PREVALENCE OF MALARIA PARASITE INFECTION AND VECTOR SPECIES ABUNDANCE IN HUYE DISTRICT, SOUTHERN RWANDA

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DECLARATION

I hereby declare that this dissertation is my original work under the guidance of the supervisors listed below and has not been submitted to the University of Nairobi or any other higher learning institution.

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DEDICATION

To my parents, brothers, sisters and my love Chibvongodze Raymond

ACKNOWLEDGEMENT

I thank God for bringing me this far.

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LIST OF ABBREVIATIONS

An.	Anopheles
CDC	Center for Disease Control and Prevention
CHUB	Butare Central University Hospital
CSP	Circumsporozoite Protein.
EIR	Entomological Inoculation Rate
ELISA	Enzyme Linked Immunosorbent Assay
GPIRM	Global Plan for Insecticide Resistance Management.
HBI	Human Blood Index
IRS	Indoor Residual Spray
ITN(s)	Insecticide treated net (s)
LLIN(s)	Long Lasting Insecticides Nets
LSM	Larval Source Management
PCR	Polymerase Chain Reaction
SOPs	Standard Operating Procedures
SPP	Species
SPSS	Statistical Package for Social Sciences
WHO	World Health Organization
MOPDD	Malaria and Other Parasitic Diseases Division
RBC	Rwanda Biomedical Center
MoH	Ministry of Health

ABSTRACT

Background: Malaria is a leading cause of mortality and morbidity in sub-Saharan Africa including Rwanda. Effective control of malaria requires knowledge of vector species but information on species distribution in Rwanda is limited.

Objectives: The main objective of this study was to determine the prevalence of malaria parasites in children under five and vector species abundance in Rukira cell, Huye district, Rwanda.

Methodology: A total of 222 children under five years randomly selected from 13 villages were examined. Data on malaria vectors and risk factors were collected. Adult mosquitoes were collected indoors by light traps and Pyrethrum Spray Catch, and outdoors by light traps. Female *Anopheles* mosquitoes were identified to species level by morphological characteristics. Screening for *Plasmodium falciparum* circumsporozoite protein and for host blood meal sources was achieved by Enzyme-linked Immunosorbent Assays. *Anopheles* larvae were sampled using dippers and raised into adult stages which were identified morphologically. Data were analyzed by IBM SPSS software version 22. In all tests, a *p-value* below 0.05 was regarded as statistically significant.

Results: Two hundred and twenty-two children were included in the study. Nearly a third (28.8%) of the children were within the age of 25-36 months. The majority (54%) of the children were female. Most of the parents/guardians were married (95.9%), nearly all (99.5%) had attended primary school and most (97.3%) were farmers. The overall *Plasmodium falciparum* prevalence in children under five was 12.2%. Children aged 1 to 12 months were 3.5 times more likely to have malaria parasites than children aged 13 to 59 months [**AOR=3.56; 95%CI=1.18-10.71; *p*=0.024**]. Children who were not sleeping under insecticide treated nets (ITNs) were 15 times more likely to be infected with malaria parasites compared to those who were sleeping under one [**AOR=15.27; 95%CI=4.42-52.82; p<0.001**]. *An. gambiae s.l* was the most dominant malaria vector 69.7% of the 567 collected *Anopheles*. Others were *An. funestus* 4.1%, *An. squamosus* 16.6%, *An. maculipalpis* 6.5%, *An. ziemanni* 1.8% and *An.coustani* 0.2%. The overall human biting index was 0.509 while Sporozoite rate was 1.9%. A total of, 661 anopheline larvae from 22 larval habitas were collected. They comprised of 2 species: *An. gambiae s.l* (89%) and *An. ziemanni* (11%). The absolute breeding index was 86.4%. The most common larval habitats were in full sunlight with still water such as rice paddies and bodies of stagnant water. The larval density was significantly associated with still water current (p=0.038).

Conclusion: *P. falciparum* infection is seen in one out of 8 children under five years of age. There is need to protect children from mosquito bites by ensuring that they sleep under ITNs. The primary potential malaria vector was *An.gambiae s.l* but secondary vectors like *An. ziemanni, An. squamosus and An. maculipalpis* may play an important role as well. The findings provide useful baseline information on malaria vectors composition that would guide integrated vector management strategies in the locality.

CHAPTER ONE: INTRODUCTION

1.1 Introduction

Malaria is one of the major human health threats globally. In the tropics and sub-tropics, the disease is rampant and has received much attention over the years due to its impact on public health (Autino et al. 2012). In 2013, malaria cases were estimated to be 198 million and 584,000 deaths worldwide, 90% of which occur in Africa (World Health Organisation 2014). In Rwanda, the whole population is at risk of malaria disease with children and pregnant women having the highest morbidity. Malaria is more prevalent in lowlands than in highlands (President's Malaria Initiative 2014a).

Human malaria parasites are transmitted by mosquitoes of the genus *Anopheles* which include 465 species of which approximately 70 are able to transmit malaria. In Africa, *An. funestus*, *Anopheles gambiae sensu stricto (s.s) and An. arabiensis* are the major malaria vectors. The first two species belong to a group called *A. gambiae* complex *(sensu lato),* which is the most efficient malaria vector worldwide (Temu et al. 2007). In Rwanda the major malaria vectors are *An. gambiaes.s, An. Arabiensis* and *An. funestus* (World Health Organisation 2014).

Historical evidence shows that an effective vector control program, requires identification of mosquito species in order to separate non-vector species from vectors. In South Africa, Kenya and Tanzania, after elimination of *An. funestus species* by indoor spraying an upsurge of '*funestus* look-alike' specimens suggested failure of the control program. However, PCR identification revealed that these mosquitoes were *An. vaneedeni*, *An. rivolurum*, *An. leesoni* or *An. parensis*, which rarely transmit human malaria (Temu et al. 2007). Although the incidence of malaria in Rwanda has declined since 2004, this achievement is fragile as potential for local malaria transmission through existing vectors remains (Bizimana et al. 2015). Reduction of the malaria burden requires knowledge of the vector species but critical indicators of malaria transmission differ widely from one locality to another due to variable climatic conditions in malaria epidemic and endemic regions (Obala et al. 2012).

This study aimed at determining malaria parasite prevalence, its associated factors and the species of malaria vector mosquitoes present at Rukira Cell of Huye sector, Huye district, Southern Province of Rwanda, their breeding sites, feeding and resting behavior. The study provided useful information for results for malaria mosquito vector control interventions.

CHAPTER TWO: LITERATURE REVIEW

2.1. Malaria burden

Malaria is a disease caused by *Plasmodium* parasites which are transmitted to humans by female anopheles mosquitoes. Five *Plasmodium* species are known to cause malaria; *Plasmodium falciparum, P. ovale, P. vivax, P. malariae,* and *P. knowlesi*. *P. falciparum* is the most pathogenic and common in Africa where it causes severe forms of malaria (Hay et al. 2011).

Malaria accounts for about (300 to 660) million clinical attacks, worldwide where about 2.2 billion individuals are exposed to infections with *P. falciparum* malaria (Hay et al. 2011). According to WHO 3.3 billion individuals were at risk of malaria infection in 2014 (World Health Organisation 2014). In 2013, there were approximately 198 million malaria cases resulting in 584,000 deaths globally, majority (90%) of which were in Africa (World Health Organisation 2014). About 78% of these deaths were in children under 5 years (World Health Organisation 2014). However, there has been a considerable success in malaria control, malaria cases and deaths have decreased by 670 million and 4.3 million respectively between 2000 and 2013 globally which was attributed to heightened prevention and control interventions (World Health Organisation 2011).

Malaria is known to mainly affect the resource poor and vulnerable communities especially in Africa where pregnant women and children under five years are at high risk. It is estimated that 20 -40% of all outpatient cases in Africa and 20% - 50% of all hospital admissions are due to malaria (World Health Organisation 2005). Most communities in Africa are faced with challenges of access to effective prevention, diagnosis and treatment. However, great progress in control of malaria has been seen in the last decade where malaria mortality in Africa has declined by 54% between 2000 and 2013 (World Health Organisation 2013b). For example there has been effective control of malaria in five North African countries of Libya, Algeria, Morocco, Egypt and Tunisia where the main etiological agent is *P. vivax* which is transmitted by anopheles mosquitoes which are easy to control (MARA/ARMA collaboration (Mapping Malaria Risk in Africa) 2002).

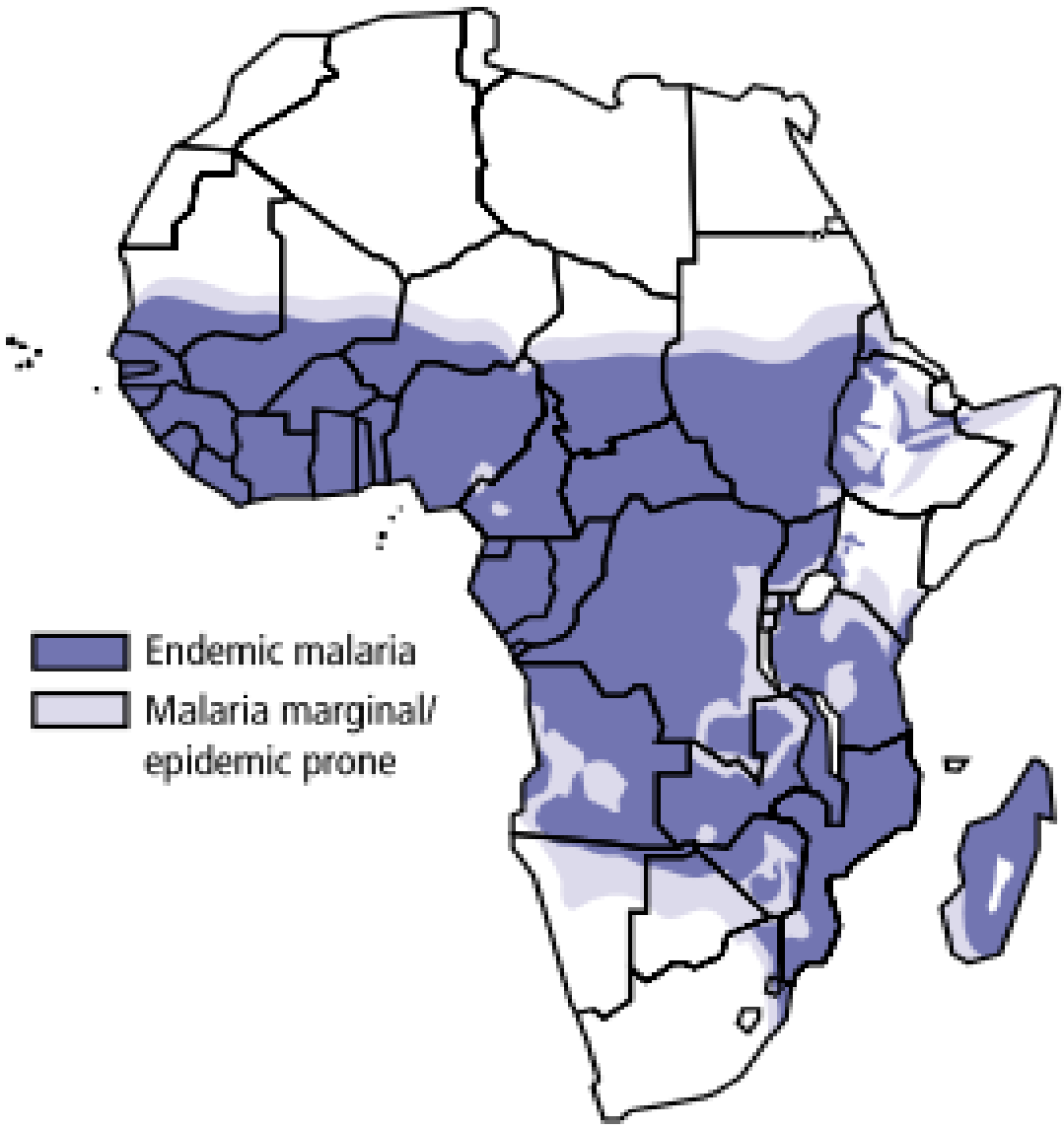

Figure 2. 1: Distribution of endemic malaria in Africa

Source: (MARA/ARMA collaboration (Mapping Malaria Risk in Africa) 2002)

2.2. Malaria in Rwanda

Rwanda is classified as a malaria endemic country by WHO (Henninger 2013). The country has four malaria ecologic zones based on microclimate, altitude and level of transmission. Nineteen (19) out of 30 districts are epidemic prone and 11 districts are endemic. Malaria transmission in Rwanda occurs with two peaks throughout the year in rainy season; May to June and November to December. In addition to the favorable climate, other factors such as proximity to marshlands, irrigation schemes and cross border movement of people influence the transmission especially in parts which include Huye District and the eastern parts of the country (President's Malaria Initiative 2014a).

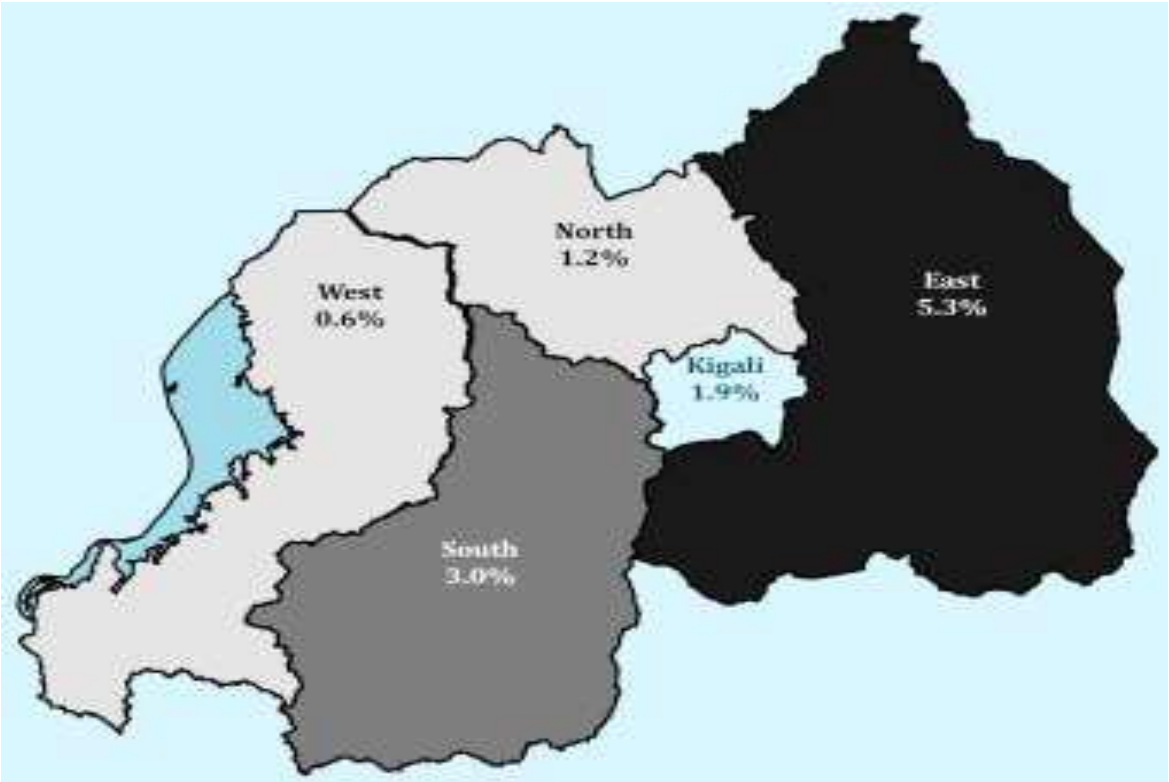

Figure 2. 2: Malaria parasite prevalence in children under five in Rwanda in 2010

Source: Rwanda Malaria Operational Plan (President's Malaria Initiative 2014a)

2.3. Current situation of Malaria in Rwanda

Use of malaria control interventions such as use of long lasting insecticide-treated mosquito nets, indoor residual house spraying with insecticides and effective treatments resulted in significant decline of malaria transmission between 2006 and 2008 in the country (Bizimana et al. 2015). Also malaria related mortality rate in children under-five years old decreased by 61 percent between 2000 and 2010, where prevalence of malaria in this age group was 1.4% in 2010 from 2.6% in 2007 (President's Malaria Initiative 2014b). However, according to WHO report in 2014, the situation changed with, admissions and confirmed malaria cases increasing twofold between 2012 and 2013 from 5,306 to 9,508 and 483,000 to 962,000 respectively (World Health Organisation 2014). In 2014 the situation worsened and malaria cases in Rwanda increased to 1,597,143. The morbidity rate in the country was 5.8% in 2012 but increased to 10% in 2013 and 14.8% in 2014. This increase is blamed on climatic change, insecticide resistance and ineffective mosquito nets (Rwanda Ministry of Health 2014).

2.4. Malaria Vectors Biology

Mosquitoes of the genus *Anopheles* are the most studied and best understood due to their vectorial capacity for malaria and lymphatic filariasis. About 537 *Anopheles* species are known and most (87%) have been named. Among these, about 20 taxa are species complexes, which contain about 115 sibling species in total. The sibling species of the complex are morphologically similar which makes it hard to identify them morphologically and can only be identified using molecular techniques such as PCR (Manguin 2013). However, they differ genotypically and in their biological characteristics such as resting and feeding behavior and susceptibility to insecticides (Manguin 2013).

The greatest burden of malaria (90%) is in Africa (MARA/ARMA collaboration (Mapping Malaria Risk in Africa) 2002). This is due to the presence of the most efficient vector species. These vectors can be grouped into two sibling species complexes; *An. funestus and An. gambiae*. The latter is the most efficient and has 8 sibling species namely; *An. gambiae* s.s, *An .bwambae, An. arabiensis, An. quadriannulatus, An. melas, An. amharicus, An. merus* and *An. coluzzii* (Sinka, Rubio-palis, et al. 2010).

An. arabiensis and *An. gambiae* s.s. have a wide geographic distribution. *An. gambiae* s.s. mainly occurs in humid savannah zones and forests while *An. arabiensis* is more successful in arid environments. The breeding sites of both sibling species are usually small, shallow, sunlit fresh water bodies without vegetation. Larvae of both species often cohabit in the same habitats. Adult *An. gambiae* s.s**.** is mainly anthropophagic whereas *An. arabiensis* is more zoophagic. However, studies show that host preference and biting behavior of the species highly vary across Africa (Tirados 2006). For instance, studies have shown that in western Africa *An. arabiensis* populations are more anthropophagic, endophilic and endophagic whereas those in the east are more zoophagic and exophilic. *An. gambiae* s.s. is mainly endophagic and endophilic with few exceptions, whereas *An. arabiensis* displays high variation in these behaviors (Tirados 2006; Coetzee et al. 2013).

Two sibling species *An. merus* and *An. melas* are found in brackish water. These species occupy areas with mangrove belts such as estuaries, lagoons and swamps. *An. merus* is found in the coast of east Africa whereas *An. melas* is found in the West African coast. *An. bwambae* occur in geothermal springs in western Uganda. The sibling species *An. quadriannulatus* is known to be mainly zoophagic hence has little impact on human malaria (Gillies M. T. 1987).

The *An. funestus* species complex has nine siblings' species. Of these, only *An. funestus* s.s. is the known vector of malaria in Africa. The others *An. rivulorum* (West-East Africa), *An. confuses* (East Africa), *An. aruni* (Zanzibar), *An. fuscivenosus* (Zimbabwe), *An. vaneedeni* (North of South Africa), *An. brucei* (Nigeria), *An. parensis* (East Africa), and *An. leesoni* (West-East Africa) are not malaria vectors, they are mainly zoophagic (Sinka, Bangs, et al. 2010).

A part from the main malaria vectors, *An. gambiae* Giles and *An. funestus* Giles, a number of others are suspected to play a role in local transmission of malaria especially following the introduction of indoor residual house spraying intervention. These include: *An. tenebrosus, An. coustani, An. ziemanni, An. pharoensis, An. squamosus, An. marshalli, An. rivulorum, An. paludis* and *An. maculipalpis* (Gillies 1964).

2.5. Current Malaria control strategy

Since the introduction of insecticides with long residual effects in 1950's, the control of vectors has relied on application of these chemicals using different innovative technologies. Initially, residual indoor spraying targeting indoor resting vectors was considered the most successful technology globally and the global community considered this as the feasible option for malaria eradication. In the 1980's the introduction of insecticide treated nets was invented and has since improved further by manufacturing long lasting insecticide treated nets (LLINs), which do not require regular re-treatment. Currently, the two technological innovations LLINS and Indoor residual house spraying, are considered as the primary methods of malaria vector control (World Health Organisation 1993).

Mosquito nets have been shown to reduce malaria transmission. A study conducted by Lengler, in 2009 showed that child mortality rate, clinical malaria and severe malaria cases in populations using mosquito nets reduced by 20%, 50% and 45% respectively (Lengeler 2009). Use of mosquito nets reduced malaria parasite incidence, incidence of severe malaria anemia and the prevalence of low birth weight by 38% ,47% and 28% respectively (Kuile et al. 2003).

In Rwanda, LLIN and IRS have been widely used. According to PMI report of 2014, the overall ITN coverage for 2013 was 83% and 74% for children under five years (President's Malaria Initiative 2014b). IRS is mainly focused in areas with high malaria burden e.g. Gisagara, Nyagatare and Bugesera. In 2013, IRS coverage rate in these three endemic areas was 99.6% which protected a total of 522,315 people of which 8,935 and 81,433 were pregnant women and children under five years, respectively (President's Malaria Initiative 2013).

The emergence of insecticide resistance to chemicals used in either IRS or on LLINs is expected to lower the effectiveness of malaria vector control interventions which might result in increased malaria incidence and more malaria related morbidity and mortality (Hemingway & Hemingway 2014). Insecticide resistance has been increasing since 2010 especially in WHO African region where resistance to pyrethroids has been detected in 78% of the countries (World Health Organisation 2014). *An. gambiae s.s* from West Africa showed stronger resistance mechanisms (Toé et al. 2015).

Management of insecticide resistance in malaria vectors requires knowledge of the susceptibility status of the local vectors. Global Plan for Insecticide Resistance Management (GPIRM) has outlined the strategies that are key in management of insecticide resistance. These include utilization of multiple insecticides with different modes of action and also rotation of different insecticides in an area. Currently insecticide combinations are being developed to counter insecticide resistance. Interventions targeting the larval stage of mosquitoes offer good alternative tools for management of insecticide resistance (Manguin 2013).

2.6. Larval control

Larval control involves elimination or reduction of mosquito breeding sites. A chemical may be applied in water to kill the larval mosquitoes. Larval source management (LSM) is thought to be practical in areas with few, fixed and accessible breeding sites. In this case the most appropriate settings are urban areas and areas with seasonal or low transmission. This requires prior evaluation of larval habitats (President's Malaria Initiative 2015b).

Chemicals recommended by WHO for use as larvicides can either be insecticide growth regulators, bio-pesticides or organophosphates (World Health Organisation Pesticide evaluation scheme (WHOPES) 2006). The effectiveness period of these chemical larvicides depends mainly on the nature of the breeding habitat and its exposure to sun light; it can take a few days in polluted water and months in clean water (President's Malaria Initiative 2015b).

Environmental modification or manipulation can also be used in larval mosquito control. Environment modification includes; drainage, deepening and filling, land grading and velocity alteration. Environmental manipulation uses methods such as shading or exposing water surface for control of breeding of certain species of mosquitoes (e.g. *An. funestus)*, water level fluctuation, salinity regulation or alteration which is effective against *Anopheles spp*. breeding, in fresh water (e.g. *An. gambiaes.s., and An. arabiensis)* and thus limited by increased salinity, while brackish water breeders (e.g. *An. merus)* will be reduced in fresh water (Service 2014).

2.7. Malaria Vector Indices

Entomological indices are used to characterize malaria transmission by a vector population. They provide a guide in vector control as well as malaria transmission. The indicators mainly focus on feeding and resting behavior of mosquitoes (World Health Organisation 2013a).

2.7.1. Indoor resting density (D)

The aim of indoor resting density as an index is to provide information on the number/ density of mosquitoes that rest indoors. The indoor resting mosquitoes are collected from sampled houses. The total number of female mosquitoes collected is divided by number of houses sampled and multiplied by the number of days to get the index. When it is done at the beginning of vector intervention especially IRS it provides important baseline data on mosquito density. When done after a few months after IRS it provides information on the success or failure of IRS. (World Health Organisation 2013a).

2.7.2. Human-biting rate

Human-biting rates are best estimated by all-night collections of mosquitoes that come to feed on a human: (number of vectors per inhabitant per night). This is calculated as:

Human biting rate = number of mosquitoes / number of collectors / number of collection hours (World Health Organisation 2013a).

2.7.3. Endophagic and Exophagic indices for biting preference

These indicators provide information on whether the vector feeds indoors (endophagic) or outdoors (exophagic). This helps in choosing the right intervention. For instance, a mosquito that feeds outdoors cannot be controlled using a mosquito net. It may require personal protective measures such as use of repellant.

Endophagic and Exophagic indices can be obtained by conducting landing catches with collectors placed indoors and outdoors. The endophagic index is then calculated as the proportion of female mosquitoes that bite indoors and exophagic index as proportion of female mosquitoes that bite outdoors (World Health Organisation 2013a).

2.7.4. Sporozoite rate(s)

Sporozoite rate provides information on the number of female mosquitoes that carry the infective stage of malaria parasites. The mosquito salivary glands which have the highest concentration of sporozoites are dissected and examined for sporozoites using a microscope or using ELISA (World Health Organisation 2013a).

2.7.5. Human Blood Index (HBI)

Malaria vectors can feed on humans (anthropophagic) or other animals (zoophagic). Most vector species are placed between the 2 extremes. If a species tends more towards feeding on humans, it is a better vector of malaria and lymphatic filariasis. HBI is obtained from mosquitoes caught using resting collection methods by analyzing the blood meals using ELISA. The HBI is then calculated as: HBI= (Not fed on Human/Total No collected) × 100 (World Health Organisation 2013a).

2.7.6. Entomological Inoculation Rate (EIR)

This rate is the number of infective bites per person per night. EIR is calculated as a product of man-biting rate (ma) and sporozoite rate (s) (World Health Organisation 2013a).

3. Problem Statement and Justification

Malaria is a major cause of morbidity and mortality in Rwanda, although different measures for its control such as ITNs, IRS and antimalarial drugs have resulted in significant malaria incidence decline (World Health Organisation 2014). As a result, malaria transmission is increasingly heterogeneous in its distribution as it differs from one village to another due to diversity in type and behavior of vectors. Contemporary species distribution information is not readily available on the African continent including Rwanda (Okara et al. 2010). This necessitates more focused studies to identify current status of the disease, risk factors, type and behavior of vectors (Badu et al. 2013). One of the main control methods in Rwanda is the use of long lasting insecticidal nets (LLINs) accompanied by monitoring and evaluation through entomological surveillance. While efforts are being made in Rwanda through sentinel sites to capture key indicators, there are a number of areas which would benefit from a survey to determine the community prevalence of *Plasmodium spp*, vector species and abundance as well as the control methods in place (Winskill et al. 2011; Rwanda Ministry of Health 2012). Previous studies in Huye district showed that malaria is seen in one out of every six children under five years (Gahutu et al. 2011). There are no published entomological studies in Huye District to guide the vector control interventions.

This study aimed at providing critical baseline data of malaria vectors in Huye District that can be used to guide vector control intervention. The study also contributed to the existing literature about malaria prevalence and its transmission.

4. Research Questions

1. What is the prevalence of malaria parasite infection in children under five in Rukira cell, Huye district, Southern Rwanda?

2. What are the malaria vector species in Rukira cell, Huye district, Southern Rwanda?

5. Objectives of the Study

5.1. Main Objective

To determine prevalence of malaria parasite infection and vector species abundance in Rukira cell, Huye District, Southern Rwanda

5.2. Specific Objectives

1. To determine prevalence of malaria parasite infection and associated factors in children under-five in the study area
2. To determine the malaria vector species present in the study area
3. To determine the resting and feeding behavior of malaria vectors in the study area
4. To determine the Sporozoite rate in malaria vectors in the study area
5. To determine breeding habitats of the malaria vectors in the study area

CHAPTER THREE: METHODOLOGY

3.1 Study site

The study was conducted in rural Rwandan villages of Rukira Cell located in Huye Sector, Huye district, Southern Province of Rwanda. Huye District has 14 sectors, 77 Cells and 509 villages. The district covers a surface of 581.5 km^2, with a population of 319,000 inhabitants and a population density of 548 inhabitants/ km^2. Rukira Cell is made of 13 villages and covers 10 km^2, with a population of 6,529 persons living in 1600 households. It is situated in the central plateau with hills of an average altitude of 1700m and an average temperature of 20°C. The average rainfall is 1160 mm annually. Wetland agriculture, irrigation and rice cultivation comprise the main economic activity. As regards health, the population of Huye has high burden of malaria, diarrhea and skin disease. Despite availability of some health facilities like referral hospital and health center the district has difficulties in providing efficient health services for the fast growing population (Republic of Rwanda Sothern Province 2013).

Huye district was chosen based on its increased risk of high incidence of malaria where a previous study showed a malaria prevalence of 5.5% (Gahutu et al. 2011), this could be due to its proximity to Gisagara district which has a high burden of malaria. In addition, the closeness to the marshland and irrigation practice in the Rukira cell provides more breeding site of malaria vector mosquito thereby increasing its transmission.

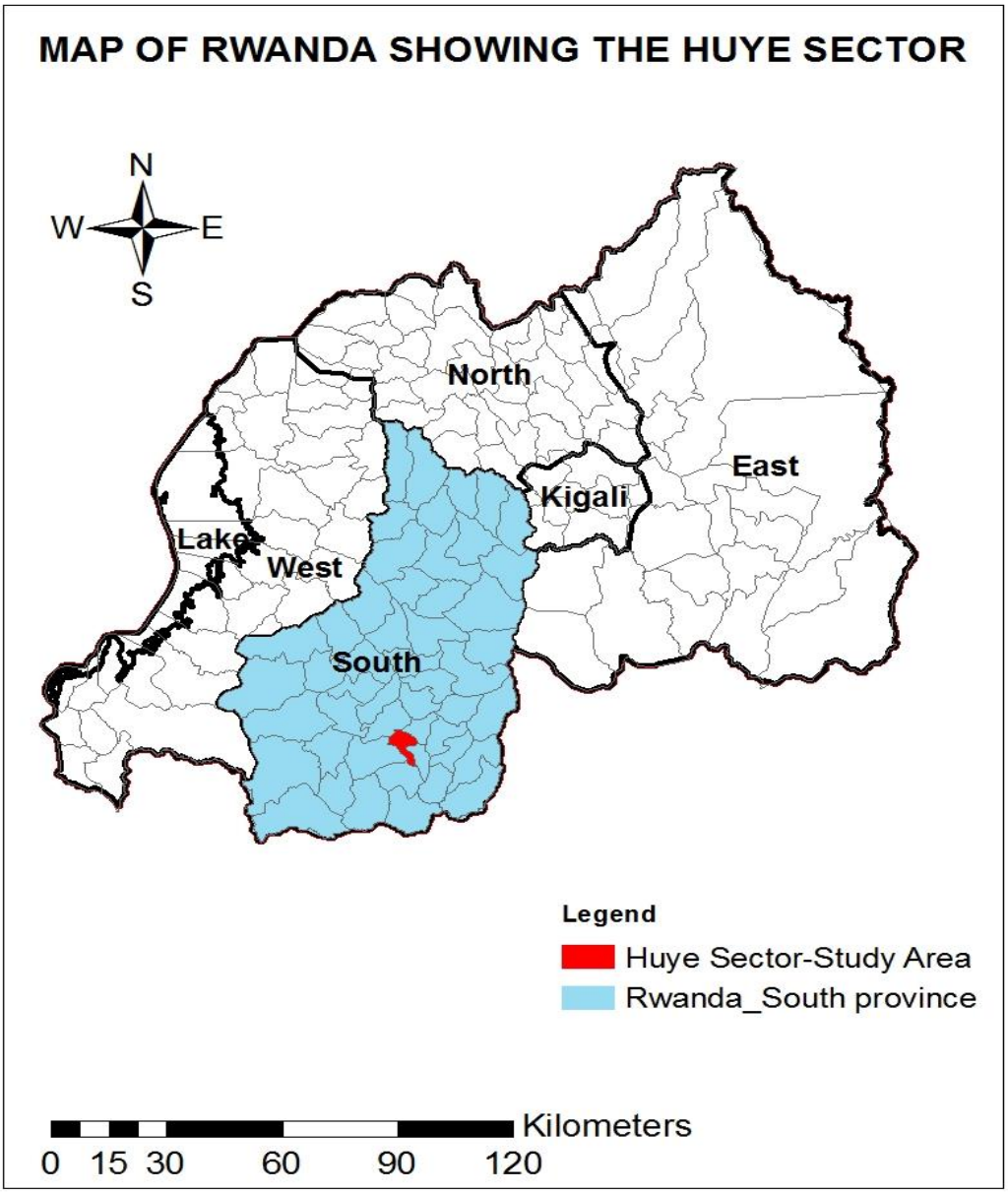

Figure 3. 1: Map of Rwanda

3.2. Study Design

This was a community based cross-sectional descriptive study.

3.3. Study Population

The study was conducted on malaria vector mosquitoes and children under 5 years in each selected household of Rukira Cell. All apparently healthy children less than five years old in Rukira cell were included in the study. Children on treatment against malaria were excluded.

3.4. Sample size determination

The sample size for malaria prevalence determination was calculated using prevalence of malaria in under 5 years children of 5.5% obtained in the study done by Gahutu and others in the southern highlands, near the district capital of Butare (Gahutu et al. 2011).

The formula of Cochran (Cochran 1953) was used to calculate sample size:

$$n = \frac{Z^2 P Q}{L^2}$$

Where n = sample size being calculated,

Z = z statistic at 95% confidence interval, 1.96

P = Estimated prevalence from the most recent previous study, 5.5%

Q = 1-P,

L = Allowable margin of error = 3 %

$$n = \frac{1.96^2 \ 0.055(1-0.055)}{0.03^2} = 221.85 \approx 222$$

Therefore, minimum sample size was 222 children.

3.5. Sampling method and Procedures

A simple random sampling method was applied to select the appropriate sample of children. The list of all children under five years of Rukira cell was obtained from the community health workers. The name of every child was written on a piece of paper which was closed and placed on the table. After mixing 222 pieces of papers with names were blindly selected and opened, children whose names were on the selected pieces of papers were recruited to participate in the study. Children whose parents or guardians declined to give consent, were excluded and their names replaced with those parents/guardians gave consent and their names appeared on the remaining pieces of papers.

During data collection at household level, informed consent was obtained in writing from the household heads. Subsequently, blood samples and demographic data were collected from and about the recruited children. Mosquitoes were also collected from their houses.

3.6. Methods of Data Collection

3.6.1. Blood Sample Collection, smear preparation and Examination

Both thick and thin blood smears were prepared from each selected child following standard operating procedures (SOPs). Middle or ring finger was selected for pricking and blood was allowed to ooze freely without squeezing the finger. Two drops of blood (about 20µl each) were collected on a clean microscopic slide. One drop was used to prepare a thick smear and the other was used to prepare a thin smear (Figure 3.2) according to Cheesbrough (Cheesbrough 1998). Finally the slides were labeled with participant code and packed into slide porter after being air dried (Warhurst & Williams 1996).

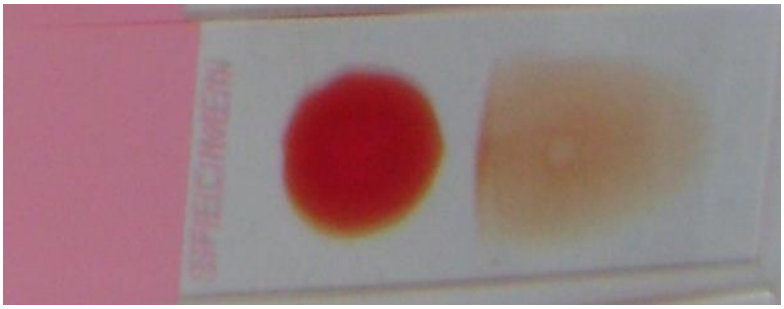

Figure 3. 2: Slide for blood smear

After collection, all slides were transported to Sovu Health Center located in the study area. The thin smear on each slide was fixed with absolute methanol and both thick and thin smears were stained with 10% Giemsa for 10 minutes according to SOPs and examined under a light microscope at Sovu Health Center laboratory.

Parasite negative results were reported based on screening of 100 microscopic fields at X100 magnification and were reported as positive or negative for *Plasmodium spp*. Malaria parasites positive results were based on the finding of the malaria trophozoite, gametocyte or schizont on thick smear. Thin smears were used for species identification.

3.6.2 Independent predictors

Data on independent predictors were collected using both questionnaire and observation methods. The family members were interviewed by researcher to obtain demographic information and risk factors for *Plasmodium spp.* infection such as utilization of mosquito/malaria control measures (e.g. ownership of ITNs, use of ITNs, who uses the nets, if they have had a child under-five with malaria fever in the household in the last two weeks, etc.). Any mosquito breeding sites near (within 2km) the house or nearby swamps were identified by observation (See study questionnaire – Appendix 5).

3.7. Mosquitoes Sample collection and procedures

All mosquito samples were collected in Rukira Cell in Rwanda and further analysis was carried out in Rwanda Biomedical Center - Malaria and Other Parasitic Diseases Division Entomology Laboratory. The Rukira cell is made up of 13 villages. Simple random sampling was applied to select 3 households in each village from which mosquitoes were collected. The list of households in each village was obtained from the Chief of each village then the names of every household was written on a piece of paper separately and after mixing all piece of papers, 3 households from each village were blindly selected from which mosquitoes specimens were collected.

3.7.1. Mosquito Collection and Preservation

Adult mosquitoes were trapped both inside and outside of selected dwellings using 2 entomological techniques namely, Pyrethrum spray catches (PSC) and CDC light traps.

Indoor collection were performed by use of both pyrethrum spray catches and CDC light traps that were placed inside houses from 1800 hours to 0600 hours.

Outdoor biting mosquitoes were trapped by CDC light traps that were set outside houses and cattle sheds.

3.7.1.1. Pyrethrum Spray Catches

Members of the household were informed of the technique prior to spraying. All the items were covered completely and white sheets were spread on floor of the rooms. All the doors and windows were closed. The rooms were sprayed using pyrethrum insecticide by two operators early in the morning from 0600hrs - 0800hrs; one inside and one outside and in opposite directions. After 10 minutes all mosquitoes knocked out on the sheets were collected and placed in a paper cup and labeled appropriately (method used, house number). This method helped to identify endophilic anopheles species and assessment of indoor resting density (World Health Organisation 1975b).

3.7.1.2. CDC Light Traps

The CDC light traps were installed both indoors and outdoors close to the houses. The traps were installed from 1800hrs to 0600hrs. In the morning, the trap collection cage was tied and then the trap switched off. This is to prevent mosquitoes from coming out of the trap. Mosquitoes collected were aspirated using sucking tube and transferred into a paper cup appropriately labeled (World Health Organisation 1975a).

3.7.2. Mosquito larvae collection and preservation

Anopheles larval collection was done to determine possible mosquito breeding sites by sampling water bodies for the presence of anopheles larvae. Pipetting method was used for collecting larvae in small water bodies like container and hoof-prints whereas dipping method was used to collect larvae in large breeding sites such as rice fields and streams (World Health Organisation 1975a). The standard dipper (500ml) was lowered slowly at an angle of 45^0 to avoid disruption and cause the water with nearby larvae to flow into the dipper. If larvae were present, ten dips

were made per site and about 30 minutes were spent at each sites. A pipette was used to collect larvae from the dipper and transferred to a well-labeled vial. The breeding sites were described in terms of permanence state (temporary, permanent, and semi-permanent), location (GPS coordinates, name of the locality), origin of the water (natural, man-made), and type of the breeding site (puddle, rice field, and ditch), water current (flowing or still), sun exposure (full sunlight or partial). Trapped larvae were reared in the entomology laboratory at 27^0c temperature and 80% of humidity and using fish meals as source of feed and the emerging adults were morphologically identified.

3.7.3. Laboratory processing of mosquitoes

3.7.3.1. Mosquito Identification

A dissecting microscope was used to morphologically identify the mosquitoes collected. The mosquitoes were grouped in to two families; Culicidae and Anopheline. The anophelines were further classified using the morphological keys available (Gillies and De Meillion (1968), Gillies and Coetzee (1987) in to two species complex, *An. funestus* and *An. gambiae* complex. Further identification into sibling species can only be done using PCR which was not done in this study.

3.7.3.2. Mosquitoes Dissection

All female anophelines collected were dissected transversely under a dissecting microscope at the thorax between the 1st and 3rd pairs of legs, the head and thorax were conserved in silica gel and cotton wool at room temperature for ELISA test for sporozoite detection. For fed mosquitoes, abdomens were used to test sources of blood meal by ELISA method. A good system for identifying and labeling samples was maintained, mosquitoes were handled with care to avoid contamination between specimens.

3.7.3.3. Infection rates in mosquitoes

The involvement of each species in malaria transmission was assessed using sporozoite ELISA tests for *Plasmodium falciparum* sporozoite infection. Sporozoite ELISA is based on immunochemical technique Circumsporozoite-ELISA (CS-ELISA) which uses antibodies to

detect specific circumsporozoite antigens of interest which cover the outer surface of the malaria sporozoites and is hence an indicator of the presence of the infective stage of the *Plasmodium* parasite (Appendix 3).

CSA begins to be expressed when the sporozoites are still in the mature oocyst stage in the mosquito midgut. Hence only the thorax and head of each female mosquito was tested to ensure that if CSA was detected, it was mostly from sporozoites that had reached the salivary glands suggesting that the female mosquito was infective. Mosquitos' specimens were preserved dry inside test tubes filled with silica gel and cotton at room temperature prior to this assay.

3.7.4. Entomological Indices Calculation

The man biting index (the number of mosquito bites per person per night), was calculated by the total number of fresh-fed and half-gravid mosquitoes caught in PSC divided by the number of persons sleeping in the house the night before the collection. Entomological Inoculation Rate (EIR), is a measure of the transmission intensity of malaria which is a product of man-biting index and sporozoite rate.

The human blood index is a total number of blood-fed mosquitoes that had fed on humans out of the total number tested. Indoor resting density was calculated as (number of females divided by number of houses) × number of days. The sporozoite index was determined as the proportion of the total number of females anopheles mosquitoes carrying infective sporozoites in the head-thorax out of the total number tested.

3.8 Quality assurance

Collection of blood samples, mosquitos' specimens, labeling, storage and transportation to the laboratory was in accordance with standard operating procedures. This was also applied to laboratory procedures. Preparation of reagents and testing procedures was carried out with regard to the kits' and equipment' manufacturer's instructions. Accuracy and quality of data were maintained at all stages. 10% of both randomly selected negative and positive smears, were sent to CHUB Referral Hospital Laboratory to be re-examined through microscopy by an independent parasitologist for quality control.

3.9 Ethical considerations

Before commencement of the study ethical clearance was obtained from Kenyatta National Hospital - University of Nairobi Ethics and Research Committee and Institution Review Board of University of Rwanda. Permission to conduct research in the area was sought from the administration of the study area and informed consent was obtained from the household heads / Parents or guardians of children before mosquito and blood smear collection from under 5 children was carried out. Children with malaria were referred to the nearest health center and the principal investigator covered the cost for appropriate treatment. Participant privacy and confidentiality were strictly observed. All data collected in hard copy were kept in a lockable cabinet accessible to the researcher only to maintain confidentiality. Information stored in soft copies was protected from access from unauthorized persons by password which was changed periodically. All records were identified by study identification number. All data collected (soft and hard) will be kept for a minimum period of 5 years.

3.10 Data management and analysis

Data were coded and double entered into computer MS-Excel. Verification and validation of the data were done by rechecking all data entries with original data forms to achieve a clean dataset that was then exported into a Statistical Package format (SPSS Version 22.0). Regular file back-up was done to avoid any loss or tampering.

Descriptive analysis using frequencies, proportions, means and standard deviation were computed. Pearson's chi-square test was used to establish the association between presence of malaria parasite and independent variables. Odds ratio (OR) and 95% Confidence interval (CI) were used to estimate the strength of association between the independent variables and malaria. All the independent variables found to be significantly associated with malaria at bivariate analysis were considered together in multivariable analysis. This was performed using binary logistic regression by specifying '*backward conditional*' method with removal at $p < 0.05$.

Kruskal Wallis and/ or Mann-Whitney U tests were used to compare mean density of larvae and physical characteristics of breeding site as the data was not normally distributed. The threshold for statistical significance was set at $p < 0.05$.

3.11 Dissemination of findings

The findings of the study will be shared with the Rwandan Ministry of Health to inform appropriate vector control measures for the community towards malaria elimination. Dissemination to national malaria partners and Malaria Vector Control Division will also be done so as to guide programming. In addition, the results will be presented through seminars, conferences and peer-reviewed publications.

3.12 Funding

The study was funded by the principal investigator as a Master of Science (MSc.) research project at the University of Nairobi.

CHAPTER FOUR: RESULTS

4.0 Introduction

This chapter presents the findings of the study. The findings are presented and interpreted based on the objectives of the study. A total of 222 parents/guardians with children less than five years gave consent for participation in the study at Rukira Cell, Rwanda. The respondents were drawn from 13 villages. Moreover, 567 mosquitoes were collected from 39 households and 22 breeding sites for mosquitoes were also included in the study. The results are presented in sections that cover: Socio-demographic characteristics of parents/guardians and children; prevalence of malaria and its associated factors; malaria vector species; sporozoite rate in malaria vectors, source of blood meal for malaria vectors and breeding habitats of malaria vectors.

Section I: Prevalence of malaria parasite and its associated factors

4.1.1 Socio-demographic characteristics of parents/guardians and children

A total of 222 children participated in the study. The distribution of selected socio-demographic characteristics among parents/guardians and children who participated in this study is shown in Table 4.1. The mean age of the children was 32.2 months. The highest percentage (28.8%, 64/222) of the children was within the age group of 25-36 months followed by (23.4%, 52/222) aged between 13-24 months. Female children were slightly more (54.1%, 120/222) than male children (45.9%, 102/222). Most of the parents/guardians were married (95.9%, 213.222) while only (4.1%, 7/222) were widowed. Nearly all the parents/guardians (99.5%, 221/221) attended primary school and most (97.3%, 216/222) were farmers.

Table 4. 1: Socio-demographic characteristics of parents/guardians and children

Variables	**n=222**	**%**
Age of child		
1-12 months	23	10.4
13-24 months	52	23.4
25-36 months	64	28.8
37-48 months	47	21.2
49-59 months	36	16.2
Mean age (±*SD) = 32.2 (±15.4)		
Sex of the child		
Male	102	45.9
Female	120	54.1
Marital status of child's parent/guardian		
Married	213	95.9
Widowed	9	4.1
Education level of child's parent/guardian		
Primary	221	99.5
Tertiary	1	0.5
Occupation of child's parent/guardian		
Farmer	216	97.3
Trader	2	0.9
Nurse	2	0.9
Teacher	2	0.9
*SD = Standard deviation		

4.1.1.1 Distribution of respondents by village

The highest proportion (13.5%, n=30) of the respondents were from Agahenerezo village followed by (11.3%, n=25) from Kubutare village as shown in Figure 4.1.

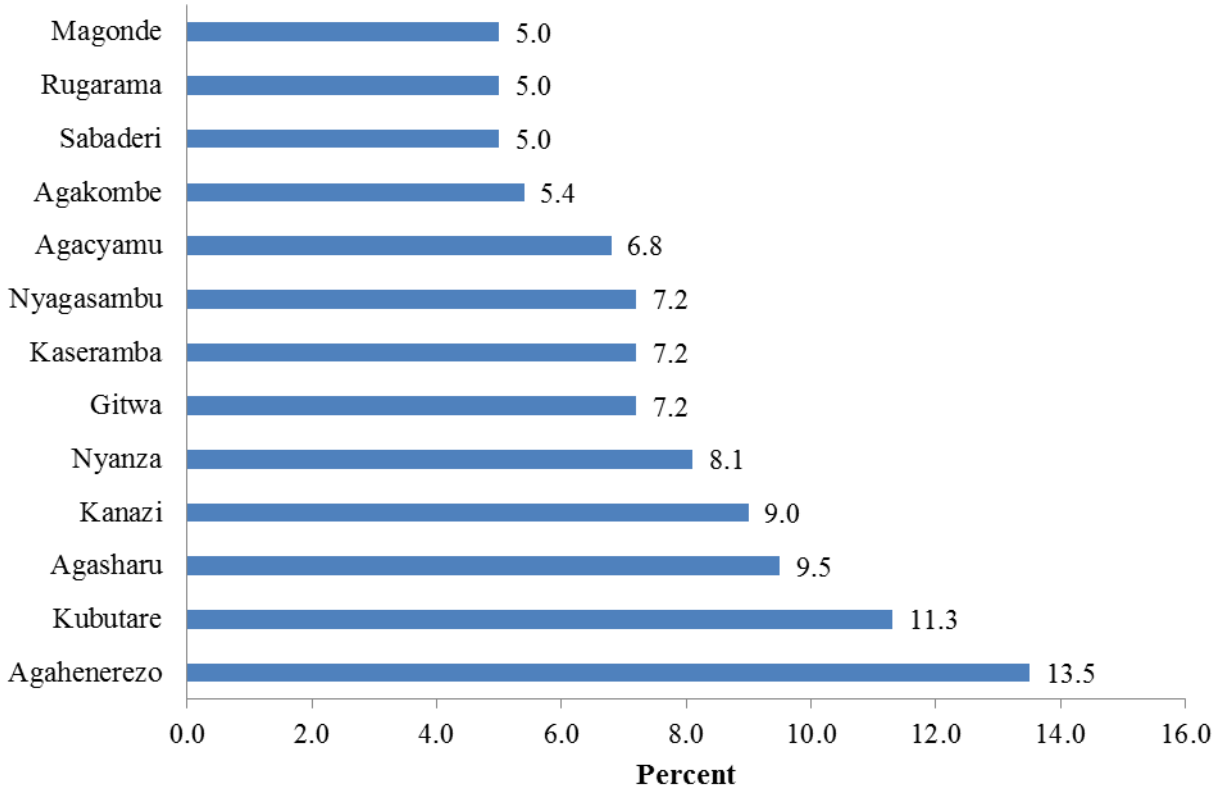

Figure 4. 1: Distribution of respondents by village

4.1.1.2 Age of children stratified by sex

Figure 4.2 shows distribution of age by sex among children. Males aged 1-12 months were more (74.0%) compared to females (26.0%) in the same age category. Figure 4.2 further shows the number of females increase with the increase of age. However, the frequency of males decreased as the age increased.

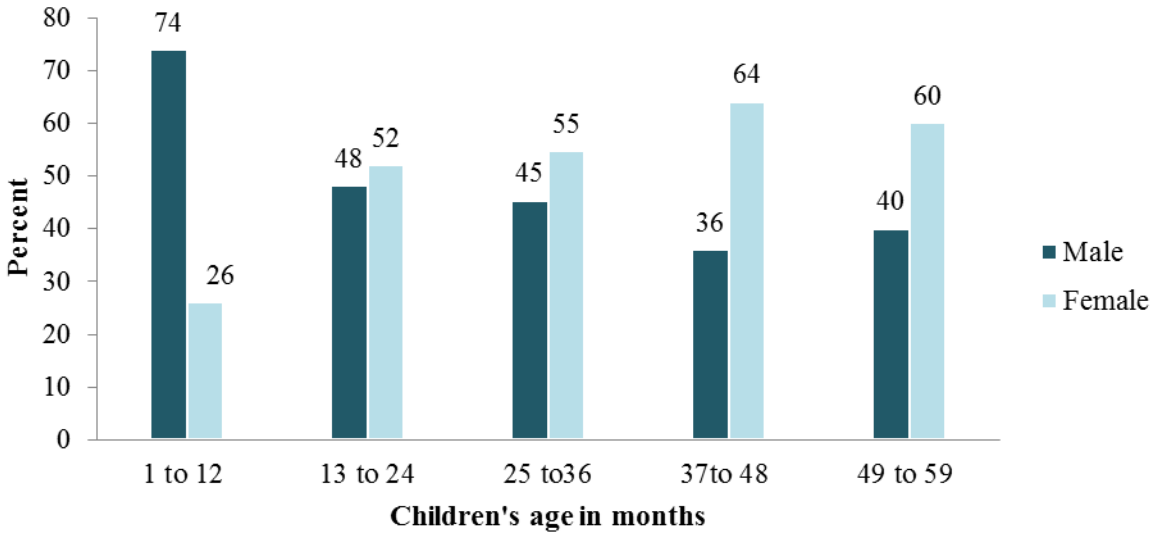

Figure 4. 2: Age of children stratified by sex

4.1.2 Malaria vector control interventions in respondents' house

A large number of the respondents (97.3%, 216/222) indicated that they possessed an insecticide-treated net while the remaining (2.7%, 6/222) reported otherwise. Most of the respondents (96.8%, 209/216), reported sleeping under insecticide-treated net whereas (3.2%, 7/216) did not. All respondents reported that they were not using house spraying for mosquito control. Similarly, nearly all respondents (98.2%. n=218/222) lived in houses with no window screens for mosquito control (Table 4.2).

Table 4. 2: Malaria vector control interventions in respondents' houses

Variables	**n=222**	**%**
Possession of insecticide treated net		
Yes	216	97.3
No	6	2.7
Sleeping under insecticide treated net		
Yes	209	96.8
No	7	3.2
Availability of screen windows		
Yes	4	1.8
No	218	98.2
House spraying for mosquito control		
Yes	0	0.0
No	222	100.0

4.1.3 Breeding sites for mosquitoes

Table 4.3 shows the mosquitoes breeding sites near the 222 households where the child less than five years old was selected from each. The breeding site near each house was examined. Most 214 (96.4%, 214/222) of them had favorable mosquito breeding sites near the house (i.e. within 2 km). The main types of breeding sites present for mosquitoes were rice paddies (32.7%, 70/214), crashed containers (30.8%, 66/214) and stagnant water (23.4%, 50/214).

Table 4. 3: Breeding sites for mosquitoes near respondents' houses

Variables	**n=222**	**%**
Presence of mosquito breeding sites near the house		
Yes	214	96.4
No	8	3.6
Types of breeding site present (n=214)		
Rice paddies	70	32.7
Crashed containers	66	30.8
Stagnant water	50	23.4
Ground pools	15	7.0
Rice paddies and stagnant water	13	6.1
Not applicable	8	

4.1.4 Malaria among children one month prior to data collection

Figure 4.3 demonstrates number of children who were reported to have suffered from malaria one month prior to data collection. Most, (95.0%, 211/222), of the children were reported to have had malaria.

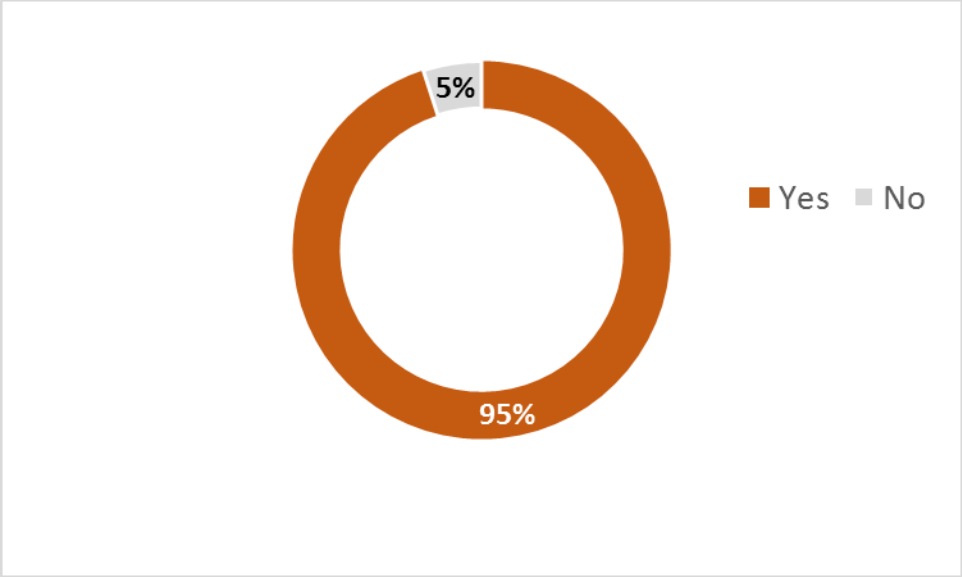

Figure 4. 3: Malaria among children one month prior to data collection by parental report

4.1.5 Prevalence of *Plasmodium* infection among children

At the time of the study, the prevalence of *Plasmodium spp.* infection among the children was 12.2% (95% CI of 7.89% to 16.51%). Microscopic examination of thin smear was used to detect the presence and type of *Plasmodium spp.* and all were *P. falciparum* (Table 4.4).

Table 4. 4: Prevalence of *Plasmodium* infection among the children by Microscopy

Variables	**n=222**	**%**
Malaria test results of the child		
Positive	27	12.2
Negative	195	87.8
Types of plasmodium species seen		
P. falciparum	27	100.0
Not applicable	195	

4.1.6 Distribution of sex by malaria status, age, sleeping under mosquito nets and presence of mosquito breeding site

Table 4.5 summarizes the distribution of sex by malaria status, age, use of mosquito nets and presence of mosquito breeding site. Malaria was reported more among males children aged 1-12 months 5 (71.4%), not sleeping under mosquito net 6 (75.0%) and living near mosquito breeding sites 18 (66.7%) than in females aged 1-12 months 2 (28.6%), not sleeping under mosquito net 2 (25.0%) and living near mosquito breeding site 9 (33.3%).

Table 4. 5 : Distribution of sex by malaria status, age, sleeping under mosquito nets and presence of mosquito breeding site

Status of Malaria	Variables	Male, n (%)	Female, n (%)
	Age		
Positive	1-12 months	5 (71.4%)	2 (28.6%)
	13-59 months	13 (65.0%)	7 (35.0%)
Negative	1-12 months	12 (75.0%)	4 (25.0%)
	13-59 months	72 (40.2%)	107 (59.8%)
	Sleeping under mosquito nets		
Positive	Yes	12 (63.2%)	7 (36.8%)
	No	6 (75.0%)	2 (25.0%)
Negative	Yes	82 (43.2%)	108 (56.8%)
	No	2 (40.0%)	3 (60.0%)
	Presence of mosquito breeding site		
Positive	Yes	18 (66.7%)	9 (33.3%)
	No	0 (0.0%)	0 (0.0%)
Negative	Yes	80 (42.8%)	107 (57.2%)
	No	4 (50.0%)	4 (50.0%)

4.1.7 Association between socio-demographic characteristics and *plasmodium* infection among children

Table 4.6 shows the relationship between socio-demographic characteristics of the parent/guardians/children and occurrence of malaria parasite among children. Children aged 1 to 12 months were more likely to have malaria infection than children aged 13 to 59 months; 7/23 (30.4%) and 20/199 (10.1%) respectively [**OR=3.92; 95%CI=1.44-10.66; p=0.005**]. The proportion of malaria infection was higher among male children compared to their female counterparts; 18/102 (17.6%) and 9/120 (7.5%) respectively [**OR=2.64; 95%CI=1.13-6.17; p=0.021**].

Table 4. 6: Association between socio-demographic characteristics and malaria parasite infection

Variable	Status of Malaria Infection		OR (95%CI)	Chi square
	Positive, n (%)	Negative, n (%)		*P value
Age of child				
1-12 months	7(30.4%)	16(69.6%)	3.92 (1.44-10.66)	**0.005**
13-59 months	20(10.1%)	179(89.9%)	Reference	
Sex of the child				
Male	18(17.6%)	84(82.4%)	**2.64 (1.13-6.17)**	**0.021**
Female	9(7.5%)	111(92.5%)	Reference	
Marital status of child's parent/guardian				
Married	26(12.2%)	187(87.8%)	1.11 (0.13-9.26)	0.922
Widowed	1(11.1%)	8(88.9%)	Reference	
Village				
Agasharu	4(19.0%)	17(81.0%)	4.47 (0.45-44.01)	0.199
Magonde	2(18.2%)	9(81.8%)	4.22 (0.33-52.90)	0.264
Rugarama	2(18.2%)	9(81.8%)	4.22 (0.33-52.90)	0.264
Agakombe	2(16.7%)	10(83.3%)	3.80 (0.31-47.21)	0.299
Kubutare	4(16.0%)	21(84.0%)	3.62 (0.37-47.21)	0.268
Agacyamu	2(13. %)	13(86.7%)	2.92 (0.24-35.29)	0.401
Kaseramba	2(12.5%)	14(87.5%)	2.71 (0.23-35.68)	0.433
Nyagasambu	2(12.5%)	14(87.5%)	2.71 (0.22-32.99)	0.433
Nyanza	2(11.1%)	16(88.9%)	2.36 (0.19-28.67)	0.496
Sabaderi	1(9.1%)	10(90.9%)	1.90 (0.11-33.7)	0.662
Agahenerezo	2(6.7%)	28(93.3%)	1.35 (0.11-16.05)	0.809
Gitwa	1(6.2%)	15(93.85)	1.26 (0.07-21.97)	0.871
Kanazi	1(5.0%)	19(95.0%)	Reference	

OR= Odds ratio; CI= Confidence Interval; * Significant p value

4.1.8 Relationship of malaria vector control interventions /breeding sites of mosquitoes with malaria parasite infection among children

Bivariate analysis of the association between preventive methods of malaria/breeding sites of mosquitoes and prevalence of malaria parasite infection among children is summarized in Table 4.7. Children who were not sleeping under insecticide-treated net were more likely to have malaria infection 8/13 (61.5%) (**OR=16.0; 95%CI = 4.76-53.80; p < 0.001**) compared to those who were sleeping under insecticide-treated net 19/209 (9.1%). However, there was no significant association between malaria infection and Possession of insecticide treated net, Window screen, Presence of mosquito breeding sites near the house and Whether suffered from malaria in the last month.

Table 4. 7: Relationship of malaria vector control interventions /breeding sites of mosquitoes with malaria parasite infection among children

Variable	Status of Malaria infection		OR (95%CI)	Chi square
	Positive, n(%)	Negative, n(%)		*p value
Possession of insecticide treated net				
Yes	26 (12.0%)	190 (88.0%)	Reference	
No	1 (16.7%)	5 (83.3%)	1.46 (0.16-13.0)	0.734
Sleeping under insecticide treated net				
Yes	19 (9.1%)	190 (90.9%)	Reference	
No	8 (61.5%)	5 (38.55)	**16.0 (4.76-53.80)**	**<0.001**
Window screen				
Yes	1 (25.0%)	3(75.0%)	2.46 (0.25-24.55)	0.428
No	26 (11.95)	192(88.1%)	Reference	
Presence of mosquito breeding sites near the house				
Yes	27 (12.6%)	187 (87.4%)	-	0.284
No	0 (0.0%)	8 (100.0%)	UD	
Whether suffered from malaria in the last month				
Yes	26 (12.3%)	185 (87.7%)	1.41 (0.17-11.43)	0.749
No	1 (9.1%)	10 (90.9%)		

OR= Odds ratio; CI= Confidence Interval; UD= Undefined; * Significant p value

4.1.9 Multivariable analysis of factors associated with malaria parasite infection among children

Binary logistic regression analysis was applied to identify the variables independently associated with malaria parasite infection among children less than five years. Three (3) factors that showed significant association (p <0.05) during bivariate analysis were considered together in multivariable analysis. Upon fitting the factors using binary logistic regression and specifying *'backward conditional'* method with removal at p<0.05, two factors remained in the final analysis as shown in Table 4.8.

Children who were not sleeping under insecticide treated net were 15 times more likely to be infected with malaria [**AOR=15.27; 95%CI=4.42-52.82; *p*<0.001**] compared to those who were sleeping under insecticide treated net.

Table 4. 8: Multivariable analysis of factors associated with malaria parasite infection among children

		95%CI		Chi square
Predictor	**AOR**	**Lower**	**Upper**	***P value**
Full model				
Age of child				
1-12 months	2.93	0.98	8.80	0.055
13-59 months	Reference			
Sex of the child				
Male	2.24	0.88	5.70	0.091
Female	Reference			
Sleeping under insecticide treated net				
Yes	Reference			
No	14.33	4.13	49.77	**<0.001**
Reduced model				
Age of child				
1-12 months	3.56	1.18	10.71	**0.024**
13-59 months	Reference			
Sleeping under insecticide treated net				
Yes	Reference			
No	15.27	4.42	52.82	**<0.001**

AOR= Adjusted odds ratio; CI= Confidence Interval; * Significant p value

Section II: Malaria vector species

4.2.1 Distribution of trapped mosquitoes by village

Five hundred and sixty- seven (567) mosquitoes were trapped from 13 villages. The highest proportion (17.3%, 98/567) of the mosquitoes were collected from Agacyamu village followed by (14.6%, 25/569) which were collected from Gitwa village as summarized in Figure 4.4.

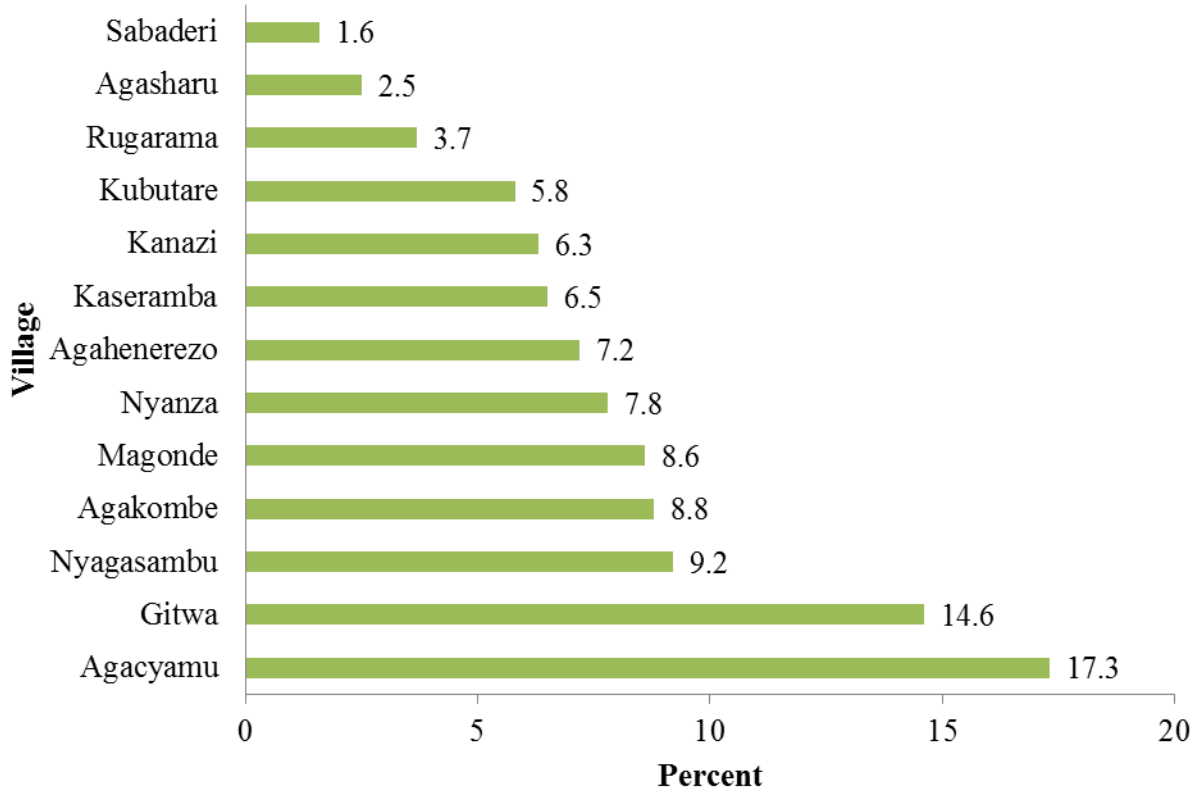

Figure 4. 4: Distribution of mosquitoes by village (n=567)

4.2.2 Collection method, place of collection, blood meal and anopheles species

Majority of the mosquitoes (67.5%, 383/567) were collected using light traps while the remaining were collected using pyrethrum spray catches (32.5%, 184/567). Similarly, majority of the mosquitoes (63.5%, 360/567) were captured indoors whereas (36.5%, 207/567) were collected from outdoors. About a quarter (28.0%, 159/567) of the mosquitoes had fed on a blood meal. Majority of the mosquitoes (69.7%, 395/567) belonged to *An. gambiae s.l.* (Table 4.9).

Table 4. 9: Collection method, place of collection, blood meal and anopheles species

Variable	**n=567**	**%**
Methods of collection		
Pyrethrum Spray Catches	184	32.5
Light Trap	383	67.5
Place of collection		
Indoors	360	63.5
Outdoors	207	36.5
Blood meal		
Fed	159	28
Unfed	408	72
***Anopheles* species**		
An. gambiae s.l	395	69.7
An. funestus	23	4.1
An. ziemanni	10	1.8
An. maculipalpis	37	6.5
An. squamosus	94	16.6
An. pharoensis	7	1.2
An. coustani	1	0.2

4.2.3 Source of blood meal (n=159)

Figure 4.5 depicts the preference of host for blood meal among those mosquitoes that were fed; nearly half fed on human blood. Human blood index was 50.9% (81/159).

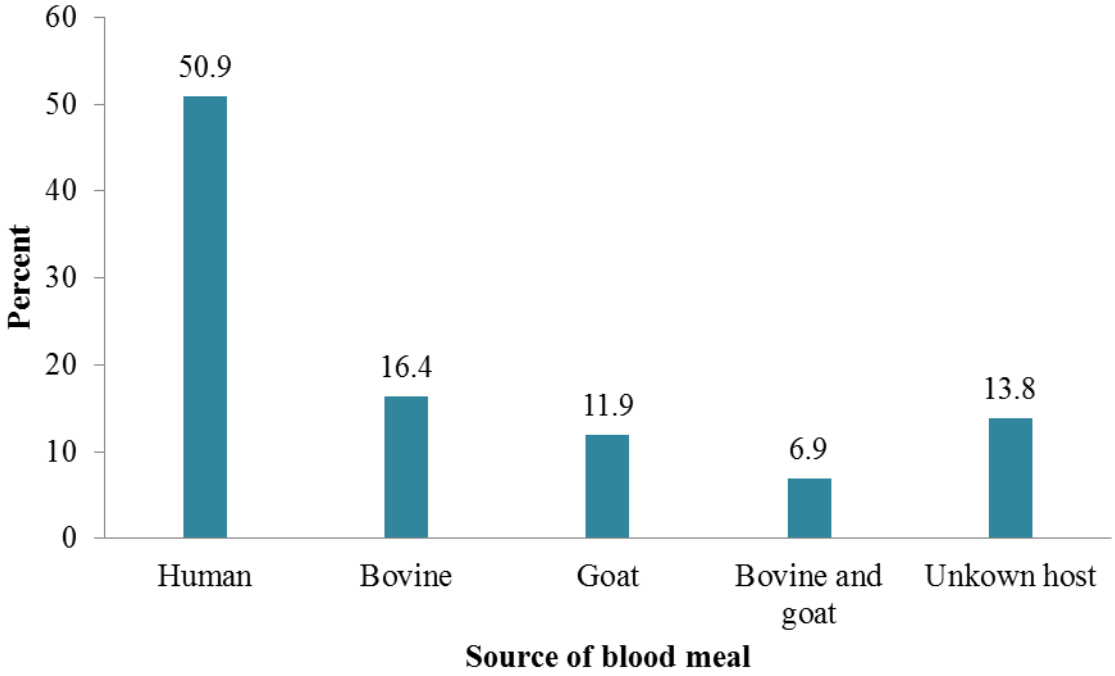

Figure 4. 5: Source of blood meal among the mosquitoes

4.2.4 Collection method, place of collection and anopheles species stratified by source of blood meal

Table 4.10 presents the collection method, place of collection and anopheles species stratified by source of blood meal. There was a statistically significant association between source of blood meal and collection method ($p<0.001$) where human blood meal was higher among mosquitoes caught by pyrethrum spray 65/84 (77.4%) than those caught by light trap 16/75 (21.3%). Similarly, mosquitoes collected indoors were more likely to have human blood meal 79/108 (73.1%) than those collected outdoors 2/51 (3.9%; $p< 0.001$). There was also significant association between the source of blood meal and anopheles mosquito species ($p<0.001$).

Table 4. 10: Collection method, place of collection and anopheles species stratified by source of blood meal

Variable	Total	Source of blood meal, (n=159)					Chi square
		Human, n(%)	Bovine, n(%)	Goat, n(%)	Bovine and goat, n(%)	Unknown host, n(%)	*p value
Method of collection							
Pyrethrum Spray	84	65(77.4%)	8(9.5%)	3(3.6%)	1(1.2%)	7(8.3%)	**<0.001**
Light Trap	75	16(21.3%)	18(24.0%)	16(21.3%)	10(13.3%)	15(20.0%)	
Place of collection							
Indoors	108	79(73.1%)	10(9.3%)	7(6.5%)	3(2.8%)	9(8.3%)	**<0.001**
Outdoors	51	2(3.9%)	16(31.4%)	12(23.5%)	8(15.7%)	13(25.5%)	
Anopheles species							
An. gambiae s.l	107	69(64.5%)	15(14.0%)	9(8.4%)	3(2.8%)	11(10.3%)	
An. funestus	10	7(70.0%)	0(0.0%)	0(0.0%)	0(0.0%)	3(30.0%)	
An. ziemanni	4	3(75.0%)	0(0.0%)	0(0.0%)	0(0.0%)	1(25.0%)	**<0.001**
An. maculipalpis	6	0(0.0%)	3(50.0%)	0(0.0%)	0(0.0%)	3(50.0%)	
An. squamosus	32	2(6.2%)	8(25.0%)	10(31.2%)	8(25.0%)	4(12.5%)	

4.2.5 Indoor resting density and Human biting rate

The overall indoor resting density was 5 mosquitoes per house. The highest indoor resting density of 10 mosquitoes per house was found in Gitwa village, followed by Kanazi and Agacyamu villages which had 9 mosquitoes per house. No mosquitoes were trapped indoors in Kaseramba village as summarized in Table 4.11. However the difference of indoor resting density between villages was not statistically significant by Kruskal Wallis test (p= 0.446).

Table 4. 11: Indoor resting density per village

Village	Mosquito caught by PSC	No of the house	Anopheles Density per house/day
Gitwa	29	3	10
Kubutare	27	3	9
Kanazi	28	3	9
Agacyamu	28	3	9
Nyagasambu	24	3	8
Magonde	12	3	4
Rugarama	11	3	4
Nyanza	10	3	3
Agasharu	5	3	2
Agakombe	4	3	1
Agahenerezo	4	3	1
Sabaderi	2	3	1
Kaseramba	0	3	0
Total	184	39	5

The number of persons sleeping in the house the night preceding the collection in the 39 selected households was 85 persons. The human biting rates (average of mosquito bites /person /night) was 0.988 (84/85) indicating that an individual would receive 0.988 mosquito bite per night.

4.2.6 *P. falciparum* Sporozoite rate and Entomological inoculation rate.

According to *P. falciparum* CSP ELISA test conducted, most (98.1%, 556 / 567) of the mosquitoes tested negative for *P.falciparum* sporozoite and only (1.9%, 11/567) tested positive as presented in Figure 4.6.

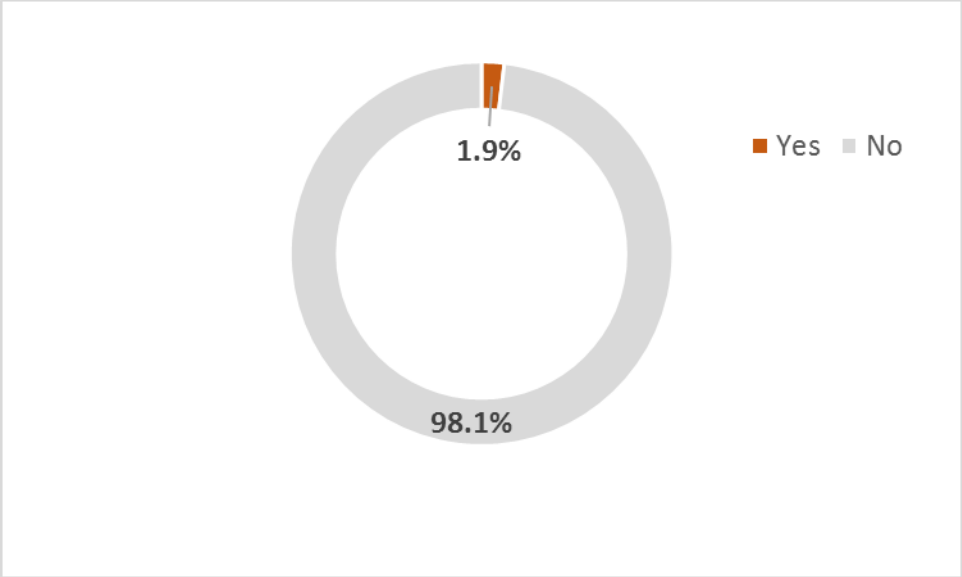

Figure 4. 6: Distribution of *P. falciparum* sporozoite test results

Entomological inoculation rate as a standard measure of transmission intensity was 0.019 (0.98x 1.9%) indicating that an individual would receive 0.019 infective bite every day and 7.068 infective bites per person per year.

4.2.7 Collection method, place of collection, blood meal and anopheles species classified by *P. falciparum* sporozoite status

Results of bivariate analysis of the association between collection methods, place of collection, blood meal and anopheles species and prevalence of positive sporozoite is shown in Table 4.12. Sporozoite positivity was significantly more among mosquitoes fed on blood meal compared to those that were not fed on blood meal (p=0.008). Similarly, the proportion of positivity was significantly higher among mosquitoes collected indoors than those collected outdoors (p=0.048).

However, there was no significant association between prevalence of sporozoites and collection methods as well as mosquito species.

Table 4. 12: Collection method, place of collection, blood meal and anopheles species classified by sporozoite test results

Variable	Total	Sporozoite test results		Chi square
		Positive, n(%)	Negative, n(%)	*p value
Method of collection				
Pyrethrum Spray Catches	184	5(2.7%)	179(97.3%)	0.352
Light Trap	383	6(1.6%)	377(98.4%)	
Place of collection				
Indoors	360	10(2.8%)	350(97.2%)	**0.048**
Outdoors	207	1(0.5%)	206(99.5%)	
Blood meal				
Fed	159	7(4.4%)	152(95.6%)	**0.008**
Unfed	408	4(1.0%)	404(99.0%)	
Anopheles species				
An. gambiae s.l	395	8(2.0%)	387(98.0%)	
An. funestus	23	0(0.0%)	23(100.0%)	
An. ziemanni	10	1(10.0%)	9(90.0%)	
An. maculipalpis	37	1(2.7%)	36(97.3%)	0.605
An. squamosus	94	1(1.1%)	93(98.9%)	
An. pharoensis	7	0(0.0%)	7(100.0%)	
An. coustani	1	0(0.0%)	7(100%)	
***Anopheles gambiae s.l* versus other species**				
An. gambiae s.l	395	8(2.0%)	387(98.0%)	0.823
Others species	172	3(1.7%)	169(98.3%)	

Section III: Breeding habitats of malaria vector

4.3.1 Distribution of breeding sites among villages (n=22)

Among the 22 breeding sites, 40.9% were found in Agahenerezo village followed by Sabaderi village (18.2%, 4/22) and Nyanza village (13.6%, 3/22) (Figure 4.7).

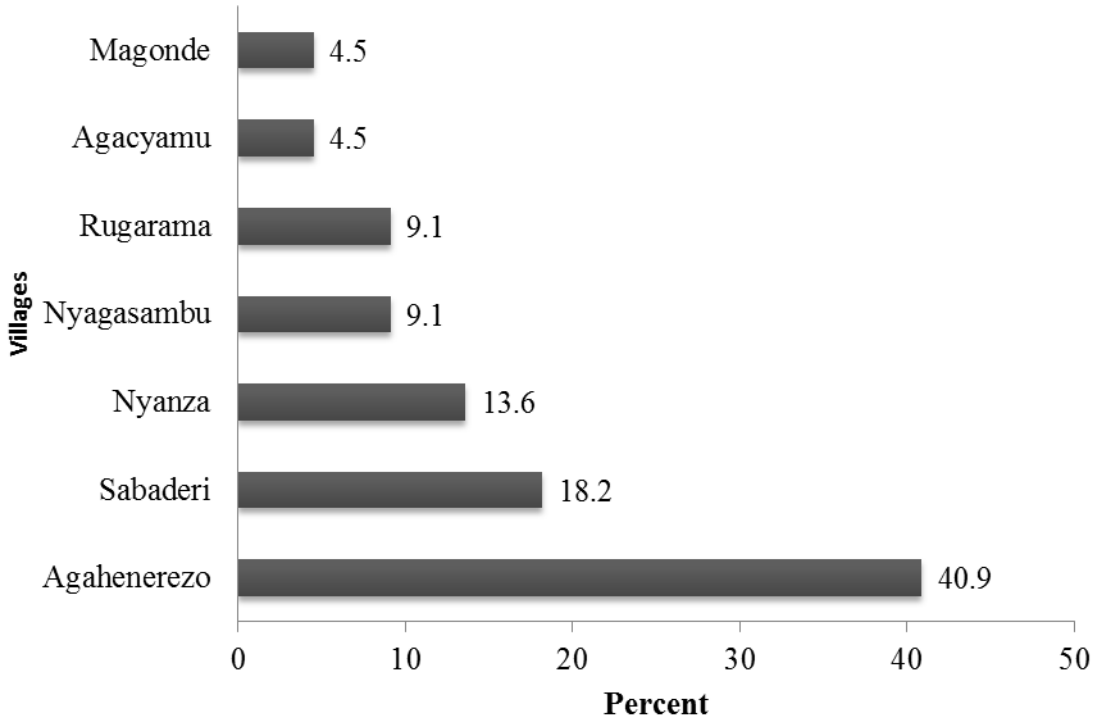

Figure 4. 7: Distribution of the breeding sites among villages

4.3.2 Physical characteristics of potential breeding sites

Table 4.13 shows the characteristics of the breeding sites included in the study. There was almost equal distribution on the types of breeding sites with (27.3%, 6/22) from rice paddies, (13.65%, 3/22) from irrigation channel borders, (22.7%, 5/22) from ground pools, (13.6%, 3/22) from stagnant water and (22.7%, 5/22) from crashed pots and other containers. Twelve (54.5%, 12/22) of the breeding sites were temporal and (45.5%, 10/22) were permanent breeding sites. The water current of most (81.8%, 18/22) of the breeding sites were still. With respect to intensity of light, majority (86.4%, 19/22) of the breeding sites had full light while the remaining (13.6%, 3/22) had partial light. About two thirds (63.6%, 14/22) of the breeding sites were of manmade origin whereas 8 (36.4%) were natural. Most of the breeding sites identified (86.4%, 19/22) had larvae.

Table 4. 13: Physical characteristics of the breeding sites

Variables	n=22	%
Type of breeding site		
Rice paddy	6	27.3
Irrigation channel borders	3	13.6
Ground pool	5	22.7
Stagnant water	3	13.6
Crashed pot and other containers	5	22.7
Breeding site state		
Permanent	10	45.5
Temporal	12	54.5
Water current		
Still	18	81.8
Slow flowing	4	18.2
Intensity of light		
Full sunlight	19	86.4
Partial	3	13.6
Origin of breeding site		
Natural	8	36.4
Man made	14	63.6
Presence of Larvae		
Yes	19	86.4
No	3	13.6

4.3.3 Counts of anopheles larvae

A total of 661 larvae of the genus anopheles were collected from 22 larvae habitat and reared to adults in the entomology laboratory insectary at a temperature of 27^{o}C and humidity of 80%. Adults were identified using morphological characteristics. They comprised of 2 species including *An. gambae s.l* and *An. ziemanni*. The counts of *An. gambae s.l* were more (589) compared to *An. ziemanni* (72) as shown in Table 4.14.

Table 4. 14: Mean of anopheles larvae counts

	Total	Minimum	Maximum	Mean	Std. Deviation
An. gambae s.l	589	0	77	31.0	24.7
An ziemanni	72	0	20	3.8	5.7
Total	661	2	82	34.9	25.2

4.3.4 Comparison of mean density of larvae with physical characteristics of breeding site

Kruskal Wallis and/ or Mann-Whitney U tests were used to compare mean density of larvae with physical characteristics of breeding sites (Table 4.15). There was statistically significant association between mean rank density of larvae and water current (p=0.038). The mean rank in still water was higher than slow flowing water. However, there was no significant association between the other variables.

Table 4. 15: Comparison of mean density of larvae with physical characteristics of breeding site

Physical characteristics	N=19	Mean rank	Kruskal Wallis/Mann-Whitney U test
			p value
Village			
Agahenerezo	8	10.0	
Nyagasambu	1	6.0	
Sabaderi	4	7.4	
Agacyamu	1	8.0	0.596
Nyanza	3	14.7	
Rugarama	2	11.3	
Magonde	1	2.0	
Type of breeding site			
Rice paddies	6	11.6	
Irrigation channel borders	2	1.8	
Ground pools	5	12.3	0.196
Stagnant water	3	10.5	
Crashed pots and other containers	3	8.0	
Breeding site state			
Permanent	9	11.9	0.152
Temporal	10	8.3	
Water current			
Still	16	11.2	**0.038**
Slow flowing	3	3.8	
Intensity of light			
Full sunlight	16	9.5	0.400
Partial	3	12.5	
Origin of breeding site			
Natural	8	10.9	0.562
Man-made	11	9.4	

The following images (picture1-5) depict different types of Anopheles breeding sites surveyed.

Picture1: Stagnant water

Picture2 : Irrigation Channel border

Picture3 : Rice paddy

Picture4 : Ground pool

Picture5 : Crashed pot

Identified breeding habits were localized with Global Positioning System. Figure 4.8 shows the location of potential mosquito breeding habits in Rukira cell of Huye sector, Huye district Southern Province of Rwanda.

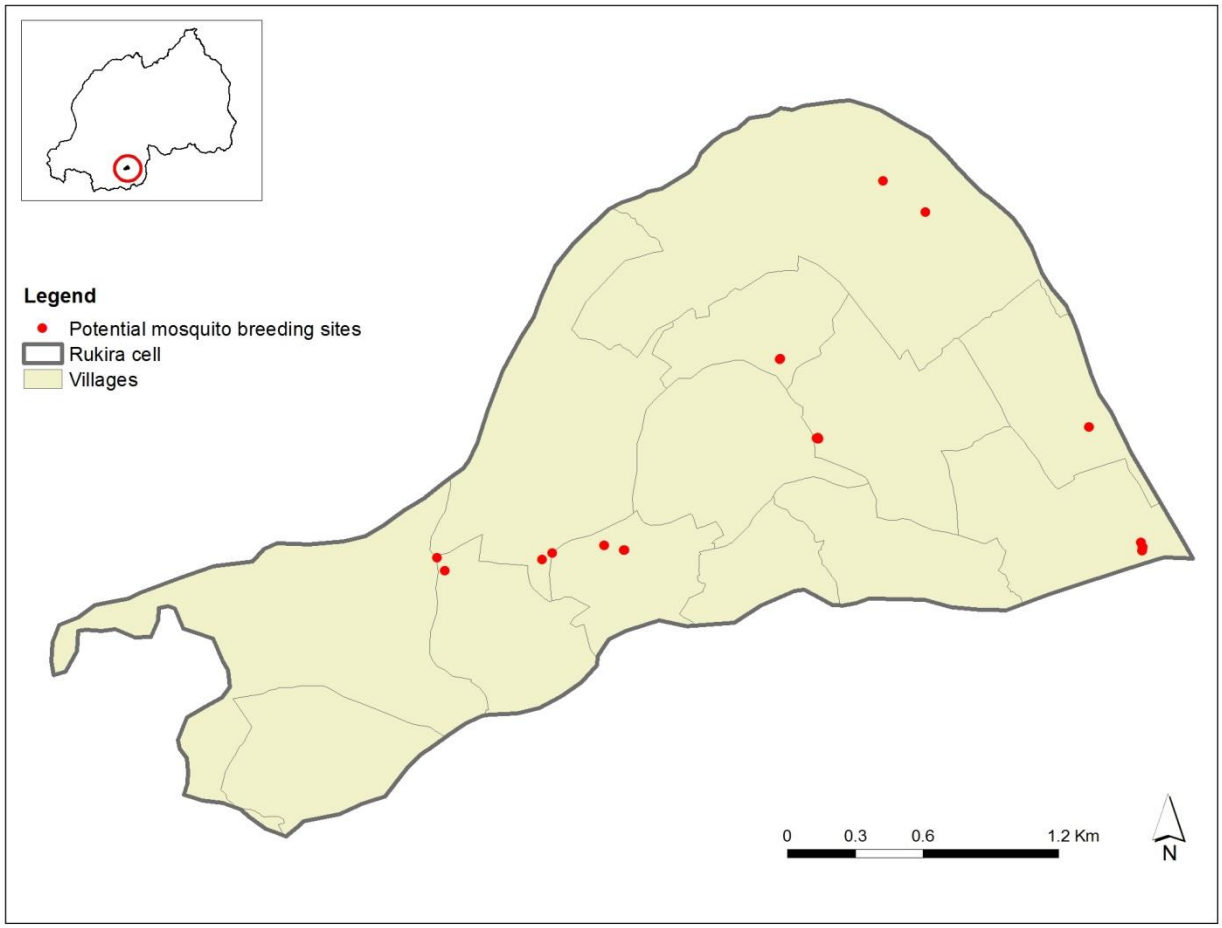

Figure 4. 8 : Map of Rukira cell showing potential mosquito breeding sites

CHAPTER 5: DISCUSSION, LIMITATIONS, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

This study was carried out in Rukira cell of Huye District, Southern Province of Rwanda aiming to determine the prevalence of malaria infection among children under-five and its associated factors as well as malaria vector abundance. This study was done in May which is a high malaria season in the study area.

5.1.1 Prevalence of malaria parasite and its associated factors among children

Malaria transmission in Rwanda varies widely with two seasonal peaks in May to June and November to December. Although the participants in the present study were apparently healthy, infection prevalence of 12.2% was observed indicating that 1 in every 8 children under five years had malaria. The prevalence was high as compared to the previous report of Malaria Operational Plan (2014) which reported a malaria prevalence of 3% in southern province of Rwanda (President's Malaria Initiative 2014a). Similarly, it was higher than the prevalence reported by Gahutu and others in the same area which indicated an overall prevalence of malaria to be 5.5% (Gahutu et al. 2011). This difference may be related to the coverage of this study which was conducted in one malaria-endemic cell of Huye district whereas other studies were conducted on a larger scale. However, it is lower than that of Malawi Malaria Indicator Survey in 2012 which reported prevalence of malaria at 28% (National Malaria Control Programme 2012). Likewise, the prevalence was lower compared to what was reported elsewhere by the Nigeria malaria fact sheet of 2010 which reported 27.6%, in the South East region of Nigeria (United States Embassy in Nigeria 2010).The difference may be related to the scale-up of Malaria control interventions in Rwanda in the past years and in part could be the different geographical characteristics.

P. falciparum which is known to cause severe malaria was the only observed *Plasmodium spp.* in this study. These findings are in agreement with previous studies which indicated that *P. falciparum* is the main species found in tropical and sub-tropical Africa (Cheesbrough 1998). Also, in agreement with this, Kenya Malaria Indicator Survey in 2010, reported that 96% of malaria infections in Kenya were due to *P. falciparum* (Division of Malaria Control 2010).

According to the findings of this study, the concept of using ITNs was considered as one of the protective factors against mosquito bites. Children who were not sleeping under ITNs were 15 times more likely to be infected with malaria compared to those who were sleeping under ITNs. This was supported by the study conducted by Lengler et al. (2009) which showed that clinical cases of malaria and severe malaria in populations using mosquito nets reduced by 50% and 45%, respectively (Lengeler 2009).

Children aged 1 to 12 months were more likely to have malaria infection than children aged 13 to 59 months. This could be due to the relative slow build-up of anti-*Plasmodium spp*. immunity which is bolstered by repeated exposure. However, it contrasts with the results of Malawi Malaria Indicator Survey of 2012 which reported that malaria prevalence was increasing by age (National Malaria Control Programme 2012).

The proportion of malaria was significantly more among male children compared to their female counterparts in bivariate analysis. However, this observation was not sustained in multivariate analysis. The reason for this may be due to the high number of male children who were between 1-12 months, many of whom were not using mosquito nets. Upon considering both age and sex, the analysis revealed that sex was no longer significant implying that sex was confounded by age.

5.1.2 Malaria vectors

The most efficient known vectors of human malaria in Rwanda are *An. gambiae* s.l. and *An. funestus* group, other mosquito species are non-vectors of malaria (President's Malaria Initiative 2015a). In this study, *An. gambiae s.l* was the most abundant species sampled in Rukira (69.7%). This could be due to the favorable larval habitats available in the area. The findings were consistent with the studies conducted in Kenya which reported that *An. gambiae* s.l was the most prevalent known vector contributing 95.4%, 96.8% respectively (Mulambalah et al. 2011; Kipyab et al. 2013). Apart from being the most prevalent species, in the present study *An.gambiae s.l* was found to coexist with other anopheles species like *An. funestus, An. ziemanni, An. squamous, An. maculipalpis*, *An. pharoensis* and *An. coustani.* Similar findings were reported in the study conducted in Kenya, Zambia, Rwanda (Kamau & Mulaya 2006; Christen M., Laura C. 2011; President's Malaria Initiative 2015a).

In the present study, mosquitoes collected indoors were more than those collected outdoors at 63.5% and 36.5%, respectively. This indicates that the malaria vectors in the study area are endophilic This concurs with a study carried out by Oyewole et al. (2005) in Nigeria where indoors collection was 53.8% and outdoors 46.8% (Oyewole, I.O, Ibidapo, C.A, Oduola, A.O. 2005). A study conducted by Yewhalaw et al. (2014) in Ethiopia also reported that indoor and outdoor collection was 98.85% and 1.15%, respectively (Yewhalaw et al. 2014).

The indoors resting density in our study varied from 0 to 10 anopheles mosquitoes/house/day and the overall indoor resting density was 5 anopheles/house/day. The indoor resting density observed was higher than that of Kirehe, Nyagatare, Gisagara and Bugesera district observed in May, 2015 which was 0.7, 0.23, 0.017 and 0 anopheles/house/day, respectively, according to the Africa Indoor Residual Spray Rwanda report (President's Malaria Initiative 2015a). It was also high compared to the densities of 0 and 0.1 anopheles/house/day observed in a study conducted in a Nigerian study (Umar A., Kabir B., Abdullahi B., et 2015). This could be due to the IRS intervention which is already available in Nigeria and not yet in the present study area.

In our study, host preference for source of blood meal was assessed only from humans, bovines and goats, although previous studies indicated that the major malaria vectors in sub-Saharan Africa readily adapt to available blood-meal hosts even if they have a preference for human hosts (Mwangangi M. Joseph , Charles M. MBOGO 2003; Hadji et al. 2013). Blood fed anopheles mosquitoes were dominant indoors (68%) as compared to outdoors (32%) despite the use of nets by the occupants. The overall human blood index was 50.9%, blood meal sources identified from primary vectors *An. gambiae s.l* were 64.5% human, 14% bovine, 8.4% goat, 2.8% from mixed bovine and goat hosts, and 10.3% from unknown hosts. Blood meal sources for *An. funestus* were human (70%) and 30% from unknown hosts. This implies that there was anthropophagic behavior of the primary malaria vectors despite the use of ITNs by people. A study in coastal Kenya reported that 98.9% of *An. gambiae s.l* and 99.4% of *An. funestus* had fed on humans (Mwangangi M. Joseph , Charles M. MBOGO 2003). The findings were also similar to the study conducted in Nigeria that reported 84.7% of An. *gambiae* s.s and 68.8% of *An. funestus* s.s fed on humans (Oyewole, I.O, Ibidapo, C.A, Oduola, A.O. 2005). This could be because primary vectors bite before people go to bed. However, it differs from the findings of Ndenga et al. (2016) who reported unusually high frequency of animal and mixed human-animal blood meals in the major

malaria vector, *An. gambiae s.s.,* in the western Kenya highlands and only 26.5 % from humans host alone (Ndenga et al. 2016). The reasons might be due to the use of LLITNs, IRS and livestock around the house which made the mosquitoes to shift from human to animal hosts.

Among secondary malaria vectors, *An. ziemanni* showed anthropophagic behavior where 75% were fed on human and 25% from unknown host. Similar findings were reported in Kenya where human-cattle mixed feeds were 47.1.0% for *An. ziemanni,* and that its human biting index was not significantly different from that of *An. arabiensis* a known efficient malaria vector (Kamau & Mulaya 2006). *An. squamosus* were more zoophagic where by 31.2% fed on goat, 25% on bovine, 25% on mixed host goat and bovine, 12.5% on unknown host and only 6.2% fed on humans. On the contrary, a study conducted by Christen et al. (2011) in Zambia reported high anthropophagic tendencies of *An. squamosus* where 65% were fed on humans (Christen M., Laura C. 2011). *An. maculipalpis* were more zoophagic, none of them had fed on human host but rather, 50% had fed on bovine and other 50% on unknown hosts.

The current study showed an overall *P. falciparum* sporozoite rate of 1.9%. It is slightly higher than that reported in a study conducted in Kenya where sporozoite rate of 1.47% (Midega et al. 2012). It is also much higher than that reported in the study by Drakeley et al. (2003) in Tanzania which reported *P. falciparum* sporozoite rate of 0.39% (Drakeley et al. 2003). In contrast, according to Yewhalaw et al. (2014) there was no mosquito found positive for *P. falciparum* CSP in Ethiopia (Yewhalaw et al. 2014). The higher positivity rate may be explained by the fact that mosquito collection was done during the malaria season and by the difference in *Plasmodium* species available in the study area. However it is slightly lower than that of Oyewole et al. (2005) who reported *P. falciparum* sprozoite rate of 2.5% in Nigeria (Oyewole, I.O, Ibidapo, C.A, Oduola, A.O. 2005). It was also far much lower than study conducted by Tasneem et al (2010) who reported 16% and 15% in Sennar and Koka state respectively in Sudan (Osman 2010). The difference may be attributed to the use of malaria control interventions.

In this study, out of 11 mosquitoes that tested positive for *P. falciparum* circumsporozoite protein, 8 were *An. gambiae s.l.* Although no *An. funestus* was found infected with sporozoites, its role in malaria transmission cannot be ruled out. The remaining 3 mosquitoes (*An. ziemanni, An. maculipalpis* and *An.squamosus)* are considered secondary vectors, suggesting their possible role as secondary malaria vector in the study area. This is supported by the study conducted by

Raymond and others reporting that *An. ziemanni* is an important local malaria vector in Ndop health district of Cameroun (Tabue et al. 2014). Similarly, a study conducted in Tanzania by Gillies in 1964, one out of 1000 *An. squamosus* was sporozoite-positive in Muheza (Gillies 1964). However, according to Christen and others, no *An. squamosus* were found to be positive for *Plasmodium* CSP in Zambia although they demonstrated anthropophagic tendencies (Christen M., Laura C. 2011).

5.1.3 Mosquito breeding site

Two malaria vector species larvae including *An. gambae s.l* and *An. ziemanni* were collected during the present study. The reasons why we did not find any breeding sites for the other mosquitoes species may be that during the survey some potential mosquito breeding habitats were dried as it was the beginning of summer, It may also be that those species were small in number therefore the probability of trapping them was small compared to the dominant species *An. gambiae s.l*. In total, 661 anopheles larvae were collected from 22 breeding habitat and reared in the laboratory and identified. The primary malaria vector *An. gambae s.l* was the dominant species (89%) being distributed in a wide range of habitats whereas the secondary vector *An. ziemanni* was the least abundant (11%). The most common larval habitats were in full sunlight with still water such as rice paddies and stagnant water. The larval density was significantly associated with still water current (p=0.038). Similar findings were reported in the study conducted by Oljira Kenea and others in Ethiopia that stagnant water was preferred by *An. gambae s.l* as larval habitat (Kenea et al. 2011). The reasons behind this could be that still water provides suitable conditions in which larvae can stay close to the surface with their spiracles open to the air for breathing. In addition, strong water current can cause physical harm to larvae.

5.2 Limitations of the Study

Identification of mosquitoes at sibling level, which requires use of molecular techniques, was not performed due to financial constraints. The specimens were preserved for identification when resources are available. In addition, mosquitoes were only tested for *P. falciparum* CSP so infection of mosquitoes with other *Plasmodium* species CSP could not be determined. Chemical parameters such as dissolved oxygen, nitrate, phosphate of anopheline mosquito larval habitats were not identified which might affect abundance and distribution of anopheles larvae.

5.3 CONCLUSIONS

This study shows that the prevalence of *P. falciparum* infection is high (12.2%) among children less than five years of age in the study area. Gender and sleeping under treated bed nets are independently associated with malaria infection. The primary potential malaria vector in the study area is *An. gambiae s.l.* However secondary vectors like *An. ziemanni*, *An. squamosus and An. maculipalpis* might also play an important role in the local malaria transmission. The indoors resting density is high ranging from 0 to 10 anopheles per house per day and the overall indoor resting density was 5 anopheles per house per day. Vectors in the study area are more anthropophagic although they can feed on other available hosts. Irrigation and other agricultural practices have significant influence on mosquito breeding habitat and some abiotic factors are associated with anopheles larval density as well. These factors should be considered when implementing larval control strategies which should be based on habitat productivity and water management.

5.4 RECOMMENDATIONS

Based on the study findings, the following recommendations were made

- The high number of indoor collection suggest that the vector is more endophilic and endophagic therefore indoor residual spray should be done in the area to reduce the vector density.
- Larval control strategies should be applied to reduce vector abundance.
- Identification of malaria vectors present in the study area at sibling level is recommended.
- Larger studies are needed for establishing the role of secondary vectors in local malaria transmission.
- Baseline study determination of the susceptibility of malaria vectors in the study area to pyrethroids used in insecticide treated nets widely distributed by the Rwanda Ministry of Health should be done.
- Chemical parameters like dissolved oxygen, nitrate, phosphate of anopheline mosquito larval habitats in Rukira cell is recommended.
- Studies on proper usage of ITNs should be carried out in the area.

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APPENDICES

Appendix 1: Informed consent form.

Respondent code: ---------- **Date: ----------**

STUDY TITLE: **STUDY OF PREVALENCE OF MALARIA PARASITE INFECTION AND VECTOR SPECIES ABUNDANCE IN HUYE DISTRICT, SOUTHERN, RWANDA**.

Investigator: Ms. Chantal NYIRAKANANI, Medical Microbiology, University of Nairobi. Cell phone: +250783539059

Supervisors: 1. Dr. MUKOKO Dunstan, Medical Microbiology, University of Nairobi

2. Dr. MASIKA Moses, Medical Microbiology, University of Nairobi

3. **Prof Kato J NJUNWA, MSc PhD** Ag. Director of Research, Innovation and Postgraduate Studies, University of Rwanda

Investigator's statement:

I am asking you to kindly participate in this study. The purpose of this consent form is to provide you with the information you will need to help you decide whether or not to participate in the study.

Introduction:

Malaria remains one of the leading cause of high mortality globally especially in Africa. Children under 5 years and pregnant women are more vulnerable. Thus the control of malaria in Rwanda is of considerable importance. Efficient and effective vector control program, require identification of mosquito species in order to separate vector from non-vector species. Thus this study seeks to determine malaria prevalence and vector species abundance in Rukira cell. You will be asked a number of questions that will take an average about 15 minutes of your time. We will collect both mosquito samples in your compound and finger prick blood sample from under 5 years child and use them for the study. The laboratory tests will be treated with utmost confidentiality. Your daily duties will not be interrupted by your agreement to participate in the study.

Benefits:

The results of this study will be useful for cost effective modes of malaria mosquito vector control and larval abatement program in Rukira Cell. Children with positive result will be referred to the nearest health center for treatment.

Risks:

The risks of participating in this study are minimal, there will be a little pain from finger prick but it will only rest for short time. There is no risk of transmitting infection because the sterile needle will be used for every child. The procedure will be done by the Principle investigate. The interview will take short time almost 15 minutes.

Voluntariness:

Participation in the study will be fully voluntary. You are free to refuse to participate or withdraw from the study at any time. There will be no financial reward to you for participating in the study.

Confidentiality:

The information obtained about you and the result of blood test will be treated with utmost confidence and identification will not be released to any person or forum without your permission. All data collected in hard copy will be kept in a lockable cabinet where the researcher only will access to maintain confidentiality. Information stored in soft copies will be protected from access from unauthorized persons by password which will be changed periodically. All records will be identified by study identification number.

Questions:

If you ever have any questions regarding the study you can contact:

- The investigator, Ms. Chantal NYIRAKANANI, Tel: +250783539059.
- Kenyatta National Hospital/ University of Nairobi Ethics and Research Committee, Tel: 020 726300-9
- University of Rwanda/College of Medicine and Health Sciences

Prof Kato J NJUNWA

Chairperson

Institutional Review Board, Fax: +250571787, phone: (+250)788490522

Participant's statement:

I the Parent (s)/ Guardian of the child after having received adequate information regarding the study research, risks, and benefits hereby AGREE that my child participate in the study with malaria prevalence and vector species. I understand that the participation is fully voluntary and that I am free to withdraw the child at any time. I agree that the researcher can collect mosquitoes in the homestead that I head. I have been given adequate opportunity to ask questions and seek clarification on the study and these have been addressed satisfactorily.

Respondent's Signature: Date _____

I declare that I have adequately explained to the Parent (s)/ Guardian, the study procedure, risks, and benefits and given him /her time to ask questions and seek clarification regarding the study. I have answered all the questions raised to the best of my ability.

Interviewer's Signature Date _____

CONSENT FORM (IKINYARWANDA VERSION)

Kode y'usubiza: ---------- Tariki: ----------

UMUTWE W'UBUSHAKASHATSI: **KWEREKANA IMPUZANDENGO YUBWANDU BWA MALARIA MUBANA BARIMUNSI YIMYAKA ITANU NOGUPIMA UBWOKO BWUMUBU UKWIRAKWIZA MALARIA MUKAGALI KA RUKIRA, HUYE.**

Umushakashatsi: NYIRAKANANI Chantal, Medical Microbiology, University of Nairobi. Numero y'itumanaho: +250783539059

Abayobozi: 1. Dr. MUKOKO Dunstan, Medical Microbiology, University of Nairobi

2. Dr. MASIKA Moses, Medical Microbiology, University of Nairobi

3. **Prof Kato J NJUNWA, MSc PhD** Ag. Director of Research, Innovation and Postgraduate Studies, University of Rwanda.

Ijambo ry'ukora ubushakashatsi: Muraho! Nitwa NYIRAKANANI Chantal, ndi umunyeshuli muri kaminuza ya Nayirobi nkaba niga ibirebana na mikorobe mu buvuzi bwa kizungu.

Nasabaga ko mwakwitabira ubu bushakashatsi. Iyinyandiko igamije kugirango usobanukirwe neza ibijyanye nubu bushakashatsi hanyuma utwemerere cg se wange kubwitabira byose nuburenganzira bwawe.

Gusobanuraubushakashatsi:

Malariya iracyari imwe mu ndwara zitera imfu nyinshi kwisi hose byumwihariko muri Afurika arinaho u Rwanda ruherereye. Malariya yibasira kandi ikazahaza abana bari munsi yimyaka itanu ndetse nabagore batwite. Bityo kwirinda malariya mu Rwanda bikaba bifite akamaro cyane. Porogaramu yuburyo bunogeye bwokurwanya imibu ikwirakwiza malariya isabakumenyaneza ubwoko bwumubu uyikwirakwiza. Ubu bushakashatsi bugamije gupima ubwoko bwumubu ukwirakwiza malariya ,ahoyororokera ndetse no kwerekana impuzandengo ya malaria mubana bari munsi yimyaka itanu.

Uri bubazwe ibibazo bike bizagufata iminota nka cumi nitanu yonyine, turafata amaraso yo mugatoki kumwana utarengeje imyaka itanu womuri uru rugo ikindi ni uko turibuze gufata imibu mu nzu no mu nkengero zayo bikazadufasha mu bushakashatsi turi gukora. Ibisubizo by' ibibazo mbabaza, Ibizamini byumwana no gupima imibu byose birakorwa neza kandi mu buryo bw'ibanga. Ikindi nuko tutariburogoye imirimo yanyu yaburi munsi kugirango mwitabire ubushakashatsi.

Inyungu:

Ibizava muri ubu bushakashatsi bizafasha abakozi b'urwego rw'ubuzima kongera ubumenyi kubijyanye nubwoko bwimibu ikwirakwiza malariya mu kagali ka Rukira, ndetse n'uburyo hafatwa ingamba ziboneye zo kurandura uwo mubu harimo no gusiba ahoyororokera. Abana bazasanganwa malaria bazoherezwa ku kigo nderabuzima bavurwe neza.

Ingaruka:

Ingaruka zo kwitabira ubu bushakashatsi ni nkeya cyane, hashobora kubaho ububabare buke butewe n'amaraso dufata mu gatoki ariko bimara akanya gato cyane, nta ngaruka zo kwandura indwara zandurira mu byuma bikomeretsa kuko buri mwana akoreshwaho urushinge rushya, kandi birakorwa nanjye nyir'ubushakashatsi. Ikiganiro tugirana kiramara nk' iminota cumi nitanu.

Ubushake:

Kwitabira ubu bushakashatsi ni ubushake gusa. Ni uburenganzira bwawe kwitabira cg kubyanga igihe ushakiye. Kandi nta mafaranga ateganijwe kugirango witabire.

Ibanga:

Nkwijeje kugira ibanga ku makuru uri bumpe kandi amakuru yatuma umuntu agusobanukirwa ntabwo azerekwa undi muntu uwo ariwe wese keretse abonye uruhushya rwawe. Amakuru yandikwa mu ikayi azabikwa mu gasanduku gafungwa n'aho ayo muri mudasobwa azafungwa n' umubare w'ibanga uzwi n'umushakashatsi gusa kandi uzajya uhindagurwa kugirango hatazagira uwumenya.

Ibibazo:

Ugize ibibazo ushaka kubaza kuri ubu bushakashatsi, wabaza nyir'ubushakashatsi Ms.NYIRAKANANI Chantal, Tel: +250783539059.

-Kenyatta National Hospital/ University of Nairobi Ethics and Research Committee,

Itumanaho: 020 726300-9

-University of Rwanda/College of Medicine and Health Sciences Institutional Review Board, Fax: +250571787, phone: (+250)788490522

Amagambo y'uwitabira:

Njyewe maze kumva neza ibijyanye nubu bushakashatsi, ingaruka, inyungu nemeye kwitabira ubu bushakashatsi, nemereye umushakashatsi gufata umwana ikizamini cy'amaraso yo mu gatoki no gufata imibu aho dutuye. Nasobanuriwe neza ko kubwitabira cg kutabwitabira ari uburenganzira bwanjye ndetse n'igihe cyose nashakira navamo. Nahawe n'uburyo busesuye bwo kuba nabaza ibibazo byo kugirango mbashe gusobanukirwa neza n' ubu bushakashatsi.

Umukono w'usubiza: Tariki _____

Jye ndemeza ko nasobanuriye bihagije uvugwa haruguru witabiriye ububushakashatsi, ibyerekeye ubu bushakashatsi, ingaruka, n'inyungu ndetse muha n'igihe ngo abaze ibibazo bishoboka byose kugirango asobanukirwe cyane n'ububushakashatsi. Nasubije neza uko nshoboye kose kugirango asobanukirwe.

Umukono w'ubaza Tariki _____

Appendix 2*: Giemsa staining Procedures*(**Warhurst & Williams 1996).**

Principle of the stain

The principle of the Giemsa stain is based on its components, Methylene blue stain cytoplasm of the parasite in blue and Eosin stain nucleus of the parasite in pink/red.

Stain Preparation

To make 50 ml, Giemsa powder: 3.8g Glycerol: 250ml Methanol: 250ml

1. Add stain and methanol-cleaned glass beads to amber glass bottle.

2. Add glycerol and methanol**,** shake vigorously and place at 37◦C for 24 hours with further frequent shaking.

3. Remove from the incubator and shake again for 24 hours; the stain is then ready for use.

4. Filter small amounts as required.

Giemsa Working solution Preparation

Dilute the Giemsa in Buffered water P.H 7.1–7.2 before use. 3% is for 30minutes and 10% for 10 minutes.

New batches of Giemsa stain should be tested with known Positive and Negative slides for malaria to ensure wuality of the stain.

Staining Procedures

1. Make a thick and thin smear and air-dry.

2. Fix the thin smear with absolute methanol for 3 – 5minutes allow to dry. **(**DO NOT FIX THICK SMEAR!!)

3. Place film in a staining rack.

4. Stain both thick and thin smears with 10% Giemsa stain and leave to stain for 10 minutes

5. Dip the slides several times in water then air dry

6. Examine under oil immersion objective.

Appendix 3*: Sporozoite ELISA Methods Procedures* (Doolan 2002).

1. Prepare the mosquito sample for ELISA testing. Label sets of 1.8-mL tubes with the corresponding mosquito sample numbers. Add 50 μL of BB–NP-40 to each vial. Using a sharp clean surgical blade, cut the mosquito between the thorax and the abdomen (normally done on a filter paper). Transfer the head–thorax with forceps to the labeled tube, and transfer the abdomen to the corresponding tube for blood meal identification if the mosquito is blood-fed. If the mosquito is not blood-fed or no blood meal analysis is required, discard abdomen.

2. Grind the mosquito in the tube using a nonabsorbent glass rod or plastic pestle. Add 200 μL of the BB to bring the total sample volume to 250 μL. To avoid contamination, clean the pestle and wipe it dry before grinding the next sample. Repeat the procedure until all samples are prepared. Arrange samples in numbered order within storage boxes and keep samples in a freezer at –20 or –70°C until testing.

3. Coat number-coded ELISA plates with monoclonal antibody (MAb). In each well, add 50 μL of the diluted capture MAb. Cover the plates with another clean ELISA plate and incubate for 30 min at room temperature in subdued light.

4. Block the plates. Using an 8-channel manifold attached to a vacuum pump, aspirate the capture MAb from the micro titer plate. Bang the plate hard on an absorbent tissue paper or gauze to ensure complete dryness. Fill each well with BB using a manifold attached to a 60-mL syringe. Incubate for 1 h at room temperature in subdued light.

5. Load the plates with mosquito samples. Aspirate the blocking buffer from the wells using the manifold attached to a vacuum pump and bang plate to complete dryness. Place 50 µL of 100, 50, 25, 12, 6, 3, 1.5, 0 pg of positive control recombinant protein in the first column wells. Into the second column, add 50 µL per well of the negative controls; normally, field-collected male Anopheles mosquitoes or culicine mosquitoes are used as negative controls. Load 50 µL of each mosquito sample to the remaining wells of the plate, checking carefully that numbered mosquito samples are placed in the wells according to the completed ELISA data form. Cover the plate and incubate for 2 h at room temperature in subdued light.

6. Add peroxidase-conjugated monoclonal antibody. After 2 h, aspirate the triturate from the wells and wash the plate two times with PBS-Tw20. Add 50 µL of the peroxidase-labeled enzyme and incubate for 1 h at room temperature.

7. Add the substrate. Aspirate the enzyme conjugate from the wells and wash three times with PBS-Twn 20. Using a multichannel pipet, add 100µL of ABTS substrate and incubate for 30 min. Positive reactions, which appear green, can be determined by reading plates at 414 nm using an ELISA plate reader; absorbance values two times the mean of negative controls provides a valid cutoff for sample positivity.

8. Record results for each tested mosquito.

Appendix 4*: Bloodmeal ELISA Methods Procedures*(Doolan 2002).

1. Prepare wild-caught half-gravid to freshly fed mosquitoes by cutting them transversely at the thorax between the first and third pairs of legs (under a dissecting microscope, ×10–20). In a labeled tube, place the posterior part of the mosquito containing the blood meal in 50 μL PBS and grind with a pestle or pipet repeatedly. Dilute sample 1:50 with PBS and freeze samples at –20°C until testing.

2. Load 96-well polyvinyl micro titer plates with mosquito blood meal samples by adding 50 μL of each sample per well. On the same plate, add 50 μL samples of positive control antisera for human and cow (diluted 1:500 in PBS), and four or more negative control unfed female mosquitoes or male mosquitoes obtained from the same field collections and handled as above. Cover and incubate at room temperature for 3 h (or overnight).

3. Wash each well twice with PBS-Tw20.

4. Add 50 μL of host-specific conjugate (anti-host IgG, H&L) diluted 1:2,000 (or as determined in control tests) in 0.5% BB containing 0.025% Tween-20, and incubate 1 h at room temperature.

5. Wash wells three times with PBS–Tw-20.

6. Add 100 μL of ABTS peroxidase substrate to each well.

7. After 30 min, read each well with an ELISA reader. Samples are considered positive if absorbance values exceed the mean plus three standard deviations of four negative control, unfed female, or male mosquitoes. The dark green positive reactions for peroxidase(or the dark yellow reactions for phosphatase) may also be determined visually

Appendix 5: Study questionnaire

HOUSEHOLD QUESTIONNAIRE FOR PARASITOLOGICAL AND ENTOMOLOGICAL MALARIA SURVEY

Study title: Malaria prevalence and vector species abundance in Huye sector

Questionnaire identification number**.....Date**……

House hold Code Number……

Part A: House hold Head

1. **Occupation of the child's parent/Guardian:**

   1. Farmer 2. Trader 3. Nurse 4. Teacher 5.other

2. **Marital status of the child's parent/Guardian:**

   1. Single 2.Married , 3.Windowed , 4.Divorced ,

??. **Sex of the child :** Female Male

??. **Age of the child** (Yrs)

5. **Highest education attained by the child's parent/Guardian:**

   1. Primary school 2.Secondary school 3.Tertiary level 4.none

6**. Do you have ITNs? 1. Yes 2.No**

7. **Have you slept under a bed net last night?**

   1. Yes 2. No

8. **Has your child ever suffered from malaria in last month?**

   1. Yes 2. No

9. **Has your child had malaria test in the last one month?**

   1. Yes 2. No

10. **Has your child been on malaria medication within the last one week?**

    1. Yes 2. No

11. **Has your house ever been sprayed for mosquito control insecticide spraying?**

    1. Yes 2.No

If Yes When --- 1. A week ago 2. A month ago 3. Year ago 4. More than year ago

12**. Presence of breeding sites near the house** 1.Yes 2. No

If yes which types: **1**. Rice paddy **2**.Ground pool **3**.Stagnant water **4**.Crashed pot and other containers

HOUSEHOLD QUESTIONNAIRE FOR PARASITOLOGICAL AND ENTOMOLOGICAL MALARIA SURVEY (IKINYARWANDA VERSION).

Nimero yibibazo..... Itariki……

Code yinzu……

Igice A: Ny'irurugo

1. **Umwuga wababyeyi bumwana.**.....

??. **Indangamimerere yababyeyi :** Arubatse , Umupfakazi , Baratandukanye , Ingaragu

??. **Igitsina cyumwana**: Gore , Gabo

??. **Imyaka yumwana**

??. **Amashuri yababyeyi bumwana** Abanza Ayisumbuye Kaminuza Ntayo

6. **Hari umwana wawe wigeze urwara malariya mukwezi gushize?**

1. Yego 2. Oya

7. Ese mufite inzitira mibu?

1. Yego 2. Oya

8. **Mwaraye muri supanete irijoro ryakeye?**

1. Yego 2. Oya

9. **Harumwana wigeze usuzumwa malaria mukwezi gushize?**

1. Yego 2. Oya

10. **Har umwana wawe wigeze ufata imiti ya malaria mu cyumweru gishize**?

1. Yego 2.Oya

11. Hari umuti wigeze uterwa munzu yanyu urwanya imibu?

1. Yego 2. Oya

Niba ari yego ni ryari? 1. Hashize icyumweru 2. Ukwezi 3. Umwaka 4.igihe kirenze umwaka

12. Aho imibu yakororokera hari hafi yurugo 1.Yego 2.Ntaho

Niba hahari nubuhe bwoko:

1. Mugishanga cyumuceli **2**.Mubidendezi byamazi **3**.Mumazi areka ahantu **4**. Munkono nibindi bicupa bishaje

Laboratory Malaria Result form

Cell:…… Village:……..

HH Code	Name	Age	Sex	Microscopy (MPsseen/No MPs seen)			
				P.f	P.o	P.m	P.v

P.f -Plasmodium falciparum, P.o-Plasmodium ovale,P.m- Plasmodium malariae, Plasmodium vivax
MPs=Malaria parasites

Appendix 6: Data collection sheets

ADULT MOSQUITO FIELD AND LAB DATA FORM

Cell………………………………………… Village……………………………………. Date………………………………. Time…

Name of Collector ……………………………..

House Hold Code	Place of Collection	Collection Method	Number of mosquitos collected	No culicine	No of Anopheline		Fed	Unfed	No Tested for Sporozoite (a)	No positive for Sporozoite ELISA (b)	Sporozoites rate b/a × 100
					Anopheles gambiae complex	*Anopheles funestus complex*					
	Indoors										
	Outdoors										
	Indoors										
	Outdoors										
	Indoors										
	Outdoors										
	Indoors										
	Outdoors										
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	Outdoors										
	Indoors										
	Outdoors										
	Indoors										
	Outdoors										
	Indoors										
	Outdoors										
Total											

Larval Collection Form –Field and Lab

Cell…… **Date……**

…… **……**

Name of Collector **……**

Village	Type of Breeding Habitat	Permanent/Temporary	Still/Moving	Light/shaded	No of Habitats	No positive	No positive for culicine	No positive for Anopheline	
								Anopheles gambiae complex	*Anopheles funestus complex*
Totals									