,000. 2,877,000 children under five years of age had the highest burden. Males had the highest incidence and mortality (Safa *et al*., 2007). In the north and eastern states transmission is low to moderate seasonal malaria with epidemic outbreaks. In the south transmission is moderate or high, with stable (per annual) transmission, figure 1.3 (World Malaria Report, 2011).

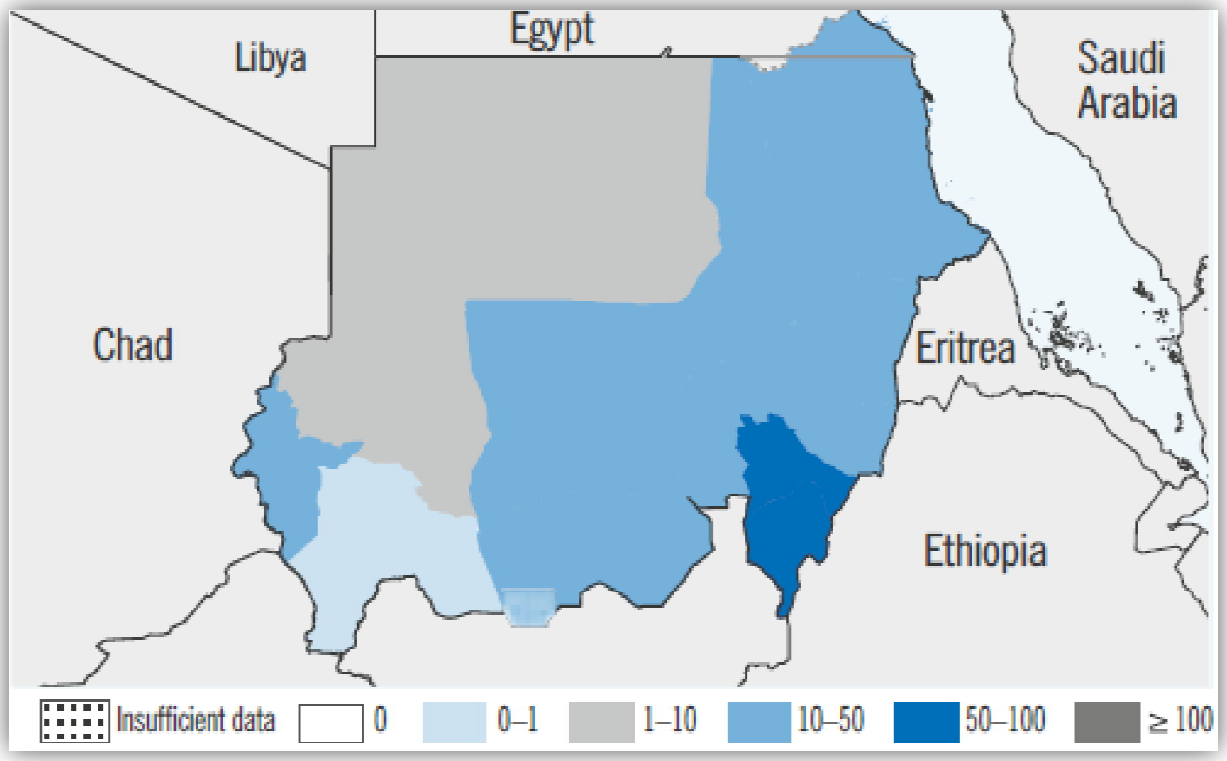

Figure 1.3: Distribution of probable and confirmed malaria cases in Sudan (per 1000 population) .Source: WHO.

1.2.3 Co- infection:

1.2.3.1 Background:

Most natural host populations are exposed to a diverse community of parasites, and co-infection of hosts by multiple parasites is common across a diverse range of systems (Petney and Andrews, 1998; Valérie *et al.*, (2005) and Cox, 2001). Two of the most prevalent types of human infection in the developing world are malaria and intestinal helminthes (Mwangi *et al.*, 2006). In the past decade, the topic of interactions between worms and malaria has generated a surge of interest with over 35 publications reporting findings in humans from different continents. Similarly, there have been over 25 publications on different animal models of co-infection between worms and malaria (Nacher, 2011 and Nacher, 2012). Because they overlap extensively in their epidemiological distributions and frequently co-infect the same individuals (Valérie *et al.*, 2005; Cox, 2001; Brooker *et al.*, 2007; Mazigo *et al.*, 2010 and Simon *et al.*, 2012). The question of whether and how these two types of parasite might interact within co-infected hosts has attracted much interest and controversy (Druilhe *et al.*, 2005; Nacher, 2006).

1.2.3.2 Malaria susceptibility and helminthes infection:

Co-infecting parasites may interact either positively (facilitation) or negatively (competition) via a range of mechanisms including resource competition, immune-mediated interactions and direct interference (Maizels *et al.*, 2004; van Riet *et al.*, 2007; Hewitson *et al.*, 2009 and Grainger *et al.*, 2010). To date, studies of intestinal helminth-malaria co-infection have focused largely on immune-mediated mechanisms, no doubt largely due to the known immunomodulatory effects of intestinal helminthes on the patient immune system (Maizels *et al.*, 2004; Riet *et al.*, 2007; Hewitson *et al.*, 2009 and Grainger *et al.*, 2010). An early study performed in (1978) described that anti-helminthes treatment of severe ascariasis in a high-transmission area was followed by an increase in symptomatic malaria (Murray *et al.*, 1978). However, several studies later supported the notion that infection with

helminthes increases the susceptibility to malaria infection. A study in Senegal revealed that the risk of clinical malaria was reduced in helminthes-free children compared to children positive for *Ascaris*, *Ancylostoma* or *Trichuris* (Spiegel *et al.*, 2003). Another study conducted in northern Senegal showed that the incidence of malaria attacks was higher in children positive for infection with *Schistosoma mansoni*, especially in subjects with the highest helminthes loads (Sokhna *et al.*, 2004). The association of intestinal helminthes infection with an increased risk for malaria incidence has been confirmed in two other studies. The first study showed a positive association of intestinal helminthes and infection with *P.falciparum* in adults in Thailand (Nacher *et al.*, 2002). Whereas the second study on mothers and children in Zaire described a positive association between infection with *Ascaris* and the occurrence of *P.falciparum* (Tshikuka *et al.*, 1996 and Hartegers and Yazdanbakhsh, 2006). The associations between malaria susceptibility and intestinal helminth infection seem to be influenced by the type of helminth infection, the intensity of infection, and the age of the population studied (Hartgers and Yazdanbakhsh, 2006). Age patterns of intestinal helminth infections and malaria are likely to be affected by exposure to infection and acquisition of immunity or a combination of both. Co-infection and co-morbidity may however not occur within the same individuals (Snow *et al.*, 1997). Much of the morbidity due to malaria is generally concentrated among young children. However, age patterns of malaria morbidity are dependent on the level of transmission within a community, which affects the age at which adequate immunity is acquired (Snow *et al.*, 1997). To interpret the plausibility of epidemiological studies of helminth-malaria interactions, it is important to consider the risk factors associated with both types of infections. The large-scale geographical distributions of malaria and helminths are determined largely by climate, which determines mosquito and helminth free-living stage survival (Hay *et al.*, 2000; Brooker and Michael, 2000). Factors that are thought to be involved in determining smaller-scale distributions

include socio-economic status and human behavior (Carme *et al.*, 1994). Several studies indicate that low education levels are associated with poor malaria prevention and access to effective anti-malarial (Varandas *et al.*, 2000), and may also determine hygienic and water contact behavior, thereby influencing exposure to helminth infective stages in the external environment (Asaolu and Ofoezie, 2003).

1.2.3.3 Immunomodulatory effects of helminthes:

It has been suggested that in malaria-helminth co-infection; helminth infection stimulates the Th2 cytokine response which possibly predominates, and down-modulates Th1 cytokines (Torre *et al.,* 2002). Inhibition of the Th1 response prevents protective effects of IFN-γ during the blood and liver stages of malaria infection (Torre *et al.,* 2002). This could explain the worsening of anemia in malaria-helminthes co-infection (Basavaraju and Schant, 2006). Helminthes infection thus creates a cytokine milieu favorable to the production of non-cytophilic antibodies, thus making individuals more susceptible to clinical malaria (Mwangi *et al.,* 2006). The presence of T regulatory cells is amplified during helminth infection, and if present in sufficient numbers, could induce a non-specific suppression (Yazdanbakhsh *et al.,* 2001), making individuals susceptible to infections such as malaria; however, malaria may also exacerbate the consequences of helminth infection (Mwangi *et al.,* 2006; Nelly *et al.,* 2010).

1.2.3.4 Anemia and co-infection:

Anemia is one of the most widespread and common health condition afflicting individuals living in the tropics, and in Africa, it contributes to 23% of nutrition-related disability adjusted life years (WHO, 2002; Brooker *et al.,* 2007). There are many hypotheses for the pathophyisiology of malaria-related anemia. These include: hemolysis or direct destruction of parasitized red blood cells both intravasculary and by sequestration in the microcirculation, mainly in the spleen, nonspecific, defective red cell production, which depresses erythropoiesis, inhibits reticulocyte

release and prematurely destructs red cells during maturation in the bone marrow; (Chigzie, 2008;Fleming, 1989; Nelly *et al.,* 2010), shortened red cell survival through specific or nonspecific immune responses; and malaria-related hypersplenism which is associated with reduction in blood cells, causing anemia, thrombocytopenia, and leucopenia (Chigzie, 2008;Fleming, 1989; Nelly *et al.,* 2010). Intestinal helminth infections, specifically infections with hookworms and *Trichuris trichura* have been demonstrated to be associated with anemia (Ndomugyenyi *et al*., 2002; Drake *et al*., 1994), and they are an important cause of anemia in developing countries (Muhangi *et al.,* 2007). Based on the distinct mechanisms by which malaria and different intestinal helminth reduce hemoglobin levels, it can be speculated that their combined presence might interact to enhance the risk of anemia. However, little is known about the impact of co-infection on anemia among different age groups (Brooker *et al.,* 2007), thus co-infection is associated with low hemoglobin level especially among primgravid women (Egwunyenga *et al.,* 2001; Nelly *et al.,* 2010).

1.2.3.5 Outcome of co-infection:

The intensity of helminth infection might be an important determinant for the outcome of immune responses to the malaria parasite (Sokhna *et al*., 2004; Valerie *et al.,* 2005; Lyke *et al.,* 2005). Different pathological outcome of co-infection has recently been noted. Enlargement of the spleen is a common observation in communities in tropical countries (Hartgers and Yazdanbakhsh, 2006).

Objectives

General objective:

- To establish epidemiological and clinical correlation in malaria- intestinal helminthes co-infection in Abu-Naama area in Sinnar State.

Specific objectives:

- To determine prevalence of intestinal helminthes and malaria infections in the study area.
- To detect intensity of intestinal helminthes infection by FECT.
- To study prevalence of other protozoan parasites infections in the study area.
- To determine relationship between intensity of intestinal helminthes infection and age groups.
- To determine relationship between malaria severity and age groups.
- To determine relationship between intestinal infections, gender and age groups.
- To determine relation between previous helminthes infection, current malaria infection, pervious intestinal helmithes infection and current co-infection.
- To compare between wet preparation and FECT in detection of intestinal helminthes and intestinal protozoa.

Chapter Two

Materials and Methods

2.1 Study design:

This study was cross sectional study.

2.2 Study area:

This study was carried out in Abu-Naama area, Sinnar State, in West coast of Blue Nile. Which consider as farms land, because ¾ of Sinnar State farms are present in Abu-Namma. Therefore the majority of populations are farmers, depending on culture and rising animals. Culture in Abu-Naama depend mainly on irrigation canals, which many of them are blocked forming collection of water that provide breeding sites for mosquitoes. Most animals (cows, goats, hens, dogs,…) raised inside houses, where their wastes may present. The area lack for swage disposable places; houses and animal wastes may present in streets, next to houses, empty irrigation canals and small water collections. The area is considered to be endemic for malaria. The peaks of the malaria transmission states are in October and continue to December. *Plasmodium falciparum* is considered to be the major malaria species in the area. *Plasmodium vivax* is also endemic in area. Mixed infection may also be present. The area is endemic with many intestinal helminthes mainly *Hymenolepis nana*.

2.3 Study population:

A total of 100 individuals with different ages and sexes were randomly selected for the purpose of this study. After informed consent was obtained, all individual included have agreed to participated in the study.

2.4 Period of study:

The study was conducted during period from November 2013 to April 2014.

2.5 Sample size:

One hundred stool samples were collected from individuals under study and 100 blood samples were collected from same individuals.

2.6 Samples collection:

2.6.1 Stool samples:

2.6.1.1 Method of collection:

Fecal specimens were collected in clean, wide mouthed containers, with tight fitting lids, free of disinfectant and labeled with patient number. All fresh fecal specimens were handled carefully. Specimens were preserved in 10% formal saline (ratio 1:9).

2.6.1.2 Method of examination:

Intestinal parasites were detected in stool sample using wet preparation and formal ether concentration technique.

Wet preparation:

Requirements:

- Light microscope.
- Slides.
- Cover glasses.
- Gloves.
- Wooden sticks.

- Normal saline.

Procedure:

A drop of normal saline was placed on slide; a small portion of stool was added by using wooden stick. It was then mixed well and covered with cover glass. Slides were examined under microscope (Olympus CX22) using x10 and x40 lenses.

Formal Ether Concentration Technique

Requirements:

- 10 % Formal Saline.

- Ether.

- Gloves.

- Pasture pipettes.

- Sieve.

- Wooden sticks.

- Beakers.

- Conical tubes.

- Centrifuges.

- Slides.

- Cover glasses.

Procedure:

One gram of stool was emulsified in 10 ml of 10% formal saline. It was then sieved in a beaker; filtrate was transferred into conical centrifuge tube (falcon tube). Equal volume of ether was added and the mixture was shaken vigorously for one minute. It was centrifuged at 3000 rpm for 5 minutes. The supernatant fluid was discharged. The sediment was mixed, then transferred to slide and covered with cover glass, the preparation was examined microscopically using x10 objective for search and screening and x40 for identification, examined all the sediment and the number of intestinal parasites were counted per 1 gram of stool.

The intensity of intestinal parasites was determined by the method of Young *et al,* (Table 2.1):

Table 2.1: Description of intestinal parasites intensity (Young *et al.*, 1979)

Intensity	Description
Many	More than 3 cysts per high-power field, or more than 20 eggs or larvae per mount.
Moderate	2 cysts per high-power field, or 10 to 19 eggs or larvae per mount.
Few	1 cyst per high-power field, or 3 to 9 eggs or larvae per mount.
Rare	Less than 1 cyst per high-power field, or less than 2 eggs or larvae per mount.

2.6.2 Blood samples:

2.6.2.1 Method of collection:

Requirements:

- Cotton.

- 70% alcohol.

- Lancet.

- Capillary tube.

- Microscopic slides.

- Spreaders.

- 10% Geimsa stain.

- Oil.

Procedure:

Blood samples were collected by standard procedure; using sterile lancet after disinfection of area collection by 70% alcohol. Blood collected using capillary tube, then thick and thin blood films were prepared.

2.6.2.2 Preparation of blood films:

To make thick smear, the collected blood was stirred with a corner of slide until an appropriate thickness was obtained. To make the thin smear, the edge of spreader was placed just in front of the drop of blood. Then it was drown back until it touches the drop of blood. The blood was allowed to run along the edge of spreader. The spreader was then pushed to the other end of the slide with a smooth movement. The slide was then allowed to dry.

2.6.2.3 Staining of blood films:

All thin and thick blood films were stained using Geimsa stain. Thin films were fixed with absolute methanol for 1-2 minutes. Then slides were covered with 10% Geimsa solution for 10 minutes. All slides were washed using clean water and allowed dry by air.

2.6.2.4 Method of examination:

The slides were examined using light microscope (Olympus CX22 x100 oil immersion lenses), using the thick film for detection and the thin film for identification of species. Then the number of parasites was counted in thick film against white blood cells using formula:

Parasitemia per µl = Parasite count x 8000/200

2.7 Data analysis:

Data was analyzed using Statistical Package of Social Sciences (SPSS) version 11.5.

2.8 Data presentation:

Data was presented in tables and graphs using Microsoft Excel after analysis by SPSS version 11.5.

2.9 Ethical consideration:

The studies adopted were approved by ethical review committees of Ministry of Health of Sinnar State. Consent was taken from each individual before being included in the study; each individual was informed on the nature of study.

Chapter Three

Results

3.1 General characteristics of studied population:

The age of the study subjects in the present study ranged between 2 months and 70 years, with mean age was 20 years. The individuals were divided into 6 groups according to age; less than 5 years, 6-12 years, 13-18 years, 19-40 years, 41-64 and more than 65 years, (Table 3.1). With 56% being females and 44% males.

Table 3.1: Frequency of age groups among gender

Age group	Frequency		Total	Mean of age
	Males	Females		
Less than 5	12	15	27 (27%)	20
6-12	4	8	12 (12%)	
13-18	7	11	18 (18%)	
19-40	15	12	27 (27%)	
41-64	4	8	12 (12%)	
More than 65	2	2	4 (4%)	
Total	100 %		100 %	

3.2 Parasitological results:

3.2.1 Intestinal helminthes:

3.2.1.1 Overall prevalence of intestinal helminthes in study area:

For detection of intestinal helminthes stool samples were collected, within these samples 15 (15%) were found positive by formal ether concentration technique, while 85 (85%) were negative, (Table 3.2).

Intestinal helminthes ova were detected by wet preparation and formal ether concentration technique, out of them 7 (7%) were found to be positive when detected by using wet preparation method, while 15 (15%) was positive when detected by using formal ether concentration technique, (Table 3.3).

When formal ether concentration technique was used, worm load (egg per gram of stool) ranged between 5-109 eggs/ g of stool, and all found to be *H.nana* eggs.

Table 3.2: Overall prevalence of intestinal helminthes in study area

	Frequency	Percentage (%)
Positive	15	15 %
Negative	85	85 %
Total	100	100 %

Table 3.3: Detection of intestinal helminthes eggs by wet preparation and Formal ether concentration technique

FECT	Wet Preparation		Total
	Positive	Negative	
Positive	7 %	8 %	15 %
Negative	0 %	85 %	85 %

3.2.1.2 Intensity of intestinal helminthes:

Out of the 100 stool samples, 15 (15%) were positive for *H.nana*.

The intensity of infection was obtained by counting the number of *H.nana* eggs per 1 gram of stool using FECT. Eggs per 1 gram of stool presented as rare, few, moderate and many (Truant *et al.*, 1981 and Young *et al.*, 1979), (Table 3.4).

Table 3.4: Intensity of Intestinal helminthes infection among age groups

Intensity / Age groups(years)	Negative	Rare	Few	Moderate	Many
Less than 5	23	0	2	0	3
6-12	10	0	0	0	2
13-18	16	0	0	2	0
19-40	25	0	0	0	2
41-64	10	0	0	0	2
More than 65	3	0	0	0	1
Total	85 %	0 %	2 %	2 %	10 %
Mean of eggs count (egg / 1 g of stool)	0	0	7	13	62

3.2.1.3 Relation between presence of *H.nana* infection and presence of clinical features:

Chi-squire test was used to determine the relation between presence of *H.nana* and appearance of clinical features; individuals where examined clinically by physician for presence of fever (p.value= 0.161), diarrhea (p.value= 0.139) and abdominal pain (p.value= 0.568), the results shown in (Table 3.5). The mean of count was determined in presence and absent of each feature as shown in (Table 3.6).

Table 3.5: Relation between presence of *H.nana* and presence of clinical features

Present of symptoms		*H.nana*		Total
		Positive	Negative	
Fever	Present	15 (15%)	75 (75%)	90 (90%)
	Absent	0 (0%)	10 (10%)	10 (10%)
Diarrhea	Present	3 (3%)	34 (34%)	37 (37%)
	Absent	12 (12%)	51 (51%)	63 (63%)
Abdominal pain	Present	7 (7%)	33 (33%)	40 (40%)
	Absent	8 (8%)	52 (52%)	60 (60%)

Table 3.6: Mean of *H.nana* density among different clinical status

Symptoms		Mean of eggs count per g of stool
Fever	Present	8
	Absent	0
Diarrhea	Present	3
	Absent	10
Abdominal pain	Present	10
	Absent	6

3.2.2 Overall prevalence of other intestinal protozoan infection:

For detection of intestinal protozoa, stool samples were collected, within these samples 18 (18%), 24 (24%) were found to be positive for *G.lambelia, E.histolytica* respectively by wet preparation, while 32 (32%), 35 (35%) were found to be positive for *G.lambelia*, *E.histolytica* respectively by FECT, (Table 3.7). Overall prevalence was considered by FECT results.

Table 3.7: Prevalence of intestinal protozoan infections in the study area

Variables	*G.lambelia*		*E.histolytica*	
	Positive	Negative	Positive	Negative
Wet preparation	18 (18%)	82 (82%)	24 (24%)	76 (76%)
FECT	32 (32%)	68 (68%)	35 (35%)	65 65%)

3.2.2.1 Intensity of intestinal protozoan infection:

The intensity of infection was obtained by counting the number of *G.lambelia* and *E.histolytica* cysts per 1 gram of stool using FECT. Infection intensity of stool presented as rare, few, moderate and many (Young *et al.*, 1979), (Table 3.8) and (Table 3.9), respectively.

Table 3.8: Intensity of *G.lambelia* infection among age groups

Intensity / Age groups(years)	Negative	Rare	Few	Moderate	Many
Less than 5	20	1	0	0	6
6 – 12	6	0	1	0	5
13 – 18	12	2	1	1	2
19 – 40	20	0	0	0	7
41 – 64	9	1	0	1	1
More than 65	1	1	0	0	2
Total	68	5	2	2	23
Mean of count (cyst / g of stool)	0	194	796	1008	10387

Table 3.9: Intensity of *E.histolytica* infection among age groups

Intensity / Age groups(years)	Negative	Rare	Few	Moderate	Many
Less than 5	21	3	0	0	3
6 – 12	8	0	0	0	4
13 – 18	8	1	0	1	8
19 – 40	19	1	0	2	5
41 – 64	7	1	0	1	3
More than 65	2	1	0	0	1
Total	65	7	0	4	24
Mean of count (cyst / g of stool)	0	326	0	1008	7708

3.2.2.2 Relation between presence of intestinal protozoan infection and presence of clinical features:

Chi-squire test was used to determine the relation between presence of intestinal protozoa and appearance of clinical features; individuals where examined clinically by physician. In *G.lambelia* infection, the results for fever, diarrhea and abdominal pain were (p.value= 0.391), (0.414), (0.600), respectively, (Table 3.10).

While in *E.histolytica* infection, fever, diarrhea and abdominal pain were (p.value= 0.727), (0.648) and (0.669) respectively, (Table 3.11). The mean of count was determined in presence and absent of each feature as shown in (Table 3.12).

Table 3.10: Relation between presence of *G.lambelia* and presence of clinical features

Present of symptoms		*G.lambelia*		Total
		Positive	Negative	
Fever	Present	30 (30%)	60 (60%)	90 (90%)
	Absent	2 (2%)	8 (8%)	10 (10%)
Diarrhea	Present	10 (10%)	27 (27%)	37 (37%)
	Absent	22 (22%)	41 (41%)	63 (63%)
Abdominal pain	Present	14 (14%)	26 (26%)	40 (40%)
	Absent	18 (18%)	42 (42%)	60 (60%)

Table 3.11: Relation between presence of *E.histolytica* and presence of clinical features

Present of symptoms		*E.histolytica*		Total
		Positive	Negative	
Fever	Present	31 (31%)	59 (59%)	90 (90%)
	Absent	4 (4%)	6 (6%)	10 (10%)
Diarrhea	Present	14 (14%)	23 (23%)	37 (37%)
	Absent	21 (21%)	42 (42%)	63 (63%)
Abdominal pain	Present	13 (13%)	27 (27%)	40 (40%)
	Absent	22 (22%)	38 (38%)	60 (60%)

Table 3.12: Mean of intestinal protozoan infection density among different clinical status

Variables		Mean of eggs count per 1 g of stool	
		G.lambelia	*E.histolytica*
Fever	Present	2654	1998
	Absent	469	1153
Diarrhea	Present	1212	2799
	Absent	3154	1293
Abdominal pain	Present	2991	2563
	Absent	2066	1480

3.2.3 Malaria

3.2.3.1 Prevalence of malaria in the study area:

Out of 100 study subjects, 73 (73 %) were harboring *P.falciparum*, (Table 3.13).

Table 3.13: The prevalence of malaria parasite in the study subjects

Samples	Frequency	Percentage (%)
Positive	73	73 %
Negative	27	27 %
Total	100	100 %

3.2.3.2 Density of malaria parasite:

Malaria parasite density was available for 73 (73%) individuals (individuals with positive results). Parasitemia expressed as number of parasite per µl of blood, and parasitemia (parasite per microleter of blood) ranged between 1000- 71520 parasite/µl, with mean of count 8437 parasite/µl of blood, (Table 3.14).

Table 3.14: Mean of parasitemia among age groups and sex

Variables		Mean of parasitemia per µl of blood
Age groups	Less than 5	9674
	6-12	8837
	13-18	7869
	19-40	7638
	41-64	6650
	More than 65	11892
Sex	Males	9613
	Females	7513

3.2.3.3 Relation between presence of malaria and presence of clinical features:

Chi-squire test was used to determine the relation between presence of malaria and appearance of clinical features; individuals where examined clinically by physician for presence of fever (p.value= 0.329), diarrhea (p.value= 0.061) and abdominal pain (p.value = 0.141), results shown in (Table 3.15), and the mean of count was determined in presence and absent of each feature as shown in (Table 3.16).

Table 3.15: Relation between presence of malaria and presence of clinical features

Present of symptoms		Malaria		Total
		Positive	Negative	
Fever	Present	67 (67%)	23 (23%)	90 (90%)
	Absent	6 (6%)	4 (4%)	10 (10%)
Diarrhea	Present	23 (23%)	14 (14%)	37 (37%)
	Absent	50 (50%)	13 (13%)	63 (63%)
Abdominal pain	Present	26 (26%)	14 (14%)	40 (40%)
	Absent	47 (47%)	13 (13%)	60 (60%)

Table 3.16: Mean of malaria parasitemia among different clinical status

Symptoms		Mean of count per µl of blood
Fever	Present	8292
	Absent	9744
Diarrhea	Present	8123
	Absent	8621
Abdominal pain	Present	8384
	Absent	8472

3.3 Co-infection

3.3.1 Intestinal helminthes - *Plasmoduim* co-infection:

The present study indicated that 13 individuals (13%) were co-infected with intestinal helminthes and malaria, (Table 3.17), with prevalence ratio= 1.22 and p.value= 0.196.

Table 3.17: Prevalence of intestinal helminthes- *Plasmoduim* co-infection in the study area

Variable		Malaria		Total
		Positives	Negatives	
Helminthes	Positives	13 %	2 %	15 %
	Negatives	60 %	25 %	85 %
Total		73 %	27 %	100 %

3.3.2 Mean of *Plasmoduim falciparum* parasitemia (per µl of blood) in relation to presence of helminthes infection:

Mean of *Plasmoduim falciparum* count (parasite per µl of blood) was determined in relation to presence of intestinal helminthes infection among malaria positive individuals, (Table 3.18).

Table 3.18: Mean of *Plasmoduim falciparum* parasitemia (per µl of blood) in relation to presence of intestinal helminthes infection:

Variable	Helminthes infection (*H.nana*)	
	Positive	Negative
Mean of parasitemia (parasite per µl of blood)	13785	7638

3.3.3 Mean of count *Plasmoduim falciparum* (parasite per µl of blood) in relation to intestinal helminthes infection intensity:

Mean of count of *Plasmoduim falciparum* was determined for each sex and among the different status of intestinal helminthes infection (*H.nana* density), (Table 3.19).

Table 3.19: Mean *Plasmoduim falciparum* parasitemia (per µl of blood) in relation to helminthes infection intensity and sex

Variable		Mean of parasitemia per µl of blood
Sex	Male	9613
	Female	7513
H.nana intensity	Rare	0
	Few	5880
	Moderate	6880
	Many	15117

3.3.4 Prevalence of co-infection among different age groups:

Among different age groups, prevalence of co-infection was obtained and results shown in (Table 3.20).

Table 3.20: Prevalence of co-infection among different age groups

Co-infection / Age groups(years)	Positive	Negative
Less than 5	4	22
6 – 12	2	10
13 – 18	2	16
19 – 40	2	25
41 – 64	2	10
More than 65	1	3
Total	13	87

3.3.5 Relationship between co-infection and fever, abdominal pain and diarrhea:

Chi-squire test was used to determine the relation between co-infection and fever (p.value = 0.179), abdominal pain (p.value = 0.627) and diarrhea (p.value= 0.058); individuals were examined clinically by physician for presence of these features, (Table 3.21).

Table 3.21: Relationship between co-infection and fever, abdominal pain and diarrhea

Variable		Co-infection		Total
		Positive	Negative	
Fever	Presence	14 (14%)	76 (76%)	90 (90%)
	Absence	0 (0 %)	10 (10%)	10 (10%)
Diarrhea	Presence	2 (2%)	35 (35%)	37 (37%)
	Absence	12 (12%)	51 (51%)	63 (63%)
Abdominal pain	Presence	6 (6%)	34 (34%)	40 (40%)
	Absence	8 (8%)	52 (52%)	60 (60%)
Total		13 (13%)	87 (87%)	100 (100%)

3.3.6 Intestinal helminthes - protozoa co-infection:

Out of 100 studied subjects, 9 (9%) were found to be co-infected with *H.nana* and *G.lambelia* (p-value=0.012), while 6 (6%) were co-infected with *H.nana* and *E.histolytica* (Table 3.22).

Table 3.22: Intestinal helminthes - protozoa co-infection

Variable		*G.lambelia*		*E.histolytica*	
		Positive	Negative	Positive	Negative
H.nana	Positive	9 (9%)	6 (6%)	6 (6%)	9 (9%)
	Negative	23 (23%)	62 (62%)	29 (29%)	56 (56%)
Total		32 (32%)	68 (68%)	35 (35%)	65 (65%)

3.3.7 *Plasmoduim* - protozoan co-infection:

Out of 100 studied subjects, 32 (32%) were found to be co-infected with *P.falciparum* and intestinal protozoa, as shown in (Table 3.23).

Table 3.23: *Plasmoduim* - Protozoan co-infection

Variable		*G.lambelia*		*E.histolytica*	
		Positive	Negative	Positive	Negative
Malaria	Positive	27(27%)	46 (46%)	26 (5%)	47 (47%)
	Negative	5 (5%)	22 (22%)	9 (9%)	18 (18%)
Total		32 (32%)	68 (68%)	35 (35%)	65 (65%)

3.3.8 Prevalence of previous intestinal helminthes and current malaria co-infection:

The present study indicated that 26 individuals (26%) with malaria had previous intestinal helminthes infection, (Table 3.24), (p.value= 0.202).

Table 3.24: Prevalence of previous intestinal helminthes and current co-infection:

Variable		Current malaria		Total
		Positives	Negatives	
Previous helminthes	Positives	26 %	6 %	32 (32 %)
	Negatives	47 %	21 %	68 (68 %)
Total		72 (72 %)	28 (28 %)	100 (100 %)

3.3.9 Relation between previous intestinal helminthes infection and current co-infection:

Chi-squire test was used to determine the relation between previous intestinal helminthes infection and current co- infection, as shown in (Table 3.25). p.value= 0.348.

Table 3.25: Relation between previous helminthes infection and current co-infection:

Variable		Current co-infection		Total
		Yes	No	
Previous helminthes infection	Yes	5 (5%)	27 (27%)	32 (32%)
	No	8 (8%)	60 (60%)	68 (68%)
Total		13 (13%)	87 (87%)	100 (100%)

3.4 Comparison of wet preparation and FECT:

Chi squire used to compare the results of intestinal parasites obtained by wet preparation technique to those obtained using FECT technique, as shown in (Table 3.26). p- value= (0.000).

Table 3.26: Comparison of wet preparation and FECT:

Variable		FECT		Total
		Positive	Negative	
Wet preparation	Positive	40	0	40
	Negative	16	44	60
Total		56	44	100

Chapter Four

Discussion

Half the world's population lives in malaria-endemic areas, with an estimated 500 million clinical cases and over one million deaths annually (WHO, 2008). In Sudan malaria has been subject of a large amount of epidemiological, entomological, and biomedical research. Malaria evidence in Sudan was estimated to be about 9 million episodes in 2002 and the number of deaths was 44000 (Abdalla *et al*., 2007). Intestinal helminth infections are major causes of morbidity in all age groups in the developing world. More than a quarter of the world population is infected with soil-transmitted helminths like hook worms, *H.nana* and Ascaris, and 200 million with schistosomiasis (Allifia and William, 2011).

Spatial congruence of both *P. falciparum* and different helminthes remains poorly defined. Preliminary analyses, however, suggest that as many as one quarter of African school children may be coincidentally at risk of *P.falciparum* and hookworm (Brooke *et al.*, 2006). This spatial coincidence of risk between these two parasite populations would suggest that co-infection is extremely common; although the public health significance of polyparasitic infection remains a topic for which there are many unknowns (Maizel *et al.*, 2004). So this study was conducted to establish association between helminth infection and acquiring malaria infection. For this purpose 200 sample (100 blood and 100 stool) were involved and examined to detect co-infection.

Helminthes infections diagnosed in Abu-Naama area, 15 (15%) were found to be positive for *H.nana* infection, due to low hygiene and bad environmental conditions. Most individuals (77 % of positive individuals); intensity of infection presented as many, with mean of count 62 eggs / 1g of stool.

The results showed that the prevalence of other intestinal parasites (*G.lambelia* and *E.histolytica*) were 32 (32 %) and 35 (35 %), respectively.

Seventy two (72%) of *G.lambelia* positive individuals; intensity presented as many with mean of count 10387 cyst/ 1g of stool. Sixty nine (69 %) of positive *E.histolytica* individuals; intensity of infection presented as many with mean of count 7708 cyst /1g of stool.

The prevalence of *P.falciparum* infection in Abu-Naama area was 73 %, due to presence of its preferred breeding sites, provided feeding and post-feeding places and favorable hosts. The parasitemia of infection show mean of count 8437 parasite/µl of blood. High parasitemia was detected among males with mean of count 9613 parasite/µl of blood, while mean count for females 7513 parasite/ µl of blood.

The prevalence of malaria-intestinal helminthes co-infection (*P.falciparum- H.nana* co-infection) was 13%. The most co-infected individuals were found to be in the less than 5 years. Although observations indicate that intestinal helminthes infection can consider as a risk factor for malaria infection (Prevalence ratio= 1.22), but the statistical results showed that there is no correlation between malaria and intestinal helminthes infection (p-value= 0.196), this results were disagree with (Andargachew *et al*., 2013; Hartgers and Yazdanbakhsh, 2006) which prove presence of correlation.

The results showed no correlation between previous helminthes and current malaria co-infection (p.value= 0.202), and also no correlation between previous helminthes infection and current co-infection (p.value= 0.348).

The mean of malaria parasitemia in helminthes positive individuals (13785 parasite/ µl of blood) found to be higher than the mean of parasitemia of non-helminthic individuals (7638 parasite/ µl of blood). The highest mean of count associated with highest worm burden as expressed by egg / 1 g of stool (mean of 15117 parasites/ µl of blood associated with mean of 62 egg / 1g of stool in co-infected patients.

The results showed no association between co-infection and fever (p.value = 0.179), diarrhea (p.value= 0.058) and abdominal pain (p.value= 0.627).

Also the results showed no association between *H.nana* and fever (p.value= 0.161) with mean of count 8 egg /1 g of stool for individuals suffering from fever, diarrhea (p.value= 0.139) with mean of count 3 egg /1 g of stool and abdominal pain (p.value= 0.568) with mean of count 10 egg /1 g of stool.

The result showed no association between intestinal protozoa and fever, diarrhea and abdominal pain with p.values (0.391), (0.414), (0.600), respectively for *G.lambelia*, and (0.727), (0.648) and (0.669), respectively for *E.histolytica.*

Results showed no association between *P.falciparum* and fever (p.value= 0.329) with mean of count 8292 parasite/ µl of blood, diarrhea (p.value= 0.061) with mean of count 8123 parasites/ µl of blood and abdominal pain (p.value= 0.141) with mean of count 8384 parasites/ µl of blood.

The results obtained by FECT were compared with those obtained using wet preparation, p-value was (0.000) indicating that FECT is better than the wet preparation in detection of intestinal parasites.

Chapter Five

Conclusions and Recommendations

5.1 Conclusions:

The study showed no epidemiological and clinical correlation between malaria and intestinal helminthes in Abu-Naama area in Sinnar State.

5.2 Recommendations:

- Further studies should be done on immunological affects of intestinal helminthes infections on malaria immune response.
- Further studies should be done with other species of *Plasmoduim*.
- Further studies should be done to find epidemiological intestinal helminthes-malaria co-infection.
- Further studies should be done in prevalence of intestinal helminthes-malaria co-infection in other endemic areas.
- Formal ether concentration technique should be used as best method for detection of intestinal helminthes eggs than wet preparation.
- Control activities should be conducted in study area to reduce infection with malaria and other intestinal parasite infections.
- Increase sample size.

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Appendix 1: List of reagents and items

Reagent or Item	Company	Origin
Geimsa stain	Crescent Diagnostics	Jeddah - K.S.A
Formalin	BIOS Europe	Europe
Di ethyl ether	BIOS Europe	Europe
Methanol	ATOM Scientific Ltd	UK
Normal saline	Pharmacological solutions industry	Jeddah, K.S.A
Latex examination gloves	SWECO S.A.	Indonesia
Microscopic Slides	CITOPLUS	UK
Spreaders	CITOPLUS	UK
Cover glass	SAIL BRAND	China
Lancets	Vitrex Medical Ltd	UK
Stool containers	JRZ	Lebanon
Transfer Pipette	CITOTEST	China
15ml Falcon tubes	JRZ	Lebanon
Cotton Roll	Medispo	China
Wooden Applicator Sticks	Fisher Scientific	U.S.A

Centrifuge	Labtech	India
Microscope	OLYMPUS	China

Appendix 2: Table of results of samples

Number	Malaria infection	Intestinal Helminthes infection	Co-ifection
1	Positive	Negative	No
2	Positive	Negative	No
3	Positive	Negative	No
4	Positive	Positive	Yes
5	Positive	Positive	Yes
6	Positive	Negative	No
7	Negative	Negative	No
8	Negative	Negative	No
9	Positive	Negative	No
10	Negative	Negative	No
11	Positive	Negative	No
12	Negative	Negative	No
13	Positive	Negative	No
14	Negative	Negative	No
15	Positive	Negative	No
16	Negative	Negative	No
17	Positive	Negative	No
18	Negative	Negative	No

19	Negative	Negative	No
20	Positive	Negative	No
21	Positive	Negative	No
22	Positive	Negative	No
23	Positive	Negative	No
24	Negative	Negative	No
25	Positive	Negative	No
26	Positive	Negative	No
27	Positive	Negative	No
28	Positive	Negative	No
29	Negative	Negative	No
30	Positive	Negative	No
31	Positive	Negative	No
32	Positive	Negative	No
33	Positive	Negative	No
34	Positive	Positive	Yes
35	Positive	Negative	No
36	Positive	Negative	No
37	Positive	Negative	No

38	Negative	Negative	No
39	Negative	Positive	No
40	Negative	Negative	No
41	Negative	Negative	No
42	Negative	Negative	No
43	Positive	Negative	No
44	Negative	Negative	No
45	Positive	Positive	Yes
46	Positive	Negative	No
47	Positive	Positive	Yes
48	Positive	Negative	No
49	Negative	Negative	No
50	Positive	Negative	No
51	Positive	Negative	No
52	Positive	Positive	Yes
53	Positive	Negative	No
54	Negative	Negative	No
55	Positive	Negative	No
56	Positive	Negative	No

57	Positive	Negative	No
58	Positive	Negative	No
59	Negative	Negative	No
60	Positive	Negative	No
61	Positive	Negative	No
62	Positive	Negative	No
63	Positive	Negative	No
64	Positive	Negative	No
65	Positive	Negative	No
66	Negative	Negative	No
67	Positive	Positive	Yes
68	Positive	Negative	No
69	Positive	Negative	No
70	Positive	Negative	No
71	Positive	Negative	No
72	Positive	Positive	Yes
73	Positive	Positive	No
74	Positive	Positive	Yes
75	Positive	Negative	No

76	Negative	Negative	No
77	Negative	Negative	No
78	Positive	Negative	No
79	Positive	Negative	No
80	Positive	Negative	No
81	Positive	Negative	No
82	Negative	Negative	No
83	Positive	Negative	No
84	Positive	Positive	No
85	Negative	Negative	No
86	Negative	Negative	No
87	Positive	Negative	No
88	Positive	Positive	No
89	Positive	Positive	Yes
90	Positive	Negative	No
91	Positive	Negative	No
92	Positive	Negative	No
93	Positive	Negative	No
94	Negative	Negative	No

95	Positive	Negative	No
96	Positive	Negative	No
97	Positive	Positive	Yes
98	Negative	Negative	No
99	Positive	Negative	No
100	Positive	Positive	Yes

Appendix 3: Ecology of intestinal parasites and *Plasmoduim*

Appendix 4: Ecology of intestinal parasites and *Plasmoduim*

Appendix 5: Abu-Naama health center.

Appendix 6: Ecology of intestinal parasites (Contamination of water).

Appendix 7: Ecology of intestinal parasites.

Appendix 8: Stool Specimens.

Appendix 9: Collection of blood samples.

Appendix 10: Thin and thick blood films

Appendix 11: Olympus microscope

Appendix 12: Centrifuge

Appendix 13: Questionnaire

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

Name:……..… **Sex:**……

Age:…….…... **Area:**……...

1- **Previous malaria infection:**

Yes ☐ **No** ☐

2- **Previous intestinal helminthes infection:**

Yes ☐ **No** ☐

3- **Previous co-infection:**

Yes ☐ **No** ☐

4- **Clinical features:**

- **Diarrhea:** **Yes** ☐ **No** ☐
- **Abdominal pain:** **Yes** ☐ **No** ☐
- **Fever** **Yes** ☐ **No** ☐

Other……