Addis Ababa University

School of Graduate Studies College of Natural Sciences

Department of Microbial, Cellular and Molecular Biology

Status of Malaria in Wau Town, Western Bahr El Ghazal, South Sudan

By

Chan Ngor Bak Matik (BSc.)

Thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment for the requirement of Master of Science Degree in Biology (Applied Microbiology)

March, 2017

Addis Ababa, Ethiopia

Dedication

To the soul of our late father Luka Ngor Bak Matik, the entire family, relatives and friends

ACKNOWLEDGEMENT

I would like to recognize the following individuals and Institutions for their help and contribution in making this research. My Advisors Mekuria Lakew (PhD) and Habte Tekie (PhD) for tirelessly advising me throughout the research process. Hon. Nhial Deng Nhial, Senior Presidential Advisor and Presidential Envoy, Republic of South Sudan for his moral and financial support without his support, this research would not have been successful. All Addis Ababa University Laboratories that helped me in analysis, especially Insect Vectors and Entomopathogen Research Laboratory, Addis Ababa University Science Library. Mr. Youhana Mudwak Achuil (MSc.), Director of Malaria Control Program (MCP), thanks for his advice and support in the field. All MCP Technicians at Wau Teaching Hospital who helped me, especially Jima Bai and Emmanuel Eugeino Bandas. Mrs. Martha Ariel, a student at the Institute of Public and Environmental Health, University of Bahr El Ghazal. Thanks to Wau Teaching Hospital, Sikka Hadid Hospital, and St. Daniel Comboni Catholic Church Hospital for providing me with necessary data, my gratitude goes to their Statistics, Monitoring and Evaluation respectively. Especially Mr. Giovanni Canton and Alexandra of St. Daniel Comboni Catholic Church Hospital. My thanks goes to Mr. Mariano Silvano and Mrs. Agnes Benjamin a Technicians in Wau Teaching Hospitals for helping in getting blood samples from participants. Thanks to Dr. Ajoui Deng Kelei Modern Laboratories. Department of Human Resources Development and Training, State Ministry of Health- Western Bahr El Ghazal State – Wau for providing me with approvals to go to the field, and their Monitoring and Evaluation Department, especially Mr. John Natale. Mohamed Farouq, PhD candidate in the Department of Microbial, Cellular and Molecular Biology, Mr. Desta, PhD candidate in the Department of Zoological Sciences for helping me. Andu Zakaria Lukwasa and Charles Mahamoud Sebit, PhD candidates in the Department of Environmental Sciences for their valuable advice. Mr. Ajou Ajou an MSc. Student in the Ethiopian Civil Service University for his valuable SPSS application he had to support me in analyzing the dat. My colleagues in the Department of Microbial, Cellular and Molecular Biology for supporting and integrating me to the Ethiopian community life and making the study enjoyable.

TABLES OF CONTENTS

Page

List of Tables

List of Figures

List of Appendices

List of photos

Abbreviations and Acronyms

ANC	Antenatal Care
CPA	Comprehensive Peace Agreement
ELISA	Enzyme Linked Immunosorbent Assay
EPI	Expanded Programme for Immunization
FBOs	Faith-based organizations
GoSS	Government of South Sudan
ICT	Immuno-combined Test
IDSR	Integrated Disease Surveillance Response
IOM	International Organization for Migration
IRS	Indoor residual spraying
ITN	Insecticide Treated Net
KAP	Knowledge, Attitude and Practice
LLINs	Long-lasting insecticidal nets
MDGs	Millennium Development Goals
MoH	Ministry of Health
NGOs	Non-Governmental Organizations
PCR	Polymerase Chain Reaction
RDT	Random Diagnosis Test
RoSS	Republic of South Sudan
SSCCSE	South Sudan Centre for Census: Statistical and Evaluation.
UN	United Nations
WHO	World Health Organization

Abstract

South Sudan, like all other East African Countries through Global Fund had implemented several control programs as per recommendation of WHO and succeeded to reduce malaria transmission from intense to interrupted transmission contributing to the implementation of the elimination/eradication program in the years to come. Investigation of the transmission pattern of the hotspot foci and understanding the dynamics of malaria transmission was essential. Therefore, this study planned Hotspot study on urban epidemiological setting of Wau town/city. Cross sectional studies of the town at 2 months intervals in the dry season and two others in wet season were planned. Sample size was determined, peripheral blood Smear and RDT serological methods were adopted as standard methods of prevalence study, positive in both were considered for determination of the prevalence. Accordingly, four (4) study sites were selected and data collection was completed in Hai Nazareth, just before the unpredicted war between the rebel and the government forces broke in the town. Hence, relying on one time cross sectional study for prevalence study and the retrospective data of the preceding year, 2015, taken from Health clinics and hospitals to establish the parasite prevalence. There was transmission throughout the year and an increase in all age groups in the rainy seasons between June and October with peak August-October. Infected individuals who visited the hospital and were found positive are larger in number in the age groups 6-15 and above 15. The result showed that 35% of infection in those under 5 years is greater than 23% of those between 6-10 years indicating that the under 5 are the high risk groups. The 16.1% in those under 1 year are most vulnerable for at that age they do not have protective immunity ready yet. *P. falciparum* was the only species diagnosed, accounting for 47.51% of all subjects who visited for any other infections (52.49%), suggesting the health significance of Malaria in the population. Primary data done in May and June showed 32.6% infection rate in the sample population of which 5.2% were children under 5 while the age groups 6-15 and above 15 years had 13 and 14.4% respectively. All were asymptomatic cases. Given the retrospective study that showed a year round transmission with a peak between June and October and then a decline to lowest level in December, the Frequency of infection in Children and age group below 15 is a high level of infection and above 15 had relatively higher infection. The demographic distribution of the study population, Metrological data of the town, age distribution of the parasite prevalence, identification of major and minor vectors were also reflected and their implications in breaking transmission were discussed.

Keywords: Hotspot foci, elimination, epidemiological setting, asymptomatic cases, parasite prevalence.

1. INTRODUCTION

South Sudan is a landlocked country bordered by six countries, Sudan to the north, Ethiopia to the east, Kenya to the southeast, Uganda to the south, the Democratic Republic of the Congo to the southwest, and the Central African Republic to the west. It lies between latitudes 3° and 13°N, and longitudes 24° and 36°E. The habitats in the country include high-altitude plateaus covered with tropical forest; central flood plains of the Sudd, Bahr el Ghazal and the Sobat and grassy savanna escarpments. The White Nile passes through the country and the capital city, Juba.

The climate of the country is similar to an Equatorial or tropical climate, characterized by a rainy season of high humidity and large amounts of rainfall followed by a drier season. The temperature on average is always high with July being the coolest month with an average temperatures falling between 20 and 30 °C (68 and 86 °F) and March being the warmest month with average temperatures ranging from 23 to 37 °C (73 to 98 °F). The country has an approximate population of 8,260,490 according to "Fifth Population and Housing Census of Sudan" in April 2008 which have been disputed and rejected by South Sudanese authorities by then, because "the central bureau of statistics in Khartoum refused to share the national Sudan raw census data with the Southern Sudan Centre for census, statistics and evaluation." (Sudan Tribune 2009). According to 2015 estimate, South Sudan population is approximately 12,340,000.

The pre-independence era of South Sudan witness a grave gap of health infrastructures it inherited since Sudan's independence from Anglo-Egyptian colonization 1956. The long history of war has destroyed not only physical and social structures but also virtually collapsed the health system (Chanda *et al*, 2013). This has contributed to the huge burden of diseases the country suffers today. Malaria incidence in Sudan in 2002 was estimated to be about 9 million episodes resulting in 44,000 deaths. This in other words was expressed as a loss of 2, 877,000 DALYs (Disability Adjusted Life Years) (Safa, 2007).

After independence, the country put a number of strategies in place to control malaria. It strived to make progress in implementing the WHO recommended malaria control interventions as set out in the 2006-2013 National Malaria Strategic Plan. (Chanda *et al*, 2013). Yet the country encountered enormous programmatic constraints including infrastructures, human and financial resources and weak health system compounded by an increasing number of refugees, returnees and internally displaced people. Hence malaria is still the leading cause of morbidity and mortality in the country. It accounts for 20 - 40% morbidity, over 20% of deaths reported at health facilities and 30% of all hospital admissions, (RoSS, 2009). At present, the country is one amongst the highest malaria burdened countries in sub-Saharan Africa. Since independence in autonomy, the Ministry of Health (MoH) has made endeavors to implement relatively planned, coordinated and monitored malaria control interventions, and has documented successful programs in the country. It was able to receive the Global Fund rounds 2, 7 and 10 for malaria control and other financial support from other funding agencies to scale-up the interventions (RoSS, 2011).

To reduce the malaria burden, case management and vector control strategies have been implemented expansively, as per the World Health Organization's (WHO) recommendations (GoSS, 2006a). This was followed by implementation of Millennium Development Goals (MDGs), which significantly reduced disease morbidity and mortality in the country. (Hay, SI 2008). Now Ministry of Health has a well-established and functioning routine information system through the Integrated Disease Surveillance Response (IDSR) and National Health Management Information System (HMIS) including sentinel site surveillance to regularly monitor the outcomes of malaria control (GoSS, 2009b) Over seven years (2006 – 2013) of implementation of recommended interventions, the country has experienced marked reduction in morbidity and mortality with heterogeneity in effectiveness of malaria control efforts (RoSS, 2009a), suggesting the need for better understanding of the various ecology and adjustment of the control strategies for maximum effectiveness of the control. More recent WHO strategy of stopping Malaria transmission and eliminating through thorough regulation of malaria hotspots during the dry and non-transmission seasons so that they will not be able to fuel malaria transmission across a wider area in peak season was also to be tried at a pilot scale. Targeting hotspots was believed to represent an efficacious strategy for eliminating malaria transmission. In preparation to that, in addition to microscopy attempt was also made to serologically define malaria hotspots of malaria transmission across the communities in the pilot study sites. Unfortunately, the study could not be continued at the selected pilot sites because irruption of war between the government and rebel forces.

It was as part of this program the present study was designed to focus on Urban Malaria and find out what it would require to implement the elimination program in cities. Wau was selected as a future control spot hoping that it would be out of the war zone and would be possible to continue implementing the hot spot strategy and boost the effort of the country.

While there are many doctors and nurses committed and working in South Sudan health system, there is also a lack of highly skilled healthcare workers in Southern Sudan which contributed to the deteriorating condition of health situation in the country that hampers the efforts being exerted by the authorities to enhance the livelihood of the community (Tim W, 2012).

1.1. Rationale

Several country wide control programs have been conducted to control malaria incidence in South Sudan as is done in many developing countries. That is conducting control programs using all available means of controls: chemotherapy, pesticide application and utilization of insecticides-treated nets (ITNs)

However, the measures taken in one year had no influence on the prevalence/incidence of the next transmission season, suggesting that the parasite maintains its life cycle in susceptible individuals, groups or small communities located in hotspots in "non- transmission seasons", and later serve as fueling agents when the peak season comes thus ending up in the same old cycle. Whereas, Malaria transmission is heterogeneous in both distribution of malaria inoculations in a population and the susceptibility of humans to infection. Often a small proportion of people tend be infected frequently and serve as source of infection to e a large proportion of the population. This heterogeneity in human–mosquito contact and human susceptibility to malaria increases the tenacity of transmission. And if the control program is to continue the same way there is no way that malaria could rollback as targeted by WHO. Every time the rainy season comes and the population of the dominant vector increases the transmission is fuelled by the parasites that stay in the human or other reservoir hosts, thus repeating the same peak transmission cycles. To break this cycle a survey of the hot spots areas and susceptible individuals that maintain the transmission at low level during the non-transmission season need to be identified and treated so that during the rainy season there would not be any parasite for the dominant vector to rapidly spread the infection to a large number of people within a short period of time as an epidemic. But it could as well be that there is a high dose and infection frequency a wave of highly heterogeneous and strong local population structure might be overwhelming the immunity gained by the population. It is to these ends of stopping transmission of malaria, through effective control and management ecology of the disease the present study was organized with the following general and specific objectives.

2. OBJECTIVES

2.1. General objectives

To establish the parasite, vector, hotspot transmission foci and other risk factors.

2.2. Specific objectives :

i. Data will be collected during the wet and dry seasons from one randomly selected cluster of houses in the purposefully selected 4 study sites

ii. Blood smear of parasites will be prepared on pairs of slides from systematically selected households and fixed with 70% Ethanol, to be stained and examined under microscope in laboratories.

iii. RDT – finger pricked blood samples will also be taken from the same subjects smear was prepared and directly tested for the presence of *P. falciparum* parasite using RDT kit for the species.

iv. Entomological survey will be conducted across potential breeding sites using scoop nets and will be sorted at the site under binocular microscope and transported to Addis Ababa University Entomological laboratory for identification.

v. Retrospective data on number of people clinically suspected to have malaria infection and sent to the hospitals and clinic laboratories in the town/city.

vi. Repeated examination of the sample population for malaria at 2 months interval for dry season and once for wet season will be conducted.

vii. Follow up of patients with repeated malaria infection will be done throughout the study period.

viii. Mosquitoes will be collected by nocking mosquitoes using indoor spraying of insecticides on white sheet.

3. LITERATURE REVIEW

3.1. Malaria Situation in South Sudan

Malaria is a serious threat worldwide. In 2010, about 219 million malaria cases and 660,000 deaths were reported globally. The greatest toll was in sub-Saharan Africa where over 80% of cases and 90% of deaths occur (WHO, 2012). The huge burden is ascribed to the efficient Afro-tropical malaria vectors with strong vectorial capacities and high levels of transmission. Combined with Environmental factors and climatic changes, population movement, deteriorated socioeconomic situation, lack of access to effective anti-malaria treatment and use of traditional anti-malarial drugs, which are common features of developing countries, thus get worse (Sinka, 2012).

South Sudan have been through chronic liberation wars from the time of Sudan's independence from Anglo-Egyptian rule in 1956 (GoSS, 2006a). The war had destroyed literarily everything: the physical infrastructures, social setting and the health system as well. During the last phase of the conflict (1983–2005), international donors, non-governmental organizations (NGOs) and faith-based organizations (FBOs) assumed the responsibility for basic health service delivery and helped building nascent health institutions, (Downie, 2012). A growing body of evidences have demonstrates that rational malaria control and prevention significantly reduce illness and death thus contributing directly to the attainment of health-specific Millennium Development Goals (MDGs). However, in South Sudan, through 2012 malaria remained endemic in many areas of the country's 10 administrative states (RoSS, 2009a).

The malaria control situation in South Sudan was threatened by the impact of refugees, returnees, internally displaced populations, and natural disasters, like flooding. These facts put added strain on an already weakened system from years of conflict and destabilized whatever gains that have been made. Given the gross constraints to access and the austerity budget announced by the government, humanitarian need remains very high. It is estimated that out of a projected population of 11.1 million people, 40% o (4.5 million people) were in urgent need of humanitarian assistance, (UN, 2013). While South Sudan is in the post-conflict phase, some volatile states of the country were experiencing active conflict with armed hostilities and inter-communal violence persisting and displacing tens of thousands of people and continue to threaten development efforts and humanitarian aid by UN agencies, IOM and NGOs. The challenging operational environment of South Sudan continues to require emergency response and protection, increased support for livelihoods and resilience, and strong coordination, (UN, 2013).

Upon the signing of the Comprehensive Peace Agreement in 2005, a national malaria strategic plan 2006-2011was developed, (GoSS, 2006). One of the key objectives of the strategy was to “increase the population coverage with effective malaria prevention as part of an integrated vector control strategy that utilizes all approaches, including long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS) and larval source management (larviciding and environmental management) when and where most suitable and sustainable”. However, due to lack of specialized technical capacity, appropriate infrastructure and sustainable financing in the post conflict environment, to date vector control has largely consisted of promoting the use of LLINs distributed through periodic mass campaigns and via routine Antenatal Care (ANC) and Expanded Programme for Immunization (EPI) services to pregnant women and infants respectively (GoSS, 2008a).As a result of these efforts since 2008, more than seven million nets have been provided free-of-charge to people across the country (GoSS, 2008b). However, household ownership, and more importantly, the use of LLINs by vulnerable groups have remained lower than is required to provide vector control benefits of the intervention. The 2009 Malaria Indicator Survey found that 53% of households in South Sudan owned an ITN; 25% of children less than five years of age and 36% of pregnant women had slept under an ITN the night before the survey (RoSS, 2009b).

The Sudan Household and Health Survey (SHHS, 2010) found a lower household ownership of LLINs at only 34.2 percent (RoSS, 2010), while a more recent survey conducted by Net-Works/Malaria Consortium in Lainya County in April 2012, found higher household ownership of ITNs (66.3%). The use of the nets was still low with only 27% of children having used the nets, more recent observations (Chanda et al., 2013)

3.2. Malaria parasite and mosquito vectors

Malaria is caused by a single-celled parasite of the genus Plasmodium whose life cycle alternates between humans and mosquitoes (Fig: 1). More than 100 different species of Plasmodium are known to exist distributed in many types of animals and birds, as well as humans (Richard M, 2007). There are five species that infect humans. Each has a distinctive appearance under the microscope, and produces different pattern of symptoms. Two or more species can live in the same area and infect a single person at the same time. (NIAID, 2007).

Plasmodium falciparum is responsible for most malaria deaths, especially in Africa. The infection can develop suddenly and produce several life-threatening complications. With prompt, and effective treatment, however, it is almost always curable. It is the most widespread parasite in South Sudan.

Plasmodium vivax, geographically most widespread of the species, produces less severe symptoms but could relapses within 3 years post exposure and cause chronic and is debilitating disease. It once was common in temperate climates. Now found mostly in the tropics, especially in Asia.

Plasmodium malariae infections not only produce typical malaria symptoms but also can persist in the blood for decades, without ever producing symptoms. A person with asymptomatic P. *malariae*, however, can infect others, either through blood donation or mosquito bites. *P. malariae* has been wiped out from temperate climates, but it persists in Africa. *Plasmodium ovale* is rare, can cause relapses, and generally occurs in West Africa. And the recent fifth Plasmodium parasite that have been discovered is *P. knowlesi* which is a major cause of malaria in Malaysia, particularly in the island of Borneo. (White, 2008).

Plasmodium parasites are usually transmitted by female Anophline mosquito. Adult females of many mosquito species will bite humans to secure the blood they require for egg production. However, only about 60 species of the genus Anopheles can transmit malaria. Anophelines generally bite at night and usually rest on surfaces such as the wall of a house before or after feeding (Kathleen, 2002). As with all mosquitoes, the immature stages are aquatic, and they prefer slow-moving or still water in which they can stay close to the water surface with their breathing orifices open to the air. Unlike some other mosquito genera, Anophelines require relatively clean water for larval development, which is why malaria transmission frequently declines with urbanization and concomitant water pollution.

The country is characterized by its two, (dry and rainy) seasons. The former is a period the parasites are maintained in the population in limited number (<20%) of highly susceptible individuals, yet transmission and incidence of malaria are relatively low because of the vectors capacity and frequency of engagement in transmission. Hence from December through April if there are cases mostly are asymptomatic. Interventions at this stage are expected to be cost and efficiency wise. This has been confirmed by clinical, parasitological and serological markers of malaria transmission studies (Kangoye *et al*, 2016). This means that the parasites during dry season are real targets that need to be considered in order to break transmission and go forward for elimination. Without them, the rainy season peak transmissions could not be effected. It is with this assumption that the present research work was designed to serve as a pilot study to words the control and eradication of malaria in the country i.e. To serve as a start-up point for decision makers to plan on how to eradicate malaria rather than trying to control it in peak rainy seasons.

Malaria Life Cycle

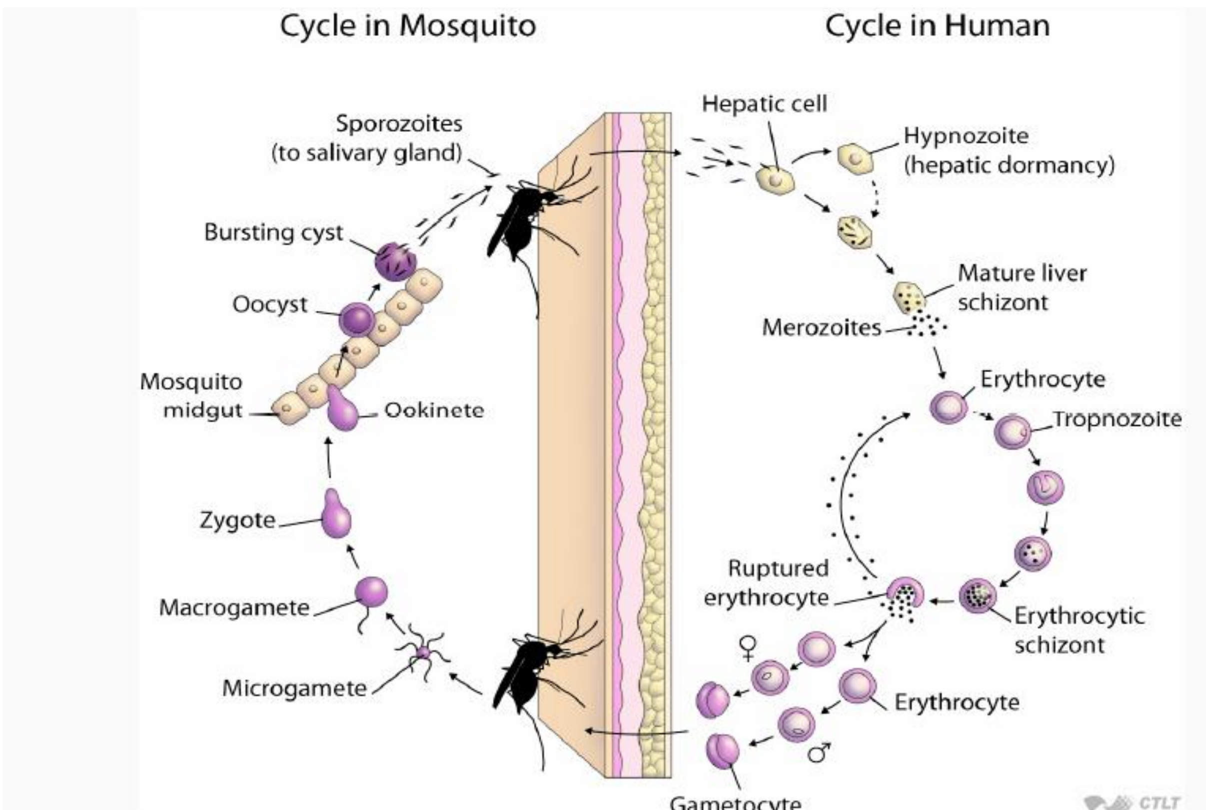

Figure 1. The life cycle of the malaria parasite (*Richard Morrow: Epidemiology and Control of Malaria- Johns Hopkins University*)

3.3. Anophelines species

The link between human and mosquito vector in the parasite life cycle is the blood meals that is needed by female mosquito for egg production. Humidity and higher temperatures contributed to the accelerated growth of parasite within the mosquito that helps the successful development of the malaria parasite in the mosquito (from the "gametocyte" stage to the "sporozoite" stage)

(CDC). And whether the Anopheles survives long enough to allow the parasite to complete its cycle in the mosquito host ("sporogonic" or "extrinsic" cycle, duration 10 to 18 days). Apart from the human host, the mosquito host does not suffer noticeably from the presence of the parasites.

Anophelines mosquitoes are found across the globe except Antarctica, different species can transmit malaria depending on the region and the environment. Those malaria are found not only in malaria-endemic areas, but also in areas where malaria have been eliminated, which make these areas to be at constant risk of re-introduction of the disease.

Like all other mosquitoes, Anophelines undergo four stages in their life cycle: egg, larva, pupa, and adult. Most of these stages are aquatic for 5-14 days except the adult, depending on the species and the temperature. Whereas, the adult stage play the role of the vector that transmit malaria when the female Anopheles mosquito is in the process of laying the eggs. The adult females can live up to a month (or more in captivity) but most probably do not live more than 1-2 weeks in nature. Adult females can lay 50-200 eggs per ovi position. Eggs are laid directly on water and are unique because they can floats on either sides. They are not resistant to drying and can hatch within 2-3 days, hatching may sometimes take up to 2-3 weeks in colder climates.

Mosquito larvae head is equipped with mouth brushes used for feeding, a large thorax, and a segmented abdomen, Lack locomotive organs. Unlike other mosquitoes, Anopheles larvae lack a respiratory siphon and for this reason position themselves so that their body is parallel to the surface of the water, Larvae breathe through spiracles located on the 8th abdominal segment and therefore must come to the surface frequently (CDC). They stay feeds most of their times on some algae, bacteria, and other microorganisms in the surface of the water. When disturbed, dive below the surface, they swim either by jerky movements of the entire body or through propulsion with the mouth brushes. Their development occur in 4 stages, instars. The metamorphose to pupae, at the end of each instar, the larvae molt, taking their exoskeleton, or skin to allow for further growth.

Larvae of *Anopheles gambiae*, the major malaria vector in Africa, can breed in diverse habitats Such as, tire tracks, rice fields and irrigation water.

The larvae occur in a wide range of habitats but clean, unpolluted water. Those of Anopheles mosquitoes are found in fresh- or salt-water marshes, rice fields, and vegetated ditches, the edges of streams and rivers, and small, temporary rain pools. Most species prefer vegetated habitats, others may not. Some breed in open, sun-lit pools while others are found only in shaded breeding sites in forests. Some species rarely breed in tree holes or the leaf axils of some plants (CDC).

The pupa is a comma-shaped that have head and thorax merged into a cephalothorax while the abdomen is curved. As with the larvae, pupae always come to the surface to breathe, which they do through a pair of respiratory trumpets on the cephalothorax. After a few days as a pupa, the dorsal surface of the cephalothorax splits and the adult mosquito comes out.

The duration of development from egg to adult is dependent on the temperature variations. Varies considerably among species and is strongly influenced by ambient temperature. Mosquitoes can develop from egg to adult in as little as 5 days but usually take 10-14 days in tropical conditions.

Figure 2. Pupa of the African malaria mosquito, *Anopheles gambiae* Giles. Photograph by Ray Wilson. (*Sabrina and Philip, 2014*)

Adult Anophelines have slender bodies with 3 sections: head, thorax and abdomen. The head is specialized for acquiring sensory information and for feeding. It contains the eyes and a pair of long, many-segmented antennae that are used detect the host odors as well as odors of breeding sites where females lay eggs. The head also has an elongate, forward-projecting proboscis used for feeding, and two sensory palps.

The thorax is responsible for locomotion. Three pairs of legs and a pair of wings are attached to the thorax. The abdomen is specialized for food digestion and egg development. It expands considerably when a female takes a blood meal. The blood that is digested over time serving as a source of protein for the production of eggs, which gradually fill the abdomen.

Anopheles mosquitoes can be distinct from other mosquitoes by having palps, which are as long as the proboscis, and presence of discrete blocks of black and white scales on the wings. Adult Anopheles can also be identified by their typical resting position: males and females rest with their abdomens sticking up in the air rather than parallel to the surface on which they are resting.

Adult mosquitoes usually mate within a few days after emerging from the pupal stage. In most species, the males form large swarms, usually around dusk, and the females fly into the swarms to mate. Males feeding on nectar and other sources of sugar, can live for about a week. Females could also feed on sugar sources for energy but usually require a blood meal for the development of eggs. After obtaining a full blood meal, the female rest for a few days while the blood is digested and eggs are developed. This process depends on the temperature but usually takes 2-3 days in tropical conditions. Once the eggs are fully developed, the female lays them and start seeking another.

The cycle repeats itself until the female dies. Females can survive up to a month (or longer in captivity) but most probably do not live longer than 1-2 weeks in nature. Their survival depend on temperature and humidity, but also their ability to successfully obtain a blood meal while avoiding host defenses (CDC, 2014).

3.4. Transmission and control

Transmission of malaria depends on some factors that affect mainly the successful completion of the parasite life cycle in vector hosts. They differ from place to places. There are areas where the transmission and prevalence of the disease is continuous at low levels and in others it lasts only for several months in a year. Such factors include, different climatic and non-climatic factors favoring transmission: temperature, rainfall and relative humidity are the main climatic variables that influence parasites life cycle of the parasites in the vector hosts (Pavan and Reddy, 2014). Non-climatic factors include parasites, vectors, activities related to human hosts: population movement or migration, urbanization and interruption of control and preventive measures.

Understanding these factors and biology and behavior of Anopheles mosquitoes can contribute in understanding of how malaria is transmitted. That in turn can help in designing appropriate control strategies. Factors that affect mosquitos' ability to transmit malaria include its innate susceptibility to Plasmodium, its host choice, and its longevity. These are factors that should be taken into account when designing a control programs among the susceptibility of malaria vectors to insecticides and the preferred feeding and resting location of adult mosquitoes.

3.5. Anopheles mosquito habitat and capacity

Anopheles gambiae Giles is the most efficient vector of human malaria in the Afrotropical Region. Thus, it is commonly called the African malaria mosquito. Members of this are found throughout tropical Africa, south of the Sahara desert, with *Anopheles arabiensis* reaching southern Arabia. *Anopheles gambiae s.s*. is distributed throughout sub-Saharan Africa, including Madagascar (CDC 2010). Both *Anopheles gambiae* and *An. Pretoriensis* are not seasonal and breed in permanent freshwater swamps and vegetated streams as such, their densities remain relatively stable throughout the year and continue to transmit the parasite to the communities living near these swampy and vegetated areas (Govere *et al*., 2009).

Anopheles nili is a widespread efficient vector of human malaria parasites in the humid savannas and forested areas of sub-Saharan Africa. It is the nominal taxon of a group of closely related species including *An. Nili sensu stricto*, *Anopheles somalicus*, *Anopheles carnevalei* and *Anopheles ovengensis*. The members of this group can be distinguished through slight morphologic diagnostic characters observable at the larval and/or adult stages. Of these four species, *An. nili s.s*. is the most important malaria vector although *An. Carnevalei* and *An. ovengensis* have been found infected with *Plasmodium falciparum* in natural conditions. *Anopheles somalicus* is mainly zoophilic and is not involved in human malaria transmission. Infection rates reaching 3% have been observed in *An. nili* and the species was shown to sustain entomological inoculation rates over 200 infected bites per man per year in villages close to fast running streams and rivers where its larvae develop.

Recent investigations of the ecological requirements of *An. nili* in Cameroon, a country in Central Africa at the core of the species range, showed that lotic rivers exposed to sunlight, with vegetation or debris were the best predictors of *An. nili* larval abundance and that habitats characterized by high water vapor pressure and rainfall, as typically observed in forest- savanna transition areas were of highest quality for the development of the species. Anopheles nili however is scarce in deep forest environments, where it is replaced by other members of the group, namely *An. Carnevalei* and *An. ovengensis*. The strong reliance of *An. nili* on permanent aquatic habitats for larval development suggests a patchy geographic distribution throughout the species' range, owing to the discontinuous nature of the hydrographic networks (Ndo *et al*., 2010).
Anopheles pharoensis occur in abundance during rainy season in many parts of Ethiopia, while it can occur together with *An. arabiensis* and *An. Coustani* in the southwest of Ethiopia.
Although both *Anopheles arabiensis* Patton and *An. marshalli* (Theobald) preferred bovine blood meal over humans regardless of higher human population sizes, but *Anopheles marshalli* usually dwell in vegetated environment and can be found in thatched houses(Massebo et al., 2015).
Although there is enormous data pertaining to the habitat, ecological niche of many anopheles species that contribute to the huge malaria transmission to the human being, but unfortunately both *anopheles rhodensiensis* and *anopheles obscurus* are of uncertain habitat.

3.6. Malaria Vaccine

As the malaria exert great threat on human life, efforts have been made to combat the disease, such as development of vaccines against the parasite as a cheap means of combating the disease. Three main strategies were adopted for developing malaria vaccines:

1. The first that would neutralize the sporozoites on entry to the circulatory system and the liver tissues, this vaccine has the potential of preventing infection completely.
2. The second one targets the blood stages of the parasite. It limits the invasion of erythrocytes and produce mild disease but would not prevent infection.

3. The scares attenuated sporozoite or immunodominant repetitive epitopes or
Circumsporozoite (CS) proteins that are capable of protecting high doses of challenges from intact viable sporozoites infections but the lesser immunogenic proteins among the vaccines

i.e.) targets to block the development of the sexual stages of the parasite in mosquito vectors by inducing prophylactic antibodies in the definitive host and inhibiting the normal development of sporogony neutralizing them with antibodies while they are within the definitive hosts. It is capable of infecting a host during a blood meal. Those directed against the sporozoite stage of the parasite are said to be best. Immune response produced by attenuated sporozoites, immunodominant repetitive epitopes. Unfortunately, not everyone is capable of raising high antibody levels against the CS protein because it is genetically restricted (Patrick, 2007).

3.7. Challenges facing Malaria Vaccine production

As the malaria become a major public health threat required a persistent search for a vaccine that could save the world. It is believed that the ideal vaccine would be cheap, extremely safe, induce life-long protection, be active against all strains of the parasite and result in nearly complete interruption of the malaria life cycle by vaccine-induced immune responses. While promising results have been obtained, particularly with subunit vaccines, progress toward a malaria vaccine has been slow, due to extensive genetic diversity in candidate vaccine antigens. As with other genetically diverse pathogens such as the influenza virus and HIV, vaccines based on polymorphic malaria proteins may not elicit responses against all variants of the target antigen circulating in the parasite population and could rather lead to an increased frequency of variants not targeted by the vaccine (Zazi, 2008).

In subunit vaccination, partial or complete antigens are identified from a pathogen's proteomic complement and used to induce protective immunity to the whole pathogen on vaccination. The approaches being followed for malaria subunit vaccine development are aimed by means of vectored, recombinant or synthetic fragments to induce anti-parasite effects against the different phases of the parasites life cycle.

The parasite life cycle is composed of a sexual stage inside the mosquito and an asexual stage inside the human host (Sherman, 1998). Firstly, a mosquito infected with the malaria parasite bites a human cell and passes cells called sporozoites into the human's bloodstream where they travel to the liver and undergo asexual reproduction; each nucleus splits to form two new cells, called merozoites that enter the bloodstream and infect red blood cells (RBCs). While in RBCs, merozoites grow and divide to produce more merozoites, causing the RBCs to rupture. Some of the newly released merozoites go on to infect other RBCs. Some merozoites develop into sex cells known as male and female gametocytes. Another mosquito bites the infected human, ingesting the gametocytes. In the mosquito, the gametocytes mature and male and female gametocytes undergo sexual reproduction, uniting to form a zygote. The zygote multiplies to form sporozoites, which travel to the mosquito's salivary glands. If this mosquito bites another human, the cycle begins again.

Sporozoites in transit from the site of mosquito inoculation to the liver are targeted by pre-erythrocytic vaccines and their effect is to prevent infection and impact clinical disease (Good, 2005). Merozoites leaving infected liver cells then which travel between ruptured liver cells and RBCs, or between a ruptured RBC and a fresh one are targeted by blood stage vaccines whose effect is to lessen the disease. Whole infected RBCs or parasites ingested by the mosquito vector are targeted by mosquito–stage vaccines. A further blood-stage subunit strategy is to induce antibodies to neutralize parasite toxins (Good, 2005).

Malaria vaccine development is an active research area with enormous challenges. As the parasite proceeds from a sporozoite through the liver stage to the replicating cycle of the blood stage, it undergoes morphological changes and displays antigenic variations. This allows the parasite to evade the protective immune responses of the host. As a result, the acquisition of long-term sterile immunity, which is often associated with recovery from many other infectious diseases, is not observed in malaria-infected individuals. Despite these difficulties, the most convincing evidence that vaccination against malaria is feasible has come from experimental studies in rodents, monkeys and human subjects in which attenuated sporozoites induced sterile protective immunity (Arama & Troy-Blomberg, 2014).

3.8. Malaria Parasite Drugs Resistance

Antimalarial drug resistance has become one of the most challenges that facing malaria control nowadays. It has been blamed for the spread of malaria to new areas and re-emergence of the illness in areas where the disease had been eradicated (Peter B, 2001). Antimalarial drug resistance can be defined as: "ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject".

There are many antimalarial drugs used for malaria treatment. Chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) are the frequently used in Africa either as first or second-line drugs for the treatment of *P. falciparum* malaria. CQ is a 4-aminoquinoline while SP is a fixed-dose combination of two antifolate compounds. Both are blood schizontocidal drugs active against *P. falciparum* with no cross-resistance. Today, in Africa resistant *P. falciparum* strains to CQ and SP are increasingly spread and intensified since they were first documented in 1979, particularly in East Africa where they have been used on a larger scale as first-line drugs (Geyer, 2001). More recently they are also detected in West Africa. As a counter measure to these trends WHO has put in place a strategy which has the following four key elements (Pascal, 2009):

1. Parallel prevention programs be run on the emergence of antimalarial drug resistance
2. Monitoring antimalarial drug efficacy and change drugs as early as possible
3. Ensuring a continuous pipeline of new antimalarial medicines
4. Containing the spread of antimalarial drug resistance once it has emerged.

With this, the WHO is trying to at least reduce drug resistance and putting the basis of malaria eradication.

4. MATERIALS AND METHODS

4.1. Study area

4.1.1. Geographical location of Wau Town/city

Wau is one of the three counties that constitute the Western Bahr el Ghazal state (Fig. 3). It is a city in north western of South Sudan, on the western bank of the Jur River, in Wau County, Western Bahr el Ghazal State. It lies approximately 650 kilometers (440 mi) North West of Juba, the capital and largest city in the country. It is located between 7° North and 28° East and elevation of 433 m (Emmanuel, 2013). With an estimated population of 151320 people (SSCCSE, 2008). It serves as the capital of Western Bahr el Ghazal State, one of the ten (10) states which constitutes the republic of South Sudan.

The city is a culturally, ethnically, and linguistically diverse center. Its residents include peoples of Fertit, Dinka, Luo, and Arab ethnicity. It is the terminus of a narrow gauge branch line of the Sudan Railways. A plan exists, as of 2008, to open a standard gauge line north from Gulu in Uganda to Wau. Through trains from Khartoum to Mombasa would be possible only if one of the lines was regauged. It has Wau Airport, which has a single paved runway which measures 2500 meters in length. The city hosts University of Bahr El-Ghazal and many secondary and primary schools, The Catholic University of South Sudan maintain a campus in the city, a soccer stadium in the middle of town, the Cathedral which is known as the largest of former Sudan.

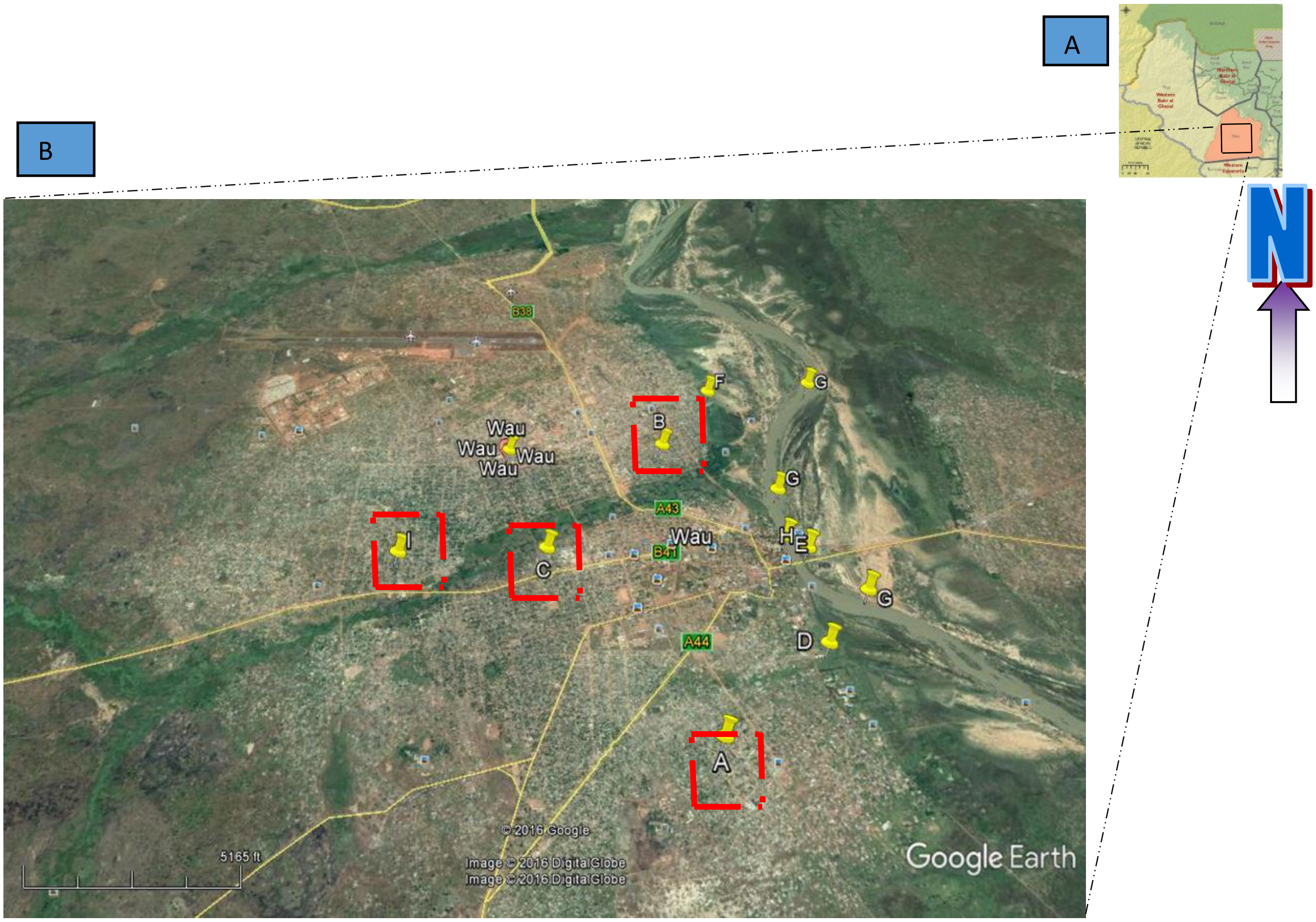

Figure 3. (**A**)Map of Western Bahr El Ghazal showing Wau Town. (*IOM, Village Assessment Survey County Profile 2013*), Image (**B**) Satellite image of the proposed data collection sites (*Google Earth image*) **A**: Hai Nazareth, **B**: Hai Zande, **C**: Hai Keresh, and **I**: Hai Lokloko residential areas, these sites were purposely selected for cross-sectional survey for the parasites.

4.1.2. Climate of Wau Town

Wau has two seasons: a dry season from November to March, and a rainy season the rest of the year. It has a humid temperate climate, the mean relative humidity varies from 26% to 77%. The warmest months are between January and May with maximum average temperature of 43.5° C, while the lower temperature is in the 38.5° C in June and November. The seasonal Jur River flow in the eastern part of the town from South to north, also there is a small stream that pass through the town dividing it into northern part and southern part along the stream found condense Mango trees that are inhibited by bats and other birds.

4.2. Demographic origin of the people

The table (Table 1) below shows the demography of the surveyed population in the study site, most of the population are originally permanent residences of Wau (62.5%) while others comes from different areas of South Sudan with those from Yirol as the list (1.0%)

Table 1. Demography of the human subject that participated in the study 2016

S/No.	State of Origin	No. of Participants	Percentage
1.	Wau	60	62.5 %
2.	Abyei	18	18.8 %
3.	Tonj	05	5.2 %
4.	Twic	06	6.3 %
5.	Gogrial	02	2.1 %
6.	Jubek	02	2.1 %
7.	Upper Nile	02	2.1 %
8.	Yirol	01	1.0 %
	Total	96	100

4.3. Parasitological Study

4.3.1. Sample size determination

Sample size for prevalence study of malaria in Wau town will be estimated on the basis of the earlier finding on malaria incidence in the area and the population size of this study in accordance to the following formula: $\mathbf{N = (z/d)^2\, p\, (1- p)}$

Where N = number of samples
P = the expected failure of participants to turn up for the study (6 %)
Z = confidence interval (95%)
d = precision (5%)

Therefore, $N = (1.96/0.05)^2\ 0.06\ (1 – 0.06)$

$= (39.2)^2 \text{ X } 0.06 \text{ X}0.94$

$= 85.7844 = 85.78$

In addition, 10% of the above outcome calculation will be added as for the failure of participants to turn up during the study period.

4.3.2. Retrospective Data on Malaria positive subjects

Data on all malaria infected subjects were obtained from the records of clinical suspects sent to hospitals' and health clinics' laboratories in outpatient departments for diagnosis. Those that were found to be smear positive during the one year study period between 2015 and 2016. The collected positive cases were categorized by age and sex into three age groups accordingly. i.e. 0 – 5 years, 6 – 15 years, and above 15 years old.

4.3.3. Malaria parasite prevalence studies

4.3.3.1. Blood Sample collection

Among the four residential areas, Hai Nazareth, Hai Keresh in the South, Hai Lokloko to the west, and Hai Zande in the Northern part of Wau, 4 study sites fairly representing the population in the area were purposefully selected. In each study site cluster of households were identified. Thin and thick blood smear samples were prepared from a drop of blood taken from the third and fourth fingers of all members of the selected households in the clustered houses and stained with Giemsa for malaria parasites examination by Microscopy. The same subjects were also examined by serologic method using Rapid Diagnosis Test kit (Photo No 4). The selection of the households was done randomly by picking every third household in the series of houses. That is the house Number three (3) was selected for survey followed by house Number Six (6), Number Nine (9), etc. to ensure representation of the area. Every head of the family (Father or Mother) in the household was asked for his/her consent and only upon voluntary acceptance to participate they were included otherwise the next house hold was considered. For those who don't write and read the consent paper was read to them and additional explanation was given following their request.

The households were selected based on the criteria that they fall within the randomly selected study site, acceptance of the head of the house to participate in the study, and all members of the household were taken to the study regardless of being with symptoms or asymptomatic with the exception that infant less than one year are excluded. Before the test samples were taken, they were asked to sign the consent sheet which was presented to them.

For serological tests, blood sample of (1-3) drops was withdrawn from the peripheral blood by finger puncture made after sterilizing the same fingers with 70% alcohol from the other hand using ICT kits (Rapid Diagnosis Test, Standard Diagnostics, Inc. Giheung-gu, Republic of Korea). The kit test was carried out to determine whether the participants were malaria positive at the time of examination or not. All malaria positive participants were advised to go tothe nearest hospital for treatment. Where the participants were miners, health assistant were assigned to see to the effect that they are treated. After these processes were completed the last question was to ask the household heads if there was/were any member of the family that had malaria prior to our visit or developed malaria symptoms.

4.3.3.2. Microscopic examination and parasite identification

The slides were taken to the Lab and fixed using 70% alcohol and stained with Giemsa and examined under microscope together with a senior laboratory technician and put in the slides boxes to be transported to Addis Ababa University for further confirmation. Prior to the survey, the ethanol and giemsa were check in a pre-test to optimize them for the best results. At the same time the investigators carried with them a safety box to contain disposed materials in order prevent any uncertainty use of these materials by the children and community in the study site.

4.4. Entomological Survey

4.4.1. Adult Mosquito Collection

A team of three persons including the principal investigator conducted the collection of mosquito vectors in “Hai Dinka” one of the randomly selected areas for the purposes of the vector collection, which is an area located along western bank of Jur River. It was at a distance of about 400 – 500 m from the River. The river bank is also used for making commercial bricks. The place, the workers use for bricks making when they leave to work in other areas it forms holes and ponds that could be very good breeding sites for mosquitoes, when the rain falls was purposely selected for sample collection for larval collection as well as the adult mosquitoes in the nearby houses mentioned above (Hai Dinka residential).

The type of houses in the clusters were of different types. Some are made of red bricks and their roofs were covered by tin sheets i.e. were relatively well constructed with windows and doors that could be protective to mosquitoes if closed and opened in good times. Five houses were picked randomly at the center of clusters were chosen for mosquito collection using spread sheet and insecticide sprays to knock the insect in the houses.

Collected mosquitoes were kept in small cups and then sorted and transported to the l laboratory before at Addis Ababa University Laboratory where they were identified into different species under binocular Microscope using Ethiopian Ministry of Public Health, Malaria Eradication Service ANOPHILINE IDENTIFICATION KEYS together with the experts in identifications (Reprinted from *Mosquito News*, Vol. 22. 1, March, 1962).

4.4.2. Collection of mosquito larvae

Larvae were collected along the banks of Jur River which flows around from South to North all the way just on the eastern part of Molem Hospital at the Southern part of the Wau town. Equipment that were used are dippers to filter larvae and a plastic cups to temporarily clean them with ethanol and sort them carefully under the binocular microscope with needles and forceps and transport them each in separate epinddorf tubes to the laboratory.

Photo No 1: Larval collection near Jur River Bridge and along River Jur Bank.

4.5. Equipment and materials used in collection and processing of adult mosquito and larva

The following equipment and materials were used in processing adult mosquito and larva: Binocular microscope, forceps, needle, Samsung GALAXY Tab4 (Photo No 2), Ethanol, and Epinddorf tubes. Both adult mosquito, larva and pupae were treated with ethanol and kept in the tubes before being transported to Addis Ababa University Insect vectors Entomopathogen Laboratory to be identified. The forceps and needles were used to handle the mosquito and larva prior to their identification under binocular microscope using the "ANOPHELINE IDENTIFICATION KEYS" of the Ethiopian Ministry of Public Health, MALARIA ERADICATION SERVICE (*Mosquito News*, Vol. 22, No. 1, March, 1962). GALAXY Tab4 was used to take picture of Mosquito under the binocular microscope.

4.6. Data Analysis

The data collected were computerized and analyzed using Excel Program (Microsoft, USA). Statistical analysis was performed using SPSS program version 22. The data that have been analyzed include the retrospective data of all malaria cases in the health facilities for the year of 2015, and sample collected from human subject during the actual study for population of 96 participants.

4.7. Ethical Clearance

This research paper received approval from the college of Natural Science Institutional Review Board (CNS- IRB) in its meeting held on 10/04/2016 minute No. IRB/021/2016. And approval from the State Ministry of Health, Western Bahr El Ghazal and the informed written consent was taken from all study participants and caregivers/ guardians of all children under 18 at Wau study site.

5. RESULTS:

5.1. Parasitological Survey

5.1.1. Retrospective Data from hospitals and clinics.

Clinically malaria suspected cases sent for laboratory diagnosis /confirmation in 2015 were collected from the three majorhospitals (Wau Teaching Hospital, Sikka Hadid Hospital, and St. Daniel Comboni Catholic Church Hospital) in the town and analyzed using (Microsoft Excel 2013, USA) to draw the graph below. Malaria suspects were found to be more prevalent in the rainy season boldly peaking in number suspected until just after the rain, from June through November especially among ages above 15 years. (Figure 4)

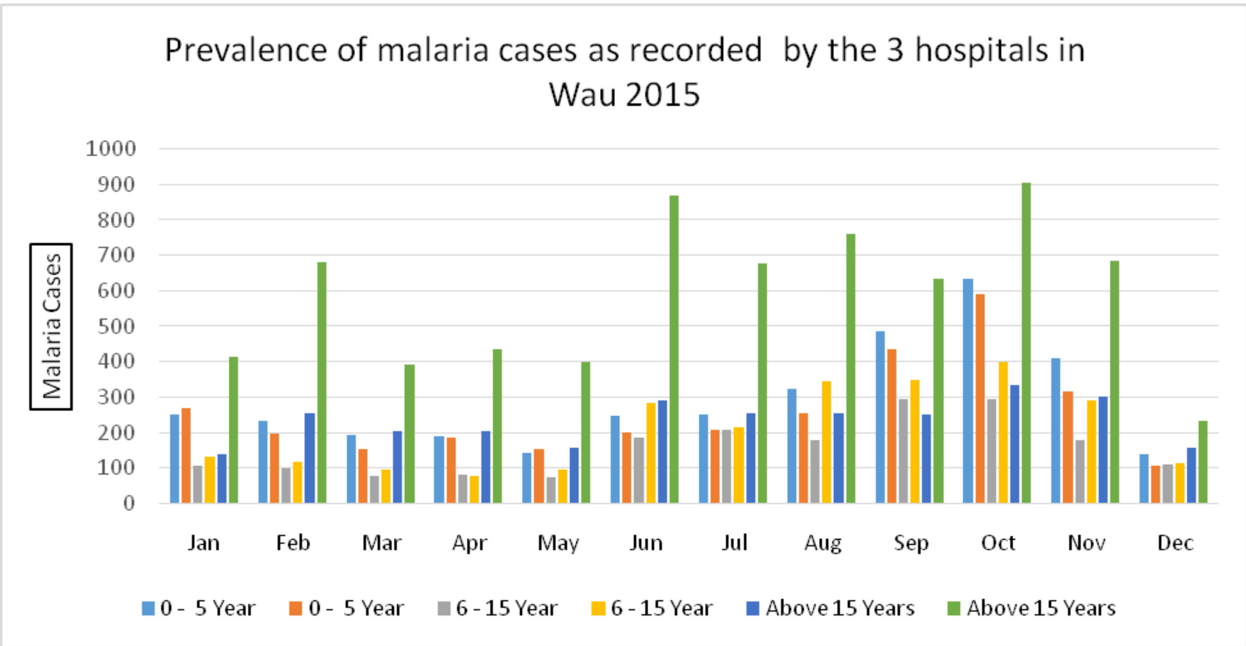

Figure 4. Prevalence of malaria suspected cases sent to health services as recorded by the 3 hospitals in Wau town in 2015

Classified by age and sex groups. It shows that the females above 15 years were the highest in all months of the years. The increase in numbers started in June and continued till November. All ages and sex groups including those in 0 – 5 age groups increased with a maximum between August – November.

Malaria prevalence among different age groups (0 -5, 6 -15, and above 15) as recorded in Sikka Hadid Hospital in 2015 (Figure 5). It was there all throughout the year. The highest number was in age groups above 15 years, with the maximum number in the rainy season as shown in (Figure. 5).

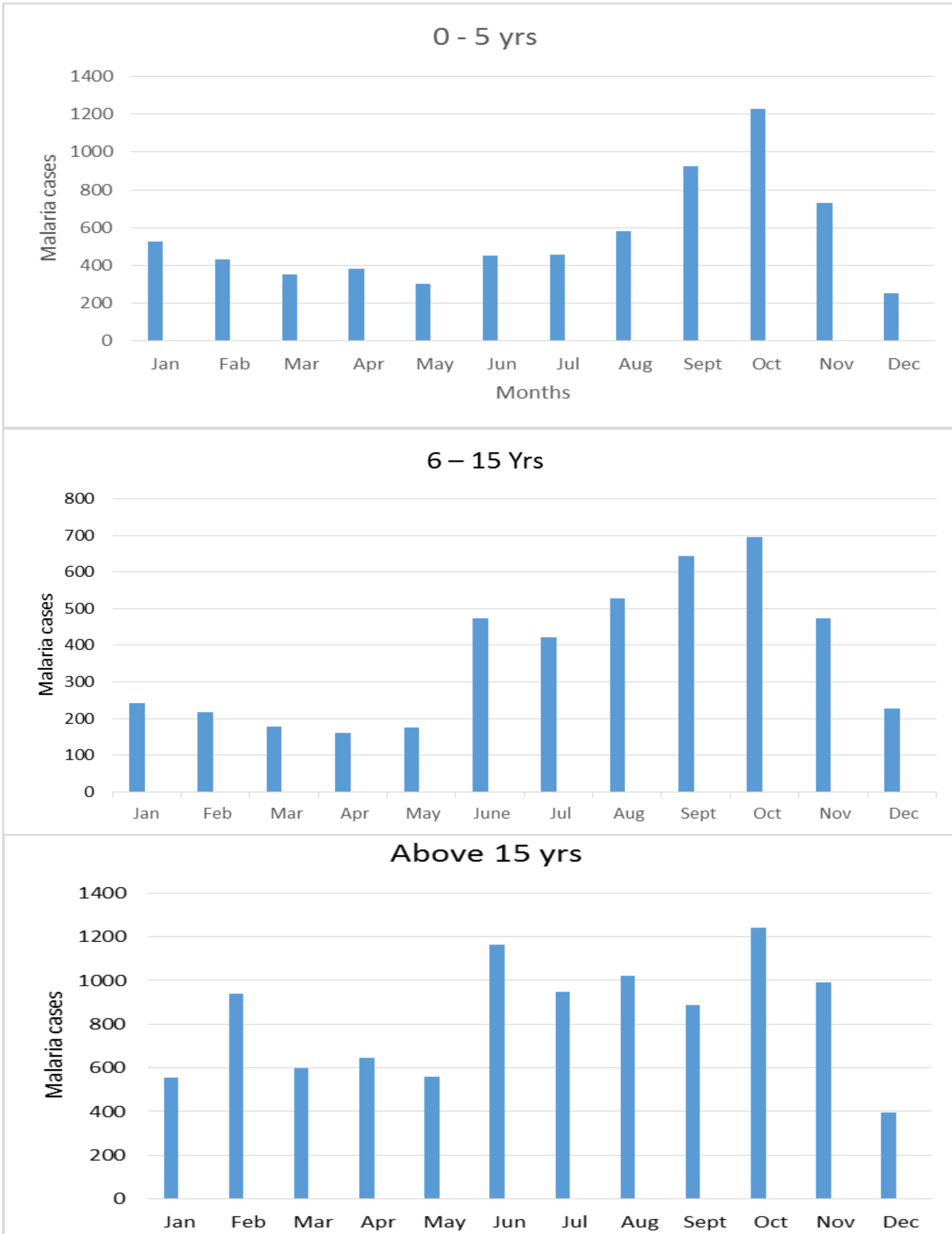

Figure 5. Malaria prevalence among different age groups (0 -5, 6 -15, and above 15) as recorded in Sikka Hadid Hospital in 2015.

Relatively organized document, these figures shows the maximum transmission was from June to November, but shows rebounce in January–February. The age groups above 15 years were experiencing relatively high number of infection (Figure 5).

Malaria prevailed among different age groups with higher prevalence among population of above 15 years old especially in the rainy season as shown in the records of Sikka Hadid Hospital in 2015 (Figure 6).

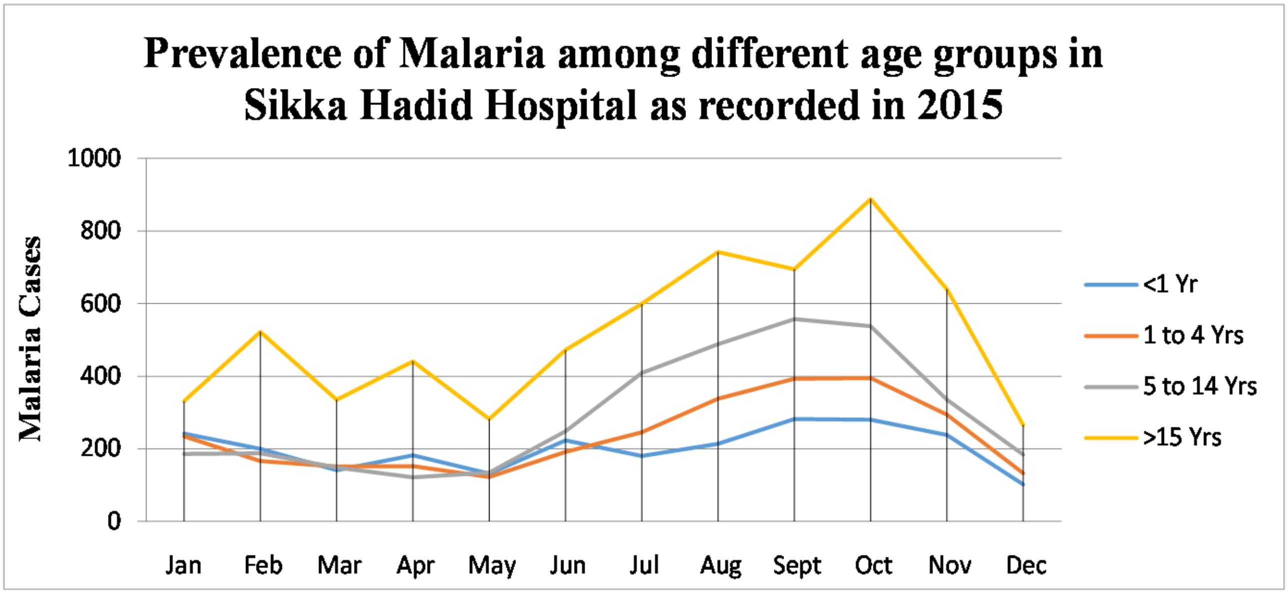

Figure 6. Prevalence of malaria among different age groups in Sikka Hadid Hospital as recorded in 2015.

Malaria is the most rampant disease in the town as it is for the whole country, as for Sikka Hadid records in 2015, malaria was with higher percentage against the other illness put together as shown in the table below.

Table 2. Percentage of malaria against other diseases in Sikka Hadid hospital in 2015

	Prevalence	**Percentage**
Malaria	14978	47.51%
Other diseases	16549	52.49%
Total	**31527**	

Distribution of malaria cases among different age groups shows that above 15 years group suffered the most with 41.5% as it can be seen in the (Table No. 3) in Sikka Hadid Hospital alone.

Table 3. Malaria prevalence in percent as seen from the records of Sikka Hadid Hospital in 2015 among different age groups

	<1 Yr	**1 to5 Yrs**	**6 to 10 Yrs**	**15 Yrs <**	**Total**
Total	2416	2813	3537	6212	14978
%	(16.1%)	(18.8%)	(23.6%)	(41.5%)	100%

Malaria is increasing with the increment of both meteorological factors in Wau town as this can be seen in (Figure 7) as per the records of 2015.

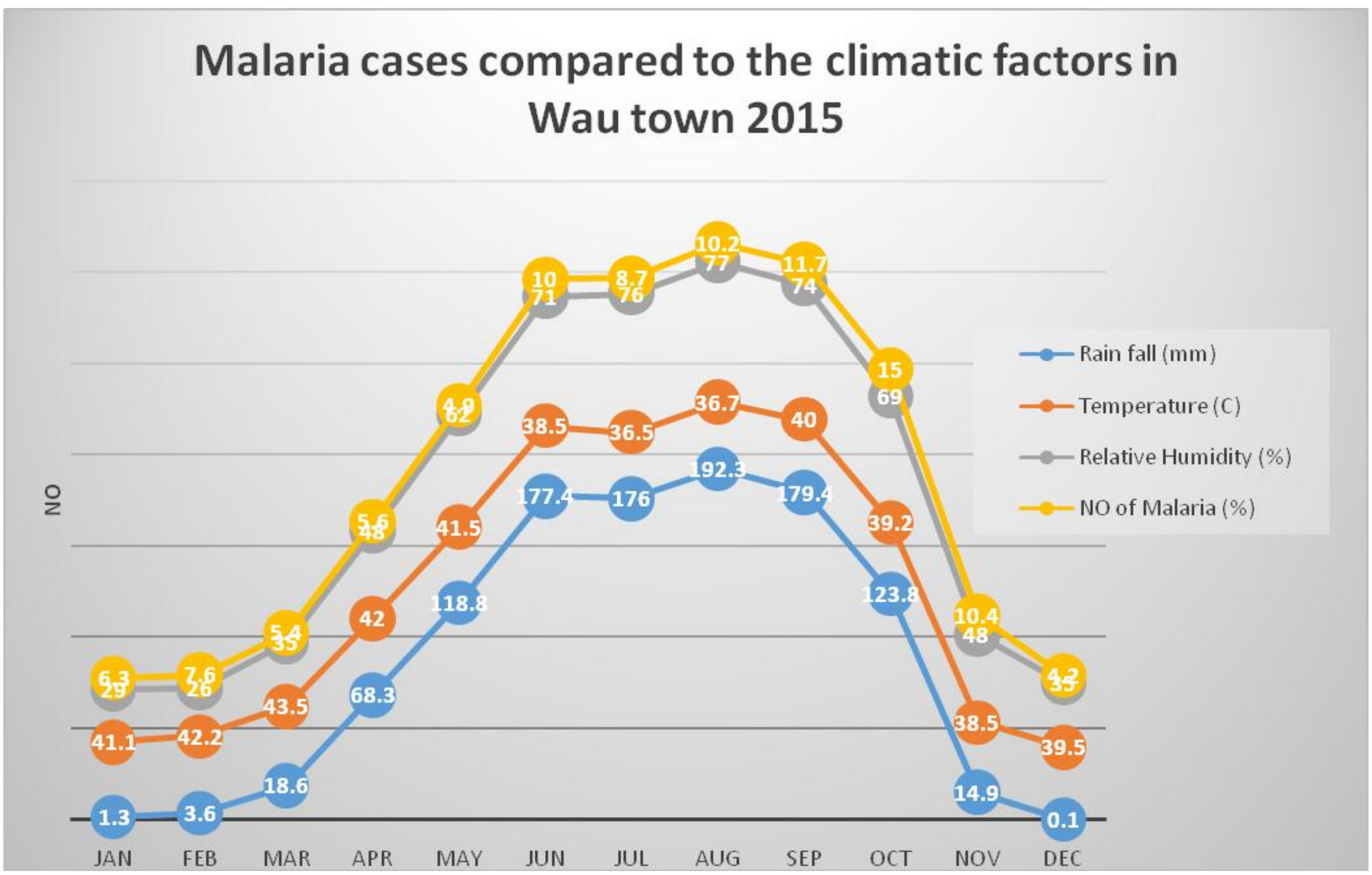

Figure 7. Malaria prevalence against climatic factors in Wau town 2015

5.1.2. Prevalence of Malaria Parasites in May - June 2016.

The human subjects that have been surveyed for malaria prevalence between May – June 2015 in a selected representative residential area in Wau town shows that female dominated the population with 59% while the rest are males (Figure 8).

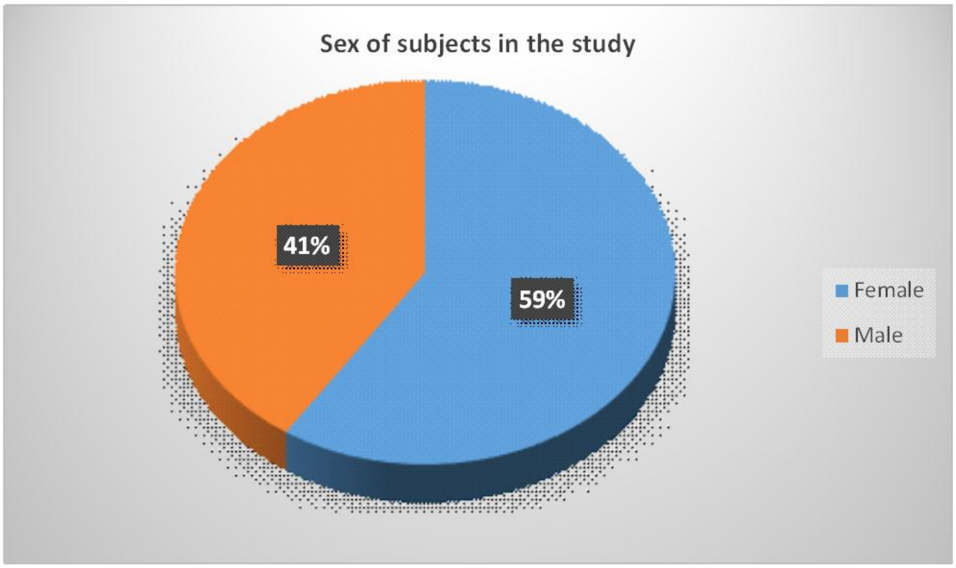

Figure 8. Sex ration of the surveyed human subject in Wau town in May – June malaria survey

The prevalence of malaria was more significance among those above 15 years in both male and female with over 40% while insignificance in children as shown in (Figure 9).

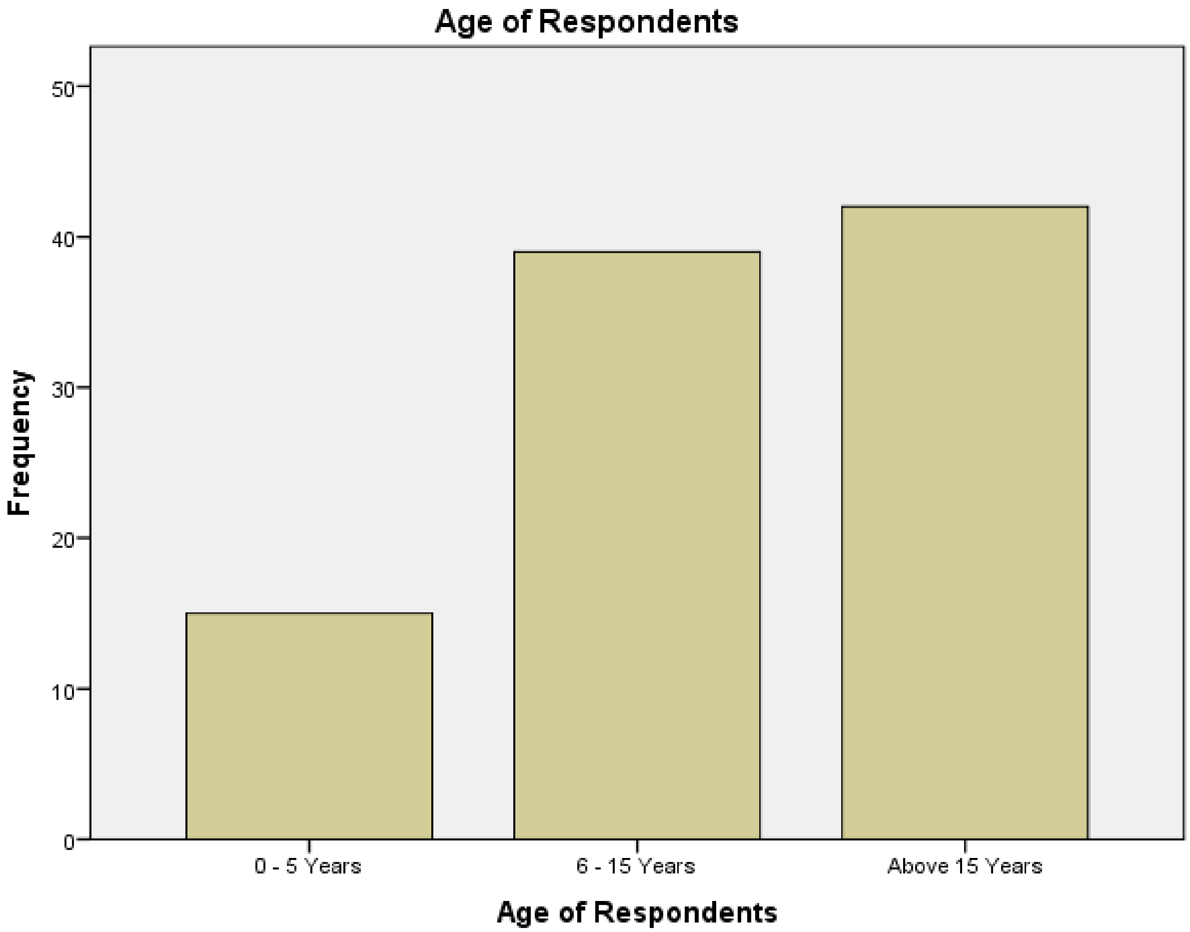

Figure 9. Incidence of malaria on age groups that have been surveyed in Wau Town (May – June 2016)

Plasmodium falciparum was found to be the major causative agent of malaria disease in the town as it is shown in the table below (Table 4) with 28% malaria positive among those surveyed in May – June 2016 in Hai Nazareth residential area alone.

Table 4. Prevalence of *P. falciparum* in Hai Nazareth residential area

		Age groups Malaria examined subjects			
		0 - 5 Yrs	6 - 15 Yrs	15< Yrs	Total
BFF	Negative	10 (10.42%)	24(25%)	35(36.5%)	69 (72 %)
M	Positive	5 (5.2%)	15(15.6%)	7(7.3%)	27 (28 %)
Total		15(15.6%)	39(40.6%)	42(43.8%)	96(100%)

5.2. Entomological Survey

5.2.1. Adult Mosquito

The specimens were taken to Addis Ababa University Insects Vector Entomopathogen Research Laboratory for examination and identifications, whereby adult mosquitoes were examined and identified under binocular microscope and the following species were identified and tabulated as follows:

Table 5. Relative abundance of Adult mosquito species collected in breeding sites around Wau Town

S/No.	Species	Number of species identified	percentage
1.	Anopheles gambiae	12	25.00%
2.	Anopheles marshalli	02	4.17 %
3.	Anopheles obscurus	05	10.42 %
4.	Anopheles rhodensiensis	01	2.08 %
5.	Anopheles pretoriensis	04	8.33 %
6.	Anopheles nili	01	2.08 %
7.	Anopheles pharoensis	01	2.08 %
8.	Unidentified/ Male Mosquito	22	45.83 %
	Total Mosquitoes	48	100 %

5.2.2. Larva and pupae

Larval instars were collected along the Jur River banks, Hai Dinka, Dier Akok and Nazareth. On the average 50 -110 larva were recovered from each breeding sites. Along western bank of Jur River larvae were collected from 3- 7 small ponds that were left by brick makers. They had relatively cleaner water than the other breeding habitats of mosquitoes. While the dirty stagnant water pools located along the residential houses were inhabited with culex mosquitoes. They lay their eggs horizontally on the water surfaces that were covered with dead plants, grasses and cattle's dungs (Table 6).

Table 6. Larvae that have been recovered from different breeding sites.

S/No	Breeding site	Description of the site	No. of larva collected	Type of larva
1.	Mullem/ Nazareth	Relatively clean but running water	37	Anophelines mosquito larvae
2.	Dier Akok	Relatively dirt standstill water with remnants of dead plants and cattle dunks	130	Culex larvae
2.	Hai Dinka	Stand-still relatively clean water in bricks ponds	170	Anophelines
3.	River Jur Bridge	Slow running clean water	28	Anophelines

6. DISCUSSION

Here the burden of malaria is higher in the 6 – 15 years age group regardless of their gender and less high in above 15 years group while it is insignificance in the young ones of 0 – 5 years old.

The prevalence of malaria is persistent throughout the year in the town which increases in the raining season from June through November and declined toward the less fall months.

The most suffering groups are females above 15 years throughout the year regardless of the season, this high infection might be conferred to the activities of these groups which are the most active due to their age whereby they carry out many duties and might go to the bed under mosquito net very late and the chances of being bitten by mosquito is high as they stayed outside for long, followed by females and males between 0 -5 years, these children are vulnerable to mosquito bite. The young ages of 6 – 15 years also suffered the disease but with less percentages unlike those above 15 years.

Out of (31527) of patients in Sikka Hadid Hospital alone malaria episode constitute (47.51 %) while the all other parasitic infections added together are responsible for (52.49%) of infection (Unpublished data of Sikka Hadid Hospital, 2015). The infants (<1 year) were less infected with malaria (16.1%), while children of (1 – 5, 6 – 10 years group) had 18.8, 23.6%. Whereas those above 15 years of age had an infection rate of 41.5%. This result shows that active groups are more likely to be infected due to their subject to the mosquito attack while still staying out of ITNs which is not a case for young and infants whom the parents and guardians took care of them and usually go to the bed earlier than adults.

The rise in meteorological factors (Temperature, rainfall and humidity) is directly correlating to the increase in malaria prevalence. The infection that was on decline starting October 2015 and went up to December,and once again in January showed a slight rise in January as a reflection of the short rain effect of November and December. Then again it declined until May which is the dry season. When the rain and all other climatic factors again came on increase the malaria infection that was kept fueled by the small rain peaked its momentum in parallel to reach the maximum in October together with the decline of the heavy rain season (June - November) and complete the cycle by declining to the lowest level in December. In general one can say ‘malaria pattern follows the rainfall, temperature and relative humidity of the town. The slight shift of malaria is a result of rainfall that requires some times hatching the mosquito's eggs and support their development to larva and release the adult mosquitoes to carry on/ initiate the transmission.

The 2016 Wau town demographic profile (Chan *et-al*., 2016, this theses, shows) It showed that 62.5% are from the indigenous inhabitants/ tribes of Wau Town, 18.8 % from Abyei area, Twic 6.3%, Tonj 5.2%, both Gogrial, Jubek and Upper Nile constitute 2.1% each and Yirol 1.0 %. The majority of the people in the study sites except that they earn their incomes from farming, they are not very different from most dwellers in the Wau town. That is to say their hygiene status, their dependence on animals and animal products, and collect water for daily use, laundering, bathing, drinking from wells, Boreholes like the people in the town. The population surveys by this group shown that the population is female dominated. It might be the latest observation among the surveyed population females constituted 59% and 41% male.

The primary study done in 2016 involved parasitological, metrological entomological and other relevant factors for malaria seasons. It was designed to be done at monthly intervals for 6 months to show malaria prevalence and incidence of the dry and wet seasons on purposefully selected representative households in Wau town. This could not be done as planned because of the eruption of war between the government and the rebel forces in the Southern Sudan. Nevertheless, the study presents here what it was able to capture just before the war broke out: The demographic, parasitological examination results from the few residential areas and the ecological, entomological, and metrological data that were secured long before the two forces met in Wau town and the surrounding areas.

Human subjects were examined for malaria infection during the less peak season in one of the cluster houses chosen in Hai Nazareth in the period from May – June. All subjects examined were in good health with no symptoms for malaria and active in their daily life. They were only taken to the study because they were member of the households selected for the study. The result showed out the 96 subjects examined, 29 (28%) positive for malaria. The age distribution into 3 groups: X, <5 – 15>, Y showed 5.2%, 15.6% and 7.3% respectively.

Plasmodium falciparum was the only species recovered from the subjects i.e. no other species was observed and the serology kit was specific for *P. falciparum*. Given this one cannot rule out the presence of other malaria species. However, knowing the fact that other workers have also come up with a similar results (Pasquale *et al*., 2013) encourages one to claim falciparum to be the major player in Wau town.

Documentation at Juba Teaching Hospital (JTH), the largest tertiary and referral hospital in the country, is often poor, limited and the official statistics of admission and mortality are sparse and their reliability is sometime questionable. Which is a case in Wau Teaching Hospital and other small health care facilities in Wau town. For this reason we collected available retrospective data from WTC, St. Mary Hospital and St. Daniel Comboni Catholic Hospital which have records that can be used and process not for malaria alone but other diseases. There is also limitations in life-saving equipment in the receiving wards, such as oxygen therapy. Simple items such as intravenous cannulae may not be found in the hospital, so the patient's relatives may look for it in the nearby pharmacies outside the hospital premises. (Tim. W, 2012). This delay in basic resuscitative measures contributed to the poorer prognosis. Emergency department in JTH as well Wau Teaching Hospital is also used as out-patient clinic, which received approximately 300 – 500 patients per day at the time doctors have a very high workload of patients to look after, and it may be difficult to recognize and focus on those who needs an urgent attention.

The major vector responsible for the transmission of the parasite is *Anopheles gambiae* with 25.0%, *Anopheles obscurus* 10.42%, *Anopheles pretoriensis* 8.33% *Anopheles marshalli* 4.17%, and both *Anopheles rhodensiensis*, *Anopheles nili* and *Anopheles pharoensis* with 2.08%. These findings are supported with previous studies of (Pasquale *et al*., 2013), except in the case of *Anopheles marshalli*, *A. pharoensis* and *A. rhodensiensis* which seem to be new species in the area.

Environmental factors that might contribute to the abundance and transmission of the parasites include the water sources like the stream that flow across the town whereby many grasses and trees grows on both side of the stream and provide the hideout for mosquito and laying position for their eggs.

The Jur River that flow from South to North on the eastern part of the town contribute very much in providing breeding sites for mosquito on its banks, especially the pockets that are left over the bricks makers whereby they became a water hold later when rain fall and the river floods, small vegetation grows extensively, this enhance the breeding of mosquito and other small insects as well as reptiles (Photo No. 2). The vehicles that transport the bricks to different destinations leave on tires tracks that will be fill with water and become good position for eggs laying by female mosquito.

The types of houses and animals that the town inhabitants used and keeps could have role in the increased transmission and prevalence of malaria. These houses are built using muds and some grasses being used as cover and arches, whereby mosquito could easily hide on these grasses inside the house during the day time and resume their activity during the night hours and early at dusk, especially the residence near the River. While having these types of houses you could see some residents keeping Cattles, Dogs, and Cats within the same compound, which raised a question that these mosquitoes might be feeding on both human and animals.

The major question of the thesis as stated in rationale was to find the malaria situation in the dry months of the year. This could not be done in a large scale it was designed involving persons from the 4 study sites due to the break of war between the government and the armed militia forces at the time of sampling, which is a major limitation of this study.

However, from the primary and secondary data generated it was possible to show that 92 (19 %) of the people (288) randomly selected and examined individuals were positive for *p. falciparum* by smear or by serological assay. None of these subject knew that they were infected or spoke of experiencing any symptoms of malaria infection, i.e. all were asymptomatic patients living with the parasite as carriers in dry season of the year. If one assume the findings as representing the population of Wau, which is (151320) people, it means in that town there are 151320 x 19/ 100 = 28751 persons which were harboring the parasite at the dry season when transmission is expected to be lowest.

This could be due to two reasons, the study town is halo – endemic one, where by because of repeated infection of the population has developed certain level of immunity that suppress the infection from causing them severe forms of the disease. The second one is that they experienced light infection in them that maintain certain immunity level that protect them from experiencing the acute symptoms at a level it forces them to stop their daily routines. As a naïve person coming in to the town, who would suffer severe forms of the disease from the lack of immunity.

The second newly explanation comes from the entomological findings as shown in (Table 5). Leaving unidentified species, there were 7 Anophelines species. *Anopheles gambiae* complex was the most dominant species accounting for 25%, *Anopheles obscurus* 10.42%, *Anopheles pretoriensis* 8.33%, *Anopheles marshalli* 4.17% and *Anopheles rhodensiensis*, *Anopheles nili*, and *Anopheles pharoensis* with 2.08% each. The former has been documented to be the major vector in South Sudan during the peak transmission (Pasquale *et al*., 2013).

The role of the other species in maintaining the transmission during the dry season combines with the identification of the asymptomatic patients and highly susceptible individuals in the population and taking care of them during the dry season is the strategy of WHO to break the transmission of malaria and eliminate/eradicate malaria from African continent.

It is however, the belief of the producer of this thesis that eradication of poverty and establishing fraternity in a nation is a mandatory element to effect elimination and eradication programs- history has witness that this is a proven track.

7. CONCLUSION

- Malaria is persistent throughout the year but peaks in the rainy season.
- Most of the human subjects that were surveyed during the period from May – June 2016 shows no symptoms, which mean that the population harbors asymptomatic subjects in the dry season.
- As far as this study goes *Plasmodium falciparum* is the only species recovered and probably the only one in circulation in the town.
- From these study it is difficult to conclusively talk about the major/responsible vectors in transmission for it requires more work on blood analysis and frequency of occurrence over the year.

8. RECOMMENDATIONS

- Transmission of asymptomatic malaria in the dry season calls for separate study of malaria hotspot situation in the town.
- The findings of this research paper indicate that more work on the vectors role in maintenance of the life cycle during the dry season should be done
- The study of this thesis must be continued to complete the work as planned in the proposal in order to serve better the elimination/eradication trials.

- There is a need for capacity building for health services workers in data recording at least at hospitals and clinics level, but also for continuous epidemiological investigations

References

"South Sudan parliament throws out census results*: retrieved from Sudan Tribune. 8 July 2009.*

Arama C. and Troye-Blomberg, M. (2014). The path of malaria vaccine development: challenges and perspectives. *J Intern Med*. **275**: 456–466.

Chanda, E., Doggale C., Pasquale, H., Azairwe R., Baba S. and Mnzava, A.(2013). Addressing malaria vector control challenges in South Sudan: proposed recommendations. *Malaria Journal*. **12**: 59

Downie R. (2012). The state of public health in south Sudan: critical condition, a report of Centre for strategic and international studies. Global Health Policy Centre. csis.org/files/publication/121114_Downie_HealthSudan_Web.pdf www.smartglobalhealth.org/publications.

Emmanuel Q. (2013). South Sudan Meteorological Services.

Good, M. F. (2005). Vaccine-induced immunity to malaria parasites and the need for novel strategies. *Immunoparasitology Series.* 29 – 34.

GoSS (2006a). Malaria control strategic plan 2006–2011. Ministry of Health, Government of Southern Sudan, Juba.

GoSS (2006b) National health policy document 2007–2011 Ministry of Health, Government of Southern Sudan, Juba.

GoSS (2008a): Neglected tropical diseases and their control in southern Sudan: situation analysis, intervention options, appraisal and gap analysis. Ministry of Health, Government of Southern Sudan, Juba.

GoSS (2008b) Strategy for Increasing Coverage of Long Lasting Insecticide Treated Mosquito Nets (LLINs) in South Sudan. Ministry of Health, Government of Southern Sudan, Juba.

GoSS (2009) Guidelines for preparedness and response to malaria epidemics. Ministry of Health, Government of Southern Sudan, Juba.

Govere J., Durrheim. D. N., Coetzee M., Hunf R. H., and la Grange J. J. (2009). Captures of mosquitoes of the Anopheles gambiae complex (Diptera: Culicidae) in the Lowveld Region of Mpumalanga Province, South Africa. University of the Witwatersrand, Johannesburg, South Africa.

Hay S. I., Smith D. L., and Snow R. W. (2008). Measuring malaria endemicity from intense to interrupted transmission. *Lancet Infect Dis*.**8**:369–378.

IOM. (2013). Village assessment survey: County profile, Western Bahr El Ghazal State.

Kangoye D. T., Abdisalan N., Janet M., Joyce M., Dora M., Polycarp M., Christine K., Pauline A., Joseph M., Chriis D., Kevin M., Philip B., and Patricia N. (2016). Malaria hotspots defined by clinical malaria, asymptomatic carriage, PCR and vector numbers in a low transmission area on the Kenyan Coast. *Malar J***15**:213

Kathleen W. (2002). A Review of Control Methods for African Malaria Vectors. US. Agency for International Development, Washington, DC.

Geyer M. (2001). ANTIMALARIAL DRUG COMBINATION THERAPY: Report of a WHO Technical Consultation. World Health Organization, Geneva. WHO.

Massebo F., Balkew M., Gebre-Michael T., and Lindtjørn B. (2015). Zoophagic behaviour of anopheline mosquitoes in southwest Ethiopia: opportunity for malaria vector control. *Parasites & Vectors*. **8**:645

National Institute of Allergy and Infectious Diseases. (2007). *Understanding Malaria*: Fighting an Ancient Scourge.

Ndo C., Christopher A., Ann C., Diego A., Pierre K., Isabelle M., Parfait H. A., Daniel C., Pierre N., Didier F., and Frederic S. (2010). Population genetic structure of the malaria vector Anopheles nili in sub-Saharan Africa. *Malaria Journa*l.**9**:161

Pascal R., Kamini M., Amy B., and Sylvia M. (2009). Strategy Paper on Management of Antimalarial Drug Resistance for the Roll Back Malaria (RBM) Board: The Global Malaria Programme (GMP). WHO.

Pasquale H., Martina J., Ahmed J., Constantino D., Bakhit S., Mark Y. L., Samson P. B., and Emmanuel C. 2013. Malaria control in South Sudan, 2006–2013: strategies, progress and challenges. *Malaria Journal*. **12**: 374

Patrick O. U. (2007). Malaria and the Challenges of Vaccine Development. Faculty of Pharmacy, University of Benin, Benin City, Nigeria.*Tropical Journal of Pharmaceutical Research*. 6 (4): 801-802

Pavan Kumar S.T.P.R.C. and Reddy N.N.R. (2014). *FACTORS AFFECTING MALARIA DISEASE TRANSMISSION AND INCIDENCE*: A SPECIAL FOCUS ON VISAKHAPATNAM DISTRICT. *International Journal of Recent Scientific Research* Vol. **5**, Issue, 1, pp.312-317.

Peter B. Bloland: Drug Resistance in Malaria. WHO. 2001.

PSI (2014). South Sudan: Pre-Harvest Anthropometric and Mortality Survey, Wau County, Western Bahr El Ghazal, South Sudan.

1. Retrieved/ https://www.cdc.gov/malaria/about/biology/mosquitoes/

Richard M. (2007). Epidemiology and Control of Malaria. The Johns Hopkins University and Richard Marrow.

RoSS (2011) Scaling-up coverage of malaria prevention and control interventions in the republic of south Sudan, SSF for consolidated R7 and R10. 2011. Ministry of Health, Republic of South Sudan, Juba.

2. *Ross, Will (9 January 2011).* "Southern Sudan votes on independence". *BBC*. Retrieved 2 April 2011.

RoSS.(2009). Malaria indicator survey report, Republic of south Sudan. Juba, Ministry of Health.

3. RoSS: Sudan Household Health Survey Report. Republic of South Sudan: Ministry of Health; 2010.

Sabrina A. W. and Phillip E. K.(2014). African Malaria Mosquito. University of Florida Publication, Florida.

Safa I. A., Elfatih M. M., and Kamil M. A. (2007). The burden of malaria in Sudan: incidence, mortality and disability– adjusted life – years. *Mala J*.**6**: 97

Sherman I. (1998). Malaria: Parasite Biology, Pathogenesis, and Protection. ASM Press.

Sinka M. E., Bangs M. J., Manguin S., Rubio-Palis Y., Chareonviriyaphap T., Coetzee M., Mbogo C. M., Hemingway J., Patil A. P., and Temperley W. H. (2012). A global map of dominant malaria vectors. *Parasit Vectors*, **5**:69.

4. *South Sudan: Retrieved from Wikipedia:* https://en.wikipedia.org/wiki/South_Sudan

SSCCSE. (2010).*South Sudan counts: tables from the 5th Sudan population and housing census, 2008. Juba*. Government of Southern Sudan: Southern Sudan Centre for Census: Statistics and Evaluation;. unstats.un.org/unsd/statcom/statcom_09/seminars/…/AruaiSSCCSE.pdf.

Tim W. (2012). The St Mary's Hospital, Isle of Wight, UK – Juba Teaching Hospital, Southern Sudan Link. *South Sudan Medical Journal*.

UN. (2013). Mid-year review of the consolidated appeal for south Sudan. UN Office of the Coordination of Humanitarian Affairs: http://reliefweb.int/report/south-sudan-republic/mid-year-review- consolidated-appeal-south-sudan-2013

White N. J. (2008). Plasmodium knowlesi: The Fifth Human Malaria Parasite. *Clinical Infectious Diseases*. **46**:172–3

WHO. (2012). Global Malaria Programme: *World malaria report 2012*. Geneva: World Health Organization; http://www.who.int/malaria/publications/world_malaria_report_2012/report/en/ (accessed Jul 27, 2013).

Zazi O. N.(2008). Why is it so difficult to develop a malaria vaccine: Challenges to malaria subunit vaccine design.

Appendices

Appendix No. 1: Malaria survey table

S/No	ID. No.	Sex	Age/Years	State of Origin	BFFM	Serology Result
9.	MN001	F	27	Twic	Negative	Negative
10.	MN002	F	23	Twic	Negative	Negative
11.	MN003	F	26	Twic	Negative	Negative
12.	MN004	M	21	Abyei	Negative	Negative
13.	MN005	F	10	Abyei	Positive	Negative
14.	MN006	F	12	Twic	Positive	Negative
15.	MN007	F	6	Abyei	Negative	Negative
16.	MN008	F	12	Abyei	Positive	Negative
17.	MN009	F	17	Twic	Negative	Negative
18.	MN010	M	20	Twic	Negative	Negative
19.	MN011	M	14	Abyei	Negative	Negative
20.	MN012	F	34	Abyei	Positive	Negative
21.	MN013	F	23	Abyei	Negative	Negative
22.	MN014	M	38	Abyei	Positive	Positive
23.	MN015	M	14	Abyei	Negative	Negative
24.	MN016	M	14	Yirol	Negative	Negative
25.	MN017	F	70	Tonj	Negative	Negative
26.	MN018	F	51	Tonj	Negative	Negative

27.	MN019	M	59	Wau	Negative	Negative
28.	MN020	F	67	Wau	Negative	Negative
29.	MN021	F	21	Wau	Negative	Negative
30.	MN022	M	5	Wau	Negative	Negative
31.	MN023	F	13	Wau	Negative	Negative
32.	MN024	M	4	Wau	Negative	Negative
33.	MN025	M	4	Wau	Negative	Negative
34.	MN026	F	8	Wau	Positive	Positive
35.	MN027	F	9	Wau	Negative	Negative
36.	MN028	M	2	Wau	Negative	Negative
37.	MN029	F	7	Wau	Negative	Negative
38.	MN030	F	73	Wau	Negative	Negative
39.	MK031	F	36	Wau	Negative	Negative
40.	MK032	M	16	Wau	Negative	Negative
41.	MK033	M	24	Wau	Negative	Negative
42.	MK034	M	18	Wau	Negative	Negative
43.	MK035	F	11	Wau	Positive	Positive
44.	MK036	M	8	Wau	Positive	Negative
45.	MK037	M	14	Wau	Negative	Negative
46.	MK038	F	28	Wau	Negative	Negative
47.	MK039	M	71	Wau	Negative	Negative
48.	MK040	M	4	Wau	Positive	Negative

49.	MK041	F	40	Wau	Negative	Negative
50.	MK042	F	9	Wau	Negative	Negative
51.	MK043	M	3	Wau	Negative	Negative
52.	MK044	F	7	Wau	Negative	Negative
53.	MK045	M	8	Wau	Negative	Negative
54.	MK046	M	64	Wau	Negative	Negative
55.	MK047	M	3	Gogrial	Positive	Negative
56.	MK048	F	28	Wau	Positive	Negative
57.	MK049	M	34	Wau	Negative	Negative
58.	MK050	M	5	Gogrial	Negative	Negative
59.	MK051	M	41	Wau	Positive	Negative
60.	MK052	M	11	Wau	Negative	Negative
61.	MK053	M	13	Wau	Negative	Negative
62.	MK054	M	18	Wau	Negative	Negative
63.	MK055	F	22	Wau	Positive	Negative
64.	MK056	M	15	Wau	Negative	Negative
65.	MK057	F	42	Jubek	Negative	Negative
66.	MK058	F	11	Jubek	Negative	Negative
67.	MK059	M	7	Wau	Negative	Negative
68.	MK060	F	28	Wau	Negative	Negative
69.	MZ061	F	70	Wau	Negative	Negative
70.	MZ062	F	29	Wau	Negative	Negative

71.	MZ063	M	13	Wau	Positive	Positive
72.	MZ064	M	11	Wau	Positive	Positive
73.	MZ065	F	7	Wau	Positive	Positive
74.	MZ066	F	3	Wau	Negative	Negative
75.	MZ067	F	35	Tonj	Negative	Negative
76.	MZ068	M	1	Wau	Negative	Negative
77.	MZ069	M	2	Tonj	Positive	Positive
78.	MZ070	M	10	Tonj	Positive	Positive
79.	MZ071	F	42	Upper Nile	Negative	Negative
80.	MZ072	F	13	Wau	Positive	Positive
81.	MZ073	M	10	Wau	Positive	Positive
82.	MZ074	F	8	Upper Nile	Positive	Positive
83.	MZ075	F	6	Wau	Negative	Negative
84.	MZ076	F	70	Wau	Negative	Negative
85.	MZ077	F	5	Wau	Positive	Positive
86.	MZ078	M	5	Wau	Positive	Positive
87.	MZ079	F	10	Wau	Negative	Negative
88.	MZ080	F	8	Wau	Negative	Negative
89.	MZ081	F	5	Wau	Negative	Negative
90.	MZ082	F	15	Wau	Negative	Negative
91.	MZ083	F	7	Wau	Negative	Negative
92.	MZ084	F	13	Wau	Negative	Negative

93.	MZ085	F	15	Wau	Positive	Positive
94.	MZ086	F	17	Wau	Positive	Positive
95.	MZ087	F	22	Wau	Negative	Negative
96.	MZ088	F	27	Abyei	Negative	Negative
97.	MZ089	F	28	Abyei	Negative	Negative
98.	MZ090	M	18	Abyei	Positive	Positive
99.	MZ091	M	21	Abyei	Negative	Negative
100	MZ092	F	7	Abyei	Negative	Negative
101	MZ093	F	7	Abyei	Negative	Negative
102	MZ094	F	9	Abyei	Positive	Negative
103	MZ095	F	4	Abyei	Negative	Negative
104	MZ096	F	28	Abyei	Negative	Negative

Appendix No. 2: Consent Sheet

Participant's enrollment form (Consent form)

1. Study Site: ……………………………………………….
2. Data Abstractor: …………………………………...

Part One: Socio Demographic Data of the participant (to be fill by household or delegate)

3. Enrollment Date (dd/mm/yyyy) …………………… Time (AM/PM) ……………
4. Identification Number: ……………………………………………………………
5. City of Residence: ………………………… Sub City: …………………………… House No: ……………………. Phone No: …………………………………..,
   …………………………………
6. Sex: 1. Female ☐ 2. Male ☐
7. Date of Birth (dd/mm/yyyy): ………………………………………
8. Age (in year): ……………………………………………………
9. State of Origin: ……………………………………………………………….
10. Inclusion and Exclusion Criteria
11. Inclusion Criteria: Tick all these requirements (Exclude if any of NO boxes is ticked)
12. Yes No
13. ☐ ☐ Volunteer Household with informed consent
14. ☐ ☐ Representative Household
15. Exclusion Criteria: tick all these requirement (exclude if any of YES box is ticked)
16. Yes No
17. ☐ ☐ Household member under age
18. ☐ ☐ Household not willing to participate

Final Decision on Inclusion/ Exclusion: Included ☐ Excluded ☐

Date registered for the research (dd/mm/yyyy): …………………………..

Appendix No. 3

Information Sheet and Consent for Participants

Information Document

Title of the project: ***Epidemiology of Urban Malaria in Wau Town, Western Bahr El Ghazal State, South Sudan***

Dear Participants,

We would like to ask your volunteer participation for the Epidemiological research which will be done by Addis Ababa University MSc. Student **Chan Ngor Bak Matik** and his advisors**Dr. Mekuria Lakew** and co-advisor **Dr. Habte Tekie**. The research mainly focuses on malaria prevalence as well as epidemiological survey on human, vector, parasite and environmental factors that favor the disease in urban settling.

We will explain the purpose of the study and the activities that are going to be done in the study in detail. You can raise questions that you want explained and clarified. After carefully listening and reading, the explanations regarding the research, if you volunteer to participate in the study, please put your signature in the space provided.

Why we need to conduct the study

Malaria is a serious thread worldwide. In 2010, about 219 million malaria cases and 660,000deaths were reported globally. The greatest toll was in sub-Saharan Africa where over 80% of cases and 90% of deaths occur (WHO, 2012). Several country wide control programs have been conducted to control malaria incidence in South Sudan, and success stories are documented. In some areas the intervention was effective that there were less death compare to similar measure the fatality was very high suggesting a detailed study of population density and parasite prevalence, which could help preventing and controlling malaria in the study area.

Objectives of the study

To conduct an epidemiological survey on the human, vector, parasite and environmental factors that favor the disease in urban settling.

Procedure

If you agree to participate in the study, small amount of blood will be drawn from your finger using finger prick lancet after being cleaned to prepare thin and thick blood smear on clean slides and then to be examined under microscope to determine whether you have malaria or not by a well-trained technician. Positive participants will be treated. You will also be asked as household to allow the researcher to spray your house by pesticides to collect mosquito that may be found in your rooms for the purpose of the study. You will be asked to answer semi-structured questionnaire.

Risks

No serious health hazard will be caused due to your participation in the study. The procedure used in blood collection is normal and may cause some minor discomfort around the punctured area, but it is easily controlled through first aid medical practice.

Incentives and payment

You will not be paid for your participation. However, any expense that you will spend because of the study will be covered by the project.

Participant's right and withdrawal from the study

Your participation in the study is entirely dependent on your willingness. Even after giving your consent to participate in the study, you have a full right to withdraw from the study at any time.

Confidentiality

We will use the data you gave for this study only according to your permission. We will not use your information for purposes other than the study. We assure that the information and data obtained from the laboratory results are confidential. If you stop to participate in the middle of the study, we will remove all your data from our laboratory. After doing the study the result will be sent for publication without exposing your identity.

Agreement

After reading and listening about the study procedures and other related issues done in the study, you will kindly be requested to put your signature of agreement. Your signature indicates that your participation is only based on your volunteer participation.

Communication

In case you have any questions, unclear ideas and doubt about the study, you can use the following addresses:

Chan Ngor Bak Matik (BSc.), Addis Ababa University MSc. Student

E-mail: chancorner2@gmail.com

Mobiles: +251 929 113 038 – Addis Ababa, Ethiopia

+211 915 503 614 – South Sudan

+211 956 996 018 – South Sudan

Mekuria Lakew (PhD), Advisor

Addis Ababa University, Department of Microbial, Cellular and Molecular Biology

E-mail: yibza2002@yahoo.com

Mobile: +251 911 217 999 – Addis Ababa

Habte Tekie (PhD), Co-Advisor

Addis Ababa University, Department of Zoological Sciences

E-mail: habte_tm@yahoo.com

Mobile: +251 911 407 319 – Addis Ababa

We thank you for consenting to take part in the study

If you agree to take part in the study, please read this form and sign the consent sheets attached.

Please tick off every box, if you agree.

1. I have read, or it was read and explained to me, the information sheet concerning this study and I understand what will be required of me if I take part in the study. ☐
2. I am aware of the possible risk and benefits of this study. ☐
3. I know that being in this study is voluntary. ☐
4. I understand that at any time I may withdraw from this study without giving a reason without affecting me. ☐
5. My questions concerning this study have been answered by ……………………
6. I agree to take part in this study. ☐

SIGNATURE: __________________________ DATE: __________________________

Name of participant or delegate

Address: _____________________________________ Phone No: __________________________

Signature of the person obtaining the information consent (Witness):

SIGNATURE: __________________________ DATE: __________________________

Appendix No. 4: Ethical Clearance Approval

COLLEGE OF NATURAL & COMPUTATIONAL SCIENCES
Addis Ababa University

የተፈጥሮና ኮምፒውቴሽናል ሣይንስ ኮሌጅ
አዲስ አበባ ዩኒቨርሲቲ

OFFICE OF THE DEAN
የዲን ጽ/ቤት

Ref. No. / ቁጥር: CNSDO/406/16
Date / ቀን: April 26 2016

To Whom It may Concern

College of Natural Science Institutional Review Board (CNS-IRB) in its meeting held on 10/04/2016 Minute No. IRB/021/2016 has reviewed MSc. thesis project proposal entitled **"Epidemiology of Urban Malaria in Wau Town, Western Bahr EI Ghazal, South Sudan"** by Chan Ngor Bak Matik from the Department of Microbial Cellular and Molecular Biology.

The proposal was approved for implementation.

With regards,

Shibru Temesgen /Dr./
Dean College of Natural & Computational Science

ስልክ/Tel.+251-11-123-94-72
ፋክስ/Fax: +251-11-123-94-69
ፖ.ሣ.ቁ/POBox 1176 Addis Ababa, Ethiopia
ኢ.ሜይል/E-mail: dean_cns@aau.edu.et
Please Quote our reference number in you correspondence
"Examine all things; hold fast that which is good"
"ሁሉን መርምሩ መልካሙን ያዙ"

Declaration

I, the undersigned, declared that this is my own original work, has not been presented for a degree to any other university and that all sources of materials used for the thesis have been fully acknowledge. I also confirm that this work has not been submitted anywhere else for the same purpose.

Chan Ngor Bak Matik

Signature ___________________

Date ________________

This thesis has been submitted for examination with our approval as University advisors.

Mekuria Lakew (Ph.D)

Signature __________________

Date ________________

Habte Tekie (Ph.D)

Signature ___________________

Date _________________