INVESTIGATION OF THE EPIDEMIOLOGY OF MALARIA

IN THE ENGELA HEALTH DISTRICT OF THE OHANGWENA REGION

IN NORTHERN NAMIBIA

A thesis submitted in fulfilment

of the requirements for the degree of

Master of Science

Of

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by

Joyce Rose Namutenya Auala

200736957

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Supervisors: Dr Davis R Mumbengegwi

Co-supervisor: Dr Ronnie Bock
ABSTRACT

Namibia has seen a decline in reported malaria cases of up to 97.4% between 2001 and 2011. The country was nominated as one of 8 countries in Southern Africa (E8) ready to move from the control phase to the elimination phase by 2020. However, interventions that were successful in bringing down malaria cases during the control phase may no longer be appropriate now that malaria transmission patterns have changed. Gaps in knowledge about infection risk factors at low malaria transmission, such as cross border movement and the possibility of localized malaria hotspots where residual malaria may still persist create an obstacle to eliminating malaria. An effective surveillance system may contribute to zero local transmission of malaria in Namibia. Currently, passive surveillance of malaria cases is conducted; however, this can be complimented by reactive case detection (RACD) which focuses on detection of additional malaria cases within the community. An RACD study was piloted in the Engela Health District of the Ohangwena region from December 2012 to July 2014. All individuals with fever testing malaria positive by rapid diagnostic test (RDT) from the 17 clinics in the district were recruited into the study and visited at their homesteads. Consenting individuals living in the case household were screened for malaria by RDT and interviewed to ascertain the presence of possible malaria risk factors; four surrounding households were also selected and recruited into the study. For the control arm, households in the enumeration area where malaria was not reported were recruited as controls for the study and their four surrounding households were also recruited. During the study period, a total of 190 confirmed malaria cases
were reported from Engela Health District of which 70 (36.5%) were local individuals residing within the district and 8 (4.2%) were asymptomatic cases discovered during RACD. From the remaining cases, 47 (24.7%) were of Angolan nationals who do not reside within the district but only crossed the border seeking medical treatment and 65 (35.2%) were regarded as untraceable cases due to various factors such as lack of or false information given at the health facility. Risk mapping and geo-locating of confirmed local cases and asymptomatic cases revealed pockets of infection in the northern regions of the district parallel to areas where clustering occurred. Increased probability of malaria infection in these areas was estimated at a mean of 2.2%, with a range of 0.04% - 28.3%. Travel, insecticide residual spraying (IRS) and mosquito net coverage were among the top significant contributors to increased risk of malaria infection. Travel was found to be more common among male individuals from case neighbourhoods with the most frequent destination being Angola. Net coverage was 4% lower in case neighbourhoods compared to control neighbourhoods with statistical analysis showing that risk of infection was much lower among net users as opposed to non-users (OR=0.89, 95% CI: 0.45-1.74). From control neighbourhoods, 67.4% of sleeping structures were not sprayed compared to 72.2% of sleeping structures from case neighbourhoods. With the presence of eaves in 70.2% of unsprayed case neighbourhood sleeping structures, risk of exposure to mosquitoes was increased due to ease of entry. Despite the need to improve the quality of information collected from patients at health facilities, RACD is a plausible method for monitoring malaria elimination and identifying asymptomatic reservoirs in the district. With RACD, the chance of finding an asymptomatic case was 8 times more likely to occur in the index household where a malaria case was initially reported while also making it possible to
identify potential hotspots of infection. Risk factors associated with the likelihood of being a confirmed case were travel, IRS and net coverage which highlighted possible reasons towards continued malaria transmission. However, the perception of low malaria risk due to significant decrease in malaria transmission results in the need to re-educate communities on the importance of continued practice and implementation of vector control strategies such as IRS and net coverage and use in order to bring transmission down to zero.
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<th>Full Form</th>
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<tbody>
<tr>
<td>ACT</td>
<td>Artemisinin-based Combination Therapy</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>Human Immuno-deficiency Virus / Acquired Immune Deficiency Syndrome</td>
</tr>
<tr>
<td>AL</td>
<td>Artemether Lumefantrine</td>
</tr>
<tr>
<td>AQAS</td>
<td>Amodiaquine-Artesunate</td>
</tr>
<tr>
<td>ASMQ</td>
<td>Artesunate Mefloquine</td>
</tr>
<tr>
<td>Be</td>
<td><em>Bacillus sphaericus</em></td>
</tr>
<tr>
<td>Bti</td>
<td><em>Bacillus thuringiensis</em></td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>DDT</td>
<td>Dichloro-diphenyl-trichloroethane</td>
</tr>
<tr>
<td>DEET</td>
<td>Diethyltouamide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EA</td>
<td>Enumeration Area</td>
</tr>
<tr>
<td>EHP</td>
<td>Environmental Health Practitioner</td>
</tr>
<tr>
<td>GPS</td>
<td>Global Positioning System</td>
</tr>
<tr>
<td>GSK</td>
<td>GlaxoSmithKline</td>
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HRP-2/pLDH  Histidine Rich Protein II / *P. falciparum* Lactate Dehydrogenase

IPTc  Intermittent Preventative Treatment for children

IPTi  Intermittent Preventative Treatment for infants

IPTp  Intermittent Preventative Treatment for pregnant women

SP  Sulfadoxine-Pyrimethamine

IRS  Indoor Residual Spraying

ITN  Insecticide Treated Nets

LLIN  Long-lasting Insecticide Net

LSHTM  London School of Health and Tropical Medicine

LSM  Larval Source Management

MDG  Millennium Development Goal

NVDCP  National Vector-borne Disease Control Programme

OR  Odds Ratio

PCD  Passive Case Detection

PACD  Proactive Case Detection

PF/pan  *Plasmodium falciparum*

PHC  Primary Health Care
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>RACD</td>
<td>Reactive Case Detection</td>
</tr>
<tr>
<td>RBM</td>
<td>Roll Back Malaria</td>
</tr>
<tr>
<td>RDT</td>
<td>Rapid Diagnostic Test</td>
</tr>
<tr>
<td>SADC</td>
<td>Southern African Development Community</td>
</tr>
<tr>
<td>SMC</td>
<td>Seasonal Malaria Chemoprevention</td>
</tr>
<tr>
<td>STAR</td>
<td>Structured Additive Regression</td>
</tr>
<tr>
<td>UCSF</td>
<td>University of California San Francisco</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organisation</td>
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DECLARATION

I, Joyce Auala, declare that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

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.................................................... Date...........................................

Joyce Rose Namutenya Auala
DEDICATION

I dedicate my work to my beloved family. You are and always will be my pillars of strength. Thank you for always supporting me, motivating me, reminding me that I have what it takes and how proud you are of me from near and far. I love and appreciate you all very much.
CHAPTER ONE: INTRODUCTION

1.1 Orientation of the Study

Malaria is both a preventable and treatable disease, yet it is still one of the major public health problems in Namibia (Ministry of Health and Social Services [MoHSS], 2009). Although the southern regions of the country are generally malaria free, low to moderate malaria transmission risk prevails in the nine northern regions of Namibia, namely: Caprivi (Zambezi), Kavango, Kunene, Ohangwena, Omaheke, Oshikoto, Omusati, Oshana, and Otjozondjupa (MoHSS, 2009) as can be seen in figure 1. Close to 70 percent of the Namibian population is located in these malarious portions of the country with majority of this population located close along the border with Angola (MoHSS, 2014).

![Map of malaria transmission risks in Namibia](MoHSS, 2014)

Figure 1 Map of malaria transmission risks in Namibia (MoHSS, 2014)
According to the SADC Elimination Framework (2000), malaria elimination involves the implementation of four programme phases in continuum which are: control; pre-elimination; elimination; certification and prevention of reintroduction. For the past years Namibia has been in the control phase in which the country has been scaling up malaria control and prevention by implementing interventions such as the use of insecticide residual house spraying (IRS), long-lasting insecticidal nets (LLIN) and prompt and effective case management with use of appropriate anti-malarials for treatment and prevention in vulnerable populations. In 2007, Namibia was identified as one of four front-line southern African countries chosen to move towards elimination of malaria by 2015 (SADC, 2000). In 2010 the National Malaria Control Program (NMCP) adopted a strategy to eliminate malaria from Namibia by the year 2020 (MoHSS, 2010).

In order for Namibia to reach its goal of zero malaria transmission, an elimination feasibility assessment needs to be carried out, which encourages the use of evidence based methods to estimate key risk factors for imported malaria infection (Pindolia et al., 2012). As malaria incidences being reported at clinics decline, it is important to account for human population movement in introducing infections to areas targeted for elimination, especially at border towns (Tatem & Smith, 2010). Between the years 2001 and 2011, reported malaria cases and deaths attributed to malaria declined by roughly 98% each, however low to moderate transmission of malaria still occurs in the northern regions bordering Angola (Gueye et al., 2014). Importation of malaria through human population movement from neighbouring countries with high malaria transmission areas is a major challenge for eliminating malaria as there is a constant
threat of imported infections within the country coming from across its border (Noor et al., 2013).

Amongst the nine malarious regions bordering Angola, Ohangwena region, with an area of 10582 km, shares the largest stretch of the border with Angola. It is the most densely populated region in the northern part of the country with the highest densities located in the west of the region. In the past, the region reported high numbers of malaria cases per 1000 population each year with 87% of the regions malaria cases being reported from the Engela District, which houses the official border post between Namibia and Angola (Gueye et al., 2014). Assessing human population movement may assist in understanding whether infections commonly originate from across the border or between rural and urban areas within borders (Pindolia et al., 2012). Other operational requirements for malaria elimination include focusing not only on clinical disease but also asymptomatic infections, identifying residual transmission foci and targeting vector control and case detection to high risk areas (Cohen et al., 2013).

1.2 Statement of the Problem

Malaria in Namibia has reduced dramatically since 2005 and as the country moves from the control phase to the elimination phase, efforts to reduce morbidity and mortality of malaria have shifted to the strengthening of control efforts and elimination of transmission foci (MoHSS, 2010). In order for the elimination phase to be executed, interventions that strengthen identification and treatment of all symptomatic cases while clearing malaria parasites from asymptomatic carriers should be identified. In addition, there is a gap in knowledge about infection risk factors at low malaria
transmission such as cross border movement and local malaria hotspots where residual malaria may still persist.

1.3 Objectives:

- To establish the feasibility of conducting reactive case detection of malaria cases in the Engela Health District
- To identify risk factors associated with being a confirmed malaria case
- To establish whether asymptomatic cases cluster around confirmed cases
- To establish whether hotspots of infection exist around confirmed cases
- To establish whether a malaria case is locally or non-locally acquired

1.4 Significance of the Study

The outcome of this study may possibly support the objectives of the SADC Malaria Elimination Framework in areas such as the preparation and implementation of a malaria eradication programme and the identification of transmission foci to begin with elimination and thereby reduce the percentage of the population at risk of malaria transmission. The information and data collected from the research, may possibly contribute greatly to the goals and objectives of the National Vector-borne Disease Control Programme (NVDCP) in identifying malaria hotspots and transmission foci and establishing effective interventions such as the strengthening of passive and active surveillance systems.
CHAPTER TWO: LITERATURE REVIEW

2.1 The Burden of Malaria in Africa

Malaria continues to be a serious health problem despite it being a preventable and curable disease. It is reported to cause more than 250-660 million infections and more than 1 million deaths yearly in Africa. The most severe form of the disease prevails in Sub-Saharan Africa where ninety percent of global malaria deaths have occurred (WHO, 2013b).

Figure 2 Estimated number of Malaria cases in 2012. Source: Kaiser Family Foundation, http://kff.org/globaldata/, based on WHO, World Malaria Report 2013; December 2013.
Pregnant women and children under 5 years of age are the worst affected by malaria (Guillebaud et al., 2013). Reduced immunity in pregnant women makes them highly vulnerable to malaria infection, they are more likely to be anaemic and give birth to babies with low birth weight or still born babies all together (Khan et al., 2014). Risk of severe malaria infection is greater in young children (77% of deaths in Africa occurred in 2012 among sub-Saharan children under five years of age) while risk of infection of pregnant women increases in their second and third trimester of pregnancy (WHO, 2012b; WHO, 2013). Apart from pregnant women and under five children, the malaria risk group also includes young children living in stable transmission areas who have not developed protective immunity against the disease, non-immune or semi-immune pregnant women living in high transmission areas, semi-immune HIV infected pregnant women, people living with HIV/AIDS, international travelers from non-endemic areas who lack immunity and immigrants from endemic areas returning to their home countries on vacation who have developed decreased or absent immunity (Okwa, 2012).

Malaria transmission in Africa is easily attributable to the climatic and ecological conditions of the continent (Teklehaimanot & Mejia, 2008). Since temperature is inversely related to altitude, vector and parasite development is limited as they rely on ambient temperatures which are generally low in high-elevated areas (Afrane, Githeko & Yan, 2011). Weather conditions in the tropical and sub-tropical regions of the continent are favorable for year round transmission of malaria, depending on altitude, creating the perfect environment for transmission of the most infective parasite *Plasmodium falciparum* by Africa’s most common vector, *Anopheles gambiae*. The
primary malaria vector in Namibia is *A. arabiensis* with the presence of two other vectors namely *A. funestus* and *A. gambiae*, having greatly been reduced in the country by the use of DDT (MoHSS, 2014). Development of the parasite inside the mosquito depends greatly on ambient temperature taking approximately 9-10 days at temperatures of 28°C but stopping at 16°C (A. Alemu, Adebe, Tsegaye & Golassa, 2011). The minimum temperature for development of *P. falciparum* and *P. vivax* parasites is approximately 18°C and 15°C respectively (Patz & Olson, 2006). At temperatures between 16°C and 36°C, survival is about 90%, while the highest proportion of vectors surviving the incubation period is observed between 28°C–32°C (A. Alemu *et al.*, 2011).

Namibia is made up of several ecoregions, namely tropical, semi-arid and desert (Wilhelm, 2012). The hottest months of the year are January and February, coinciding with the summer rainfall while limited showers are also experienced in October continuing until April (MoHSS, 2010). Northern parts of Namibia have been experiencing excessive long lasting rains over the years of 2008/2009 and 2010/2011 (Wilhelm, 2012). The average annual temperature is 22°C and regularly increases to temperatures of 35°C and higher during germination season (Dreber & Esler, 2011). The peak malaria season in the country varies from region to region and usually occurs between January to May following the rainy season (MoHSS, 2014). Malaria risk projections based on the mapping and predicting of potential redistribution of malaria vectors and consequent transmission risk especially in the context of climate change may provide policy makers with the opportunity to prevent
malaria outbreaks in vulnerable communities (Tonnang, Tchouassi, Juarez, Igweta & Djouaka, 2014).

2.2 Malaria Treatment Options

2.2.1 Artemisinin-based Combination Therapy (ACT)

In 2006, WHO recommended that the use of artemisinin-based combination therapy (ACT) be adopted as first-line treatment for *P. falciparum* malaria in virtually all malaria-endemic countries (O’Brien, Henrich, Passi & Fidock, 2012; Starzengruber et al., 2014). In Namibia, ACTs were introduced in 2005 after an increase in chloroquine resistance was observed and later rolled out nationwide in 2009 (MoHSS, 2014). Artemisinin was first isolated from *Artemisia annua* in 1972 (Sadiq, Hayat & Ashraf, 2014). Derivatives of the isolate such as artemunate, artemether, arteether and artemisone (Crespo-Ortiz & Wei, 2011; Yin et al., 2014) are highly effective malaria parasite killers (Taylor & Juliano, 2014). Combining artemisinin with drugs that have longer half-lives and different modes of action provides protection against re-infection and limits the development of drug resistance (Douglas, Anstey, Angus, Nosten & Price, 2010; Li et al., 2014).

Several artemisinin-based combinations have been developed, the most common type being artemether-lumefantrine (AL), amodiaquine-artesunate (AQAS), artesunate-mefloquine (ASMQ) (Banek, Lanek, Staedke & Chandramohan, 2014) and artemunate + sulphadoxine-pyrimethamine (AS+SP) used to treat uncomplicated malaria (Elamin et al., 2005). Introduction of the combination therapy drug managed to curb the malaria
parasites drug resistances to chloroquine (Plowe, 2007). In addition, intermittent preventive treatment with Sulfadoxine-Pyrimethamine (SP) is also recommended by WHO for pregnant women living in high transmission areas. Pregnant women are required to receive it at each scheduled antenatal visits after the first trimester. Chemoprophylaxis is available for individuals travelling to malarious regions for short periods of time. The treatment helps prevent malaria by suppressing the blood stage of malaria infections in the body.

2.2.2 Intermittent Preventive Treatment

Intermittent preventative treatment involves the administration of a full course of an anti-malarial treatment at specified time intervals to populations at higher risk of contracting malaria irrespective of their infection status (Patouillard et al., 2011; Tine et al., 2011). With malaria being a major cause of anemia in pregnant women, infants and other children (Grobusch, Egan, Gosling & Newman, 2007), WHO currently recommends the use of three preventive therapies to target these population groups who are at risk of malaria. The three therapies include intermittent preventive treatment for pregnant women (IPTp), intermittent preventive treatment for infants (IPTi) and seasonal malaria chemoprevention (SMC). IPTp for pregnant women involves giving them a curative dose of Sulfadoxine-Pyrimethamine (SP) at least twice during pregnancy, whether they have malaria or not. Despite the growing resistance of *P. falciparum* to SP, it is still the most effective drug for IPT (Anchang-Kimbi et al., 2014). It is known to have good safety profile in pregnancy and good programme feasibility, with the opportunity for delivery as a single dose treatment under direct observation (Hill & Kazembe, 2006). WHO recommends month apart doses of IPTp-
SP for all pregnant women at each of their scheduled ANC visits from as early as the second trimester until the time of delivery (WHO, 2014).

For pregnant women in Africa, 40 countries have adopted the policy of IPTp alongside the distribution of insecticide treated nets. Of the 40 countries promoting implementation of these strategies, all the countries in the SADC region are involved, except for four (Botswana, Lesotho, Seychelles and Swaziland) that do not have explicit policies related to the execution of the strategies (Van Eijk et al., 2011).

Women living in malaria endemic areas naturally have high levels of immunity towards malaria infections and may not experience fever or other symptoms of malaria (Diala, Pennas, Marin & Belay, 2013). In pregnant women however, immunity is somewhat altered and they are more vulnerable to complicated and severe malaria (Amoran, Ariba & Iyaniwura, 2012; Tan et al., 2014). In high malaria transmission areas, *P. falciparum* parasite prevalence is increased in placental and peripheral blood, which is higher in first, compared to later pregnancies (Brabin et al., 2008). In pregnancy, *P. falciparum* infection causes low birth weight in infants associated with parasitisation of the placenta, maternal deaths due to malaria related anemia and it also causes high blood pressure in babies (Diala et al., 2013; Tagbor, Bruce, Agbo, Greenwood & Chandramohan, 2010).

Infants at risk of *P. falciparum* infection in countries with moderate to high malaria transmission should receive a dose of SP along with DPT2, DPT3 and measles vaccine (WHO, 2013b). IPTi provides partial protection in the first year of life against clinical malaria and anemia, and reduces hospital admission associated with malaria parasitaemia (WHO, 2013b). It has been known to decrease malaria episodes in infants
by 22-59% (Beeson, Rogerson, Mueller, Richards & Fowkes, 2011). In areas of high seasonal malaria transmission, infection has been found to be more common amongst the older children as opposed to infants (Wilson, 2011).

SMC, formerly known as intermittent preventive treatment of malaria in children (IPTc), was designed for regions with seasonal malaria transmission whereby children are given two to three doses of a combination treatment of amodiaquine and SP during the high malaria transmission season (Konaté et al., 2011). IPTc appears to be highly effective at reducing episodes of clinical malaria in children under 5 with up to 69% protective efficacy against symptomatic malaria or higher (Beeson et al., 2011). In studies conducted on children under 5 years of age, a 69% reduction in malaria incidence was seen in the children from Mali while a 86% malaria incidence reduction was seen in children from Senegal (Cisse et al., 2009; Dicko et al., 2011). Children that live in areas where malaria transmission is high, acquire immunity to malaria earlier in their childhood, thereby reducing the risk of clinical attacks and deaths by the time they are of school going age (Clarke et al., 2008). However, some children may still remain asymptomatic, harboring parasites which could later cause anemia (Clarke et al., 2008).

2.2.3 Malaria prophylaxis

Malaria prophylaxis is the use of anti-malarial medication to prevent occurrence of malaria symptoms when travelling to malaria endemic areas (Schlagenhauf, Adamcova, Regep, Schaerer & Rhein, 2010). Travelers to Sub Saharan Africa are most at risk of contracting malaria with recent estimates indicating an attack rate of 302/100
000 travelers to West Africa, 49/100 000 to Southern Africa and lower rates of 5.4/100 000 in East Asia and 1/100 000 in the Americas (Schlagenhaus et al., 2010).

People living in malaria endemic areas acquire semi-immunity to malaria infection after repeated infections, resulting in reduced symptoms and disease severity. Immunity can however be lost when long periods of time are spent in non-endemic countries (Castelli, Odolini, Autino, Foca & Russo, 2010). Despite some countries in the Europe Region having successfully eliminated local malaria and being classed as ‘malaria free’, an estimated 10–15 million international travellers from Europe visit malaria endemic areas and 12000–15000 cases end up being imported into the European region with an average fatality rate of 0.3–3% (Odolini, Gautret & Parola, 2012; Ordanovich & Tatem, 2014).

Some drugs or drug combinations that are currently suggested for malaria prophylaxis are chloroquine, atovaquone/proguanil, mefloquine, doxycycline and primaquine (Maria, Vasiliki & George, 2008). Although all these drugs have similar prophylactic effectiveness, their side effects, contraindications and costs vary (Widmer et al., 2010).

Table 1 Prophylactic medications used to prevent malaria, their side effects and contraindications. Available at: http://www.cumc.columbia.edu/student/health/pdf/T-Z/Malaria%20medications1.pdf
<table>
<thead>
<tr>
<th>Medication</th>
<th>Side effects**</th>
<th>Contraindications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroquine</td>
<td>Nausea, vomiting, headache, dizziness, blurred vision and itching. May exacerbate psoriasis, porphyria.</td>
<td>Allergy to chloroquine</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>Dizziness, headache, insomnia and vivid dreams.</td>
<td>History of depression, anxiety, major psychiatric disorder, seizures, arrhythmia. Allergy to mefloquine.</td>
</tr>
<tr>
<td>Malarone (Atovaquone 250/ Proguanil 100)</td>
<td>Abdominal pain, nausea, vomiting and headache</td>
<td>Pregnancy, breastfeeding, severe renal impairment. Allergy to Malarone.</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>Sun sensitivity, nausea and abdominal pain</td>
<td>Pregnancy. Allergy to doxycycline</td>
</tr>
</tbody>
</table>

**Most travelers do not discontinue medication due to side effects.

Chloroquine is a 4-aminoquinoline known to interfere with parasite heme detoxification (Castelli et al., 2010). In combination with proguanil, it is a fast acting blood schizonticide recommended in areas dominated by *P. falciparum* and *P. vivax* (Castelli et al., 2010). Atovaquone and proguanil are known to act on the parasites in the liver tissue preventing blood stage infection (Delves et al., 2012; Schlagenhauf et al., 2010). In combination, atovaquone-proguanil act against the mitochondrial cytochrome *bc1* complex (Rosenthal, 2013). Proguanil is safe for use in pregnancy but
is not recommended when in combination with atovaquine (Irvine, Einarson & Bozzo, 2011; Schlagenhaufer et al., 2010). Mefloquine and doxycycline are blood schizonticidal drugs, acting on parasites in the blood stream that invade erythrocytes. Mefloquine can inhibit heme detoxification by binding to heme (Petersen et al., 2010) while doxycycline inhibits protein synthesis (Briolant et al., 2010). Doxycycline is not recommended for pregnant or breast feeding women and children under 8yrs old (Schlagenhaufer et al., 2010). Primaquine is an 8-aminoquinoline (8-AQ) antimalarial drug with activity against the liver stage parasites of *P. falciparum* (gametocytes) and *P. vivax* (hypnozoites) (Li et al., 2014).

Despite these drugs being highly efficacious at preventing malaria, there are some limits to their effectiveness such as adverse effects, cost, difficulty in monitoring daily compliance and none of them prevent development and relapse of *P. vivax* and *P. ovale* hypnozoites (Nasveld et al., 2010). Resistance to most of these drugs has developed in some countries such as South East Asia, where the efficient drugs are now only doxycycline and primaquine (Maria et al., 2008).

A new drug called Tafenoquine, is currently being developed as a replacement for primaquine and mefloquine and has the potential to protect against all the malaria species, acting on all stages of the parasite (Li et al., 2014; Nasveld et al., 2010). Tafenoquine is a 5-phenoxyl derivative of primaquine currently being co-developed by GlaxoSmithKline (GSK) and the Walter Reed Army of Research (Nasveld et al., 2010). A study conducted on Thai soldiers deployed to an area where both *P. vivax* and multi-drug resistant *P. falciparum* are endemic has shown that Tafenoquine is efficacious in preventing malaria infection by the two *Plasmodium* parasites for as
long as 6 months (Walsh et al., 2004). The protective efficacy of Tefanoquine at a dose of 200mg per day for three days followed by weekly 200mg maintenance doses in semi-immune residence of Ghana and Kenya were 86% each (Dow et al., 2014). Tafenoquine exhibits side effects of nausea, abdominal discomfort and diarrhea, with the lowest dose exhibiting side effects similar to primaquine and doxycycline (Nasveld et al., 2010; Prashar & Paul, 2009).

2.3 Malaria Vector Control

The main way to reduce malaria transmission from high levels to close to zero at the community level is through vector control. Such methods constitute control through the use of insecticide treated nets (ITNs), indoor residual spraying (IRS) and, in some specific settings, larval control. Other methods include, chemoprevention for the most vulnerable populations, particularly pregnant women and infants, confirmation of malaria diagnosis through microscopy or rapid diagnostic tests (RDTs) for every suspected case and timely treatment with appropriate antimalarial medicines (ACTs) (WHO, 2013b).

For individuals, personal protection against mosquito bites represents the first line of defense for malaria prevention. Deet (Diethyltouamide) has widely been used in insect repellent products for use on skin to prevent insect bites (Goodyer et al., 2010). Other personal forms of protection include electric insecticide vaporisers, essential oil candles, and coils (Goodyer et al., 2010).
The three forms of vector control that are more commonly implemented are, insecticide treated nets (ITN) / Long lasting insecticide nets (LLIN), indoor residual spraying (IRS) and larviciding.

2.3.1 *Insecticide treated Nets/ Long Lasting Insecticide Nets*

LLINs are considered best practice for malaria vector control because of how effective, reliable, robust and relatively simple to deliver they are even in remote regions (N’Guessan et al., 2014). WHO recommends total coverage of all at risk populations with the best way of achieving this through free distribution of LLIN. Nets have four effects which include: killing of mosquitoes that land on them, repelling of mosquitoes and possible diversion to a non-human blood host, direct protection for the individual sleeping under the net and reduced transmission from infected individuals sleeping under the net to susceptible mosquitoes (Briët & Chitnis, 2013; Griffin et al., 2010; Maia et al., 2012).

According to WHO, the number of LLINs delivered to Sub Saharan Africa by manufacturers in recent years has increased dramatically from 6 million in 2004 to 145 million in 2010 (WHO, 2013b). Nearly 300 million LLINs were delivered to African countries between 2008 and the end of 2010 (WHO, 2013a). Meanwhile, the number of people protected by IRS in the WHO African Region increased from 10 million in 2005 to 78 million in 2010 (WHO, 2013a). WHO also recommends that 1 LLIN should be distributed for every 1.8 persons in a household. However, net allocation in some countries differs from the recommendation given by WHO, in Sudan one net for each person except children sleeping with their mothers is given, in Uganda 1-4 per family is given, in Niger, Mozambique and Ghana one net was allocated per mother or
household with one or more under 5 children while in Ethiopia and Eritrea, an average of two nets per household is given with special emphasis being given to children and pregnant women in Eritrea and Nigeria (Kilian, Wijayanandana & Ssekitoleeko, 2010). In Namibia, one net per two persons is distributed, with NVDCP having set a goal to achieve 95% net coverage of the entire population at risk of malaria transmission by 2014 (Gueye et al., 2014).

### 2.3.2 Insecticide Residual Spraying

When it comes to IRS, WHO currently advises that malaria control programmes employ the use of 12 different insecticides in rotation or as a mosaic alongside DDT in IRS operations to combat malaria in areas where mosquitoes are DDT-resistant, and to slow the evolution of resistance (Appendix A). Assuming that insecticides of different modes of action are used, mosquitoes resistant to one could still be killed by other insecticides thus delaying selection of resistant mutants in the mosquito population (Okumu & Moore, 2011).

A series of alternative insecticides such as the pyrethroids, permethrin and deltamethrin; the carbamates, bendiocarb, propoxur and the organophosphates, malathion, fenitrothion, pirimiphos-methyl are alternatives to DDT (Aïzoun et al., 2013; David, Ismail, Chandor-Proust & Paine, 2013; Protopopoff et al., 2013). The insecticides are commonly sprayed on the interior walls of homes, more commonly sleeping structures. Vector resistance to pyrethroids and DDT has been reported in parts of Africa including, West Africa, Burkina Faso and Cote de’Ivoire (Tangena et al., 2013). This gives rise to a need to evaluate alternative insecticides that can be used in place of pyrethroids and DDT. A nationwide survey
conducted in 2011 indicated susceptibility of most *A. gambiae* populations to carbamates and organophosphates (Nkya *et al*., 2014). Although the use of DDT has been banned in most developed countries, its low cost and persistence has made it an exception in resource poor countries needing essential public health interventions (Ratovonjato *et al*., 2014).

After feeding, mosquitoes such as *A. gambiae* and *A. funestus* tend to rest on nearby surfaces like walls (Williams *et al*., 2011). If walls are coated with insecticides, mosquitoes that may have picked up the parasite die before they are able to transfer it to another victim when they feed again (Griffin *et al*., 2010).

The success of insecticide treated bed nets coupled with insecticidal residual spraying is important in the eradication of the disease (Takken & Knols, 2009). The killing effect of IRS primarily depends on the indoor resting behaviour (endophilic) of the species (Griffin *et al*., 2010). The fact that many of the important malaria vectors are endophilic gives rise to the effectiveness of IRS as an intervention in that when vectors come into contact with the sprayed surfaces, absorption of lethal doses of the insecticide shortens their lifespan (WHO, 2013a).

**Table 2 Table listing the breeding place, biting and resting habits of three main malaria vectors A. gambiae, A.arabiensis and A.funestus** (Williams *et al*., 2011)
<table>
<thead>
<tr>
<th>Vector</th>
<th>Breeding Sites</th>
<th>Biting Habits</th>
<th>Resting habits</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. gambiae</em></td>
<td>Sunlit temporary pools,</td>
<td>Endophagic (bites indoors), bite late at night</td>
<td>Mainly endophilic (rest indoors after feeding).</td>
</tr>
<tr>
<td></td>
<td>rice fields</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>A. arabiensis</em></td>
<td>Temporary pools, rice fields</td>
<td>Endophagic and exophagic (bite outdoors), outdoors after anthropophilic (bites feeding) and humans) and Zoophily endophilic (bites animals). Bite late at night.</td>
<td></td>
</tr>
<tr>
<td><em>A. funestus</em></td>
<td>Semi-permanent and permanent water, especially within vegetation, swamps, slow streams, ditch edges.</td>
<td>Endophagic, bite mainly late at night</td>
<td>Endophilic</td>
</tr>
</tbody>
</table>

Poor people bear the highest risk of malaria infection as their living standards may offer them little to no protection from mosquitoes without the ability to afford protection methods such as insecticide treated nets (ITNs) (Kimani, Vulule, Kuria & Mugisha, 2006). Low income households regard immediate needs such as food,
clothing, etc., as a greater priority compared to purchasing a mosquito net (Matovu, Goodman, Wiseman & Mwengee, 2009). Net affordability is often not the only factor affecting net ownership or use: other factors include, design of the houses, relatively high temperatures at night (Toé et al., 2009), type, size and availability of sleeping facility and sleeping arrangements (Graves et al., 2011). Given some of these factors, houses might be built too small to use a net, sleeping structures may not have means of hanging up a net or they may be too overcrowded to use a net, areas where temperatures are high at night may make the heat inside sleeping rooms unbearable driving some members of households to sleep side (Kimani et al., 2006).

### 2.3.3 Larval Source Management

Another vector control strategy is larval source management (LSM) which refers to the management of mosquito larval habitats in order to disrupt their development from immature stages to adult stages (Arifin, Madey & Collins, 2013). LSM can be implemented as either the modification of a habitat by permanently altering the land and water by means of landscaping, surface water drainage, land reclamation, coverage of water containers, wells and potential breeding sites; manipulation of habitats by shading or exposing them to sun depending on the vectors ecology, clearing drains and flushing streams; use of biological means of control such as predatory larvivorous fish; or larviciding by use of chemical or biological insecticide to control mosquitoes breeding in aquatic habitats (Fillinger & Lindsay, 2011; Jacups, Jurucz, Whitters & Whelan, 2011; Walker & Lynch, 2007; Walshe, Garner, Abdel-hameed Adeel, Pyke & Burkot, 2013). What makes larviciding a great intervention is the inability of immature eggs, larval and pupal stages of mosquitoes to escape their insecticide treated
habitat in which they live by flying away, thereby preventing adult mosquitoes from emerging in the first place (Killeen, 2014). However, the varied requirements of larval habitats between different species of mosquitoes, their ecology, relatively long flight ranges, and high vectorial capacity makes it difficult to implement this intervention successfully across a wide geographic area (Roll Back Malaria Partnership, 2008; Russell, Beebe, Cooper, Lobo & Burkot, 2013). This makes this intervention appropriate in settings where mosquito breeding sites are easy to identify, map and treat (WHO, 2012c). With the increased use of interventions such as ITN an LLIN, mosquitoes are becoming increasingly resistant to insecticides (Silva & Marshall, 2012). The use of biological control tools can aid in lowering development of insecticide resistance (Bukhari, Takken & Koenraadt, 2013) including the use of microbial agents of bacterial origin, such as Bacillus thuringiensis (Bti) and B. sphaericus (Be) which only attack the larvae of mosquitoes and have no negative effect on non-target organisms or humans (Mboera et al., 2014).

Between 2000 and 2012, the scale-up of interventions helped to reduce malaria incidence rates by 25% globally, and by 31% in the WHO African Region. The global malaria mortality rate was reduced by 42% during the same period, while the decrease in the WHO African Region was 49%. Between 2000 and 2012, a scale-up of malaria interventions saved an estimated 3.3 million lives. 90%, or 3 million, of these are in the under-five age group in sub-Saharan Africa.
2.4 The Four Phase Elimination-Continuum

According to the SADC elimination Strategic Program, in 2007, the Southern African Development Community (SADC) pledged to eliminate malaria from Southern Africa by the year 2015. Countries exhibiting the greatest potential to eliminate malaria were then chosen and split into two main groups. The first group, considered as the frontline countries are: Botswana, Namibia, South Africa and Swaziland. Their northern neighbors with moderately higher malaria transmission were considered the second line countries, and these are: Angola Mozambique, Zambia and Zimbabwe. In order for these countries to achieve a malaria free status, a four phase continuum needs to be implemented. The phases are: control; pre-elimination; elimination; certification and prevention of reintroduction.

Figure 3 Flow diagram showing the four phases of the elimination framework, the level of malaria incidence required before progressing onto the next phase and the status of the chosen countries in the SADC region
2.4.1 Control

Malaria control is the first phase of the continuum. It involves a country or area wide control of the malaria vector, with the main aim of reducing morbidity and mortality and the burden of malaria. Successful implementation of a vector control strategy involves following a few guiding factors such knowledge of micro-epidemiology of malaria including ecology and behavior of the vector, social and cultural characteristics of the human population, and changes therein due to interventions or developments (Hiwat, Hardjopawiro, Takken & Villegas, 2012). Main strategies include use of indoor residual house spraying (IRS), distribution of insecticide treated mosquito nets (ITNs) (Russell et al., 2013), prompt and effective management of cases as they come into the health facilities, use of appropriate anti-malarials for treatment and prevention in vulnerable populations and the use of intermittent preventative treatment using Sulfadoxine-Pyrimethamine (IPTp-SP) for pregnant women (Patouillard et al., 2011) and to reduce malaria cases to a level where malaria is no longer a major public health problem. Once a country is able to reduce the transmission intensity of malaria and bring incidence down to less than 5 indigenous cases per 1000 population per year, then they are considered ready to move on to the pre-elimination phase of the continuum.

2.4.2 Pre-Elimination

Once malaria incidence has been brought down, strategies for pre-elimination can be implemented in target areas. This phase mainly involves strengthening malaria surveillance and health information system with cooperation from all health-care providers, identifying transmission foci and reducing onward transmission from
existing cases (WHO, 2012a). Strategies for this phase include: preparations towards implementing an elimination programme, identifying and eliminating malaria foci, concentrating IRS and ITN coverage in target areas and reducing the population exposed to malaria transmission. In order for pre-elimination to be successful, efforts also need to be concentrated on intensifying inter-country and cross border collaborations between countries with varying levels of transmission (Yangzom et al., 2012), improving ease of access of population whether local or non-local to private and/or public health-care facilities to increase health coverage (Smith, Brugha & Zwi, 2001), reorientation of public and private health service staff to the new goals of malaria elimination and including provision of free treatment and diagnostic services to further reduce malaria cases to less than 1 per 1000 population at risk per year.

2.4.3 Elimination

Transition from pre-elimination to elimination is only achievable once malaria incidence in an area has been brought down to less than 1 indigenous case per 1000 population in a year or to approximately 100 cases per district yearly. This phase of the continuum mainly involves reducing locally acquired cases to zero (Cohen, Moonen, Snow & Smith, 2010). Strategies aimed at achieving this, involve the rapid identification, locating and elimination of any malaria transmission through a monitoring and surveillance strategy (Kelly, Tanner, Vallely & Clements, 2012), strengthening vector control efforts to reduce human–vector contact, improving on personal protection and environmental management methods and further strengthening surveillance systems to one that is able to detect future malaria incidences.
2.4.4 Certification and prevention of re-introduction

Once a country has succeeded in having zero locally acquired malaria cases for three consecutive years, a request to certify its malaria free status can be sent to WHO. Certification can only be granted if the country can provide sufficient proof that malaria transmission has been fully interrupted in the entire country. This includes demonstrating surveillance and health systems that are strong to convince a skeptical observer that there is no longer any malaria transmission occurring in the country (Cohen et al., 2010). Strategies are then redirected from elimination to preventing re-introduction of malaria in the area. This can be achieved by reducing onward transmission from imported cases, targeting vector control to areas of probable transmission, screening of immigrants for malaria when importation of malaria is highly suspected.

Figure 4 Categorisation of countries as malaria free, eliminating malaria, or controlling malaria, 2012 (Cotter et al., 2013)
2.5 Malaria in Namibia

Namibia is currently in the process of moving from the pre-elimination phase to the elimination phase. Having exceeded targets set in Abuja at the World Health Assembly by the Millennium Development Goal (MDG) and Global Roll Back Malaria (RBM) to reduce malaria morbidity and mortality by 2010, Namibia is now in the position of moving towards the elimination of locally transmitted malaria (MoHSS, 2010). The targets that were set by MDG and RBM include: reducing malaria burden by 75% between 2000 and 2015, reducing malaria deaths by 50% in 2010 to near zero preventable deaths in 2015, achieving and sustaining universal coverage with locally appropriate interventions for prevention and case management until coverage can gradually be targeted to high risk areas and seasons only, eliminating malaria in 8-10 countries by 2015 and in the long term eradicate malaria worldwide (Roll Back Malaria Partnership, 2008).

Malaria trends in the country have shown a significant drop in incidence between 2002 and 2013 from 249.7 per 1000 population to 2.1 per 1000 population in 2013. Between 2012 and 2013 however, an increase in incidence from 1.4 to 2.1 was seen mainly due to an increase in cases reported from the Kavango and Zambezi regions (MoHSS, 2014).
Further malaria control and elimination will require that interventions also target the identification and treatment of sub-patent, symptomatic and asymptomatic cases. Also, cross-border movement creates the possibility of importation of infections from Angola into Namibia. This too creates a challenge in Namibia’s goal to eliminate malaria by 2020 (Pindolia et al., 2012).

2.6 Risk Factors in a low Transmission Settings

As specific countries begin to make substantial progress toward malaria elimination, they need to account for cross border malaria transmission from neighbouring countries proving less successful in malaria elimination (Bhumiratana, Intarapuk, Sorosjinda-Nunthawarasilp, Maneekan & Koyadun, 2013; Pindolia et al., 2014). Some Latin American countries experiencing such challenges include Argentina, Paraguay.
and Brazil and in Africa, Zimbabwe-South Africa, Mozambique-Swaziland and Angola-Namibia (Gueye et al., 2012). For this reason, the Namibian Government signed the Trans-Zambezi Malaria Control Initiative in 2006, in partnership with Angola, Zambia, Zimbabwe and Botswana, to undertake cross-border malaria control along the Zambezi River including coverage of the Caprivi and Kavango Regions (Southern Africa Roll Back Malaria Partnership Network [SARN], 2011). In 2011, Namibia also signed the Trans-Kunene Malaria Control Initiative with Angola to scale-up control efforts along the Kunene River for the Angolan districts and coverage of the Ohangwena and Omusati Regions in Namibia (SARN, 2012). Individuals moving from areas of high malaria transmission to areas where low transmission was achieved can lead to the re-introduction of infections (Pindolia et al., 2012). This can result in imported infections, challenges to health systems and onward transmission of malaria cases which if not identified and treated quickly and vector populations are not monitored, can lead to a high risk of outbreaks. Human population movement can be due to various reasons ranging from scarce resources, immediate income opportunities, natural disasters or conflict to vacationers or individuals travelling for shopping purposes or to visit family (Pindolia et al., 2012; The World Bank, 2009).

Housing quality also plays a role in risk for malaria transmission. A study in Gambia showed that children sleeping in mud huts with open eaves and absent ceilings were more likely to be malaria infected (Silva & Marshall, 2012). Traditional huts in rural areas are often built with spaces between the roof and the wall which allows mosquito’s free movement in and out of the house. Previous studies have shown that traditional grass thatched houses with open eaves and lacking ceilings provided more
favourable resting places for mosquitoes and put the occupants at risk of contracting malaria more than houses with closed eaves, iron corrugated/asbestos covered roofs, and having ceilings (Chirebvu, Chimbari & Ngwenya, 2014)

2.7 Malaria Case Investigation

As countries transition from malaria control to elimination, malaria surveillance methods need to be changed from the traditional method of passive case detection (PCD) to methods that allow for the detection of both symptomatic and asymptomatic cases (Gueye et al., 2013). As malaria transmission decreases, relying on PCD, which is when malaria infected individuals present themselves at health facilities for treatment, is no longer sufficient in locating and capturing malaria infections. Decrease in malaria cases means that malaria surveillance systems need to be strengthened in order to detect all malaria infections and prevent them from causing secondary cases by ensuring that they are promptly and effectively treated (WHO, 2012c). Active case detection is a method recommended by WHO which is defined as, “The detection by health workers of malaria infections at community and household level in population groups that are considered to be at high risk. Active case detection can be conducted as fever screening followed by parasitological examination of all febrile patients or as parasitological examination of the target population without prior fever screening.”

Investigation of cases reported from health facilities makes it possible to identify potential transmission foci in order to implement the necessary preventative measures. In a low transmission setting, passively detected cases can be used to identify infectious groups of a population through reactive case detection (Littrell et al., 2013).
The ability to identify and treat local/imported cases as soon as they are presented is key to preventing onward transmission as some populations who do not seek treatment may be asymptomatic carriers. When bitten by a mosquito, asymptomatic carriers can still transmit the parasite, providing a reservoir of infection in areas of low transmission and thereby contributing to continuous low-grade transmission of the disease and possibly leading to devastating epidemics. (Sturrock, Hsiang, et al., 2013; Wickremasinghe, Fernando, Thillekaratne, Wijeyaratne & Wickremasinghe, 2014).

WHO currently recommends active case detection as a crucial strategy for achieving malaria elimination prompting many countries worldwide to make use of the strategy in their malaria control and elimination programs. Two types of methods are defined under the active case detection strategy, these include reactive case detection (RACD) and proactive case detection (PACD) (Sturrock, Hsiang, et al., 2013). RACD involves the screening of households or individuals within a pre-determined radius around a locally acquired case identified via PCD. Individuals are screened with the aim of identifying additional infections, symptomatic or asymptomatic and treating them accordingly and thereby halting malaria transmission (Gueye et al., 2013) RACD takes advantage of the fact that the malaria parasite tend to cluster spatially and temporally which means malaria infections are more likely to be found in households close to passively detected cases (Moonen, Cohen, Snow, et al., 2010). PACD, involves the targeting and mass screening of high-risk populations, targeting all subjects or febrile individuals with the aim of finding symptomatic or asymptomatic without relying on information from passively detected cases (Gueye et al., 2013; Sturrock, Novotny, et al., 2013b). Figure 6 shows the flow in which PCD and ACD are carried out starting
from the point a febrile case is presented at the health facility to visitation of their household should they be found malaria positive.

**Figure 6 Passive case detection and reactive case detection procedures.**

As malaria transmission begins to decline, and the country moves from the control phase to the elimination phase, there is a need to implement strategies that strengthen the collection of epidemiological information on malaria transmission in a low transmission setting. By being able to better identify the true origin of malaria infections in individuals diagnosed in local health facilities as well as identification of secondary cases yet to present with symptoms, hotspots of infection can be established along with factors possibly contributing to continued malaria transmission in those communities. By gathering relevant information through the implementation of tools such as RACD, informed decisions can be made and appropriate interventions can be put in place to further reduce transmission of malaria and support the NMC’s goal of achieving malaria elimination in Namibia by 2020.
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Area

Ohangwena region is one of fourteen regions in Namibia. The region has borders with four other regions: Kavango West, Oshikoto, Oshana and Omusati while it shares a border with two provinces of Angola: Cunene and Cuando Cubango. With an area of 10 706 km², Ohangwena region shares the largest stretch of the border with Angola and has a population of approximately 245 446 people (23.20 people per km²). The region is made up of eleven constituencies, Okongo (which makes up the largest portion of the east of the region), Omundaungilo, Epembee, Eenhana (which houses the main town of the region, Eenhana), Ondobe, Omulonga, Oshikango, Ohangwena, Ongenga, Engela and Endola. Ohangwena region is split into three Health districts, Engela, Eenhana and Okongo.

Figure 7 Map of Namibia showing the Ohangwena region and the eleven constituencies of the region.
Engela Health District was selected as the pilot study area. This study was carried out over the months, December 2012 – July 2014. The health district has 17 clinics (Figure 9) spread out over 7 of the 11 constituencies in the region, namely: Ongenga, Engela, Endola, Oshikango, Ohangwena, Ondobe and Omulonga.

Figure 8 A map of the Ohangwena Region showing the 7 constituencies that make up the Engela Health District.

Figure 9 Distribution of the 17 clinics in the Engela Health District.
3.2 Study Design
The study focused on all malaria cases confirmed by rapid diagnostic test (RDT) that were reported from all 17 clinics in the Engela Health District. A pilot case-control study was then carried throughout the health district from December 2012 – July 2014 to investigate the reported cases and compare them to randomly selected controls. This allowed for the identification of risk factors that possibly lead to continued malaria transmission, the establishment of probable imported cases and the mapping of possible malaria hotspots and transmission foci in the district.

3.3 Case - Control Study
The case control study was conducted with two arms: the case investigation arm and the control arm. The case investigation arm is preceded by passive case detection and then followed by the control arm.

3.3.1 Passive Case Detection
Individuals who visited any of the clinics reporting a fever were immediately tested for malaria via rapid diagnostic test (RDT). For all patients who tested positive for malaria, a malaria case investigation form (a surveillance tool used as per recommendation in the Republic of Namibia, MoHSS, National Policy on Malaria Brief, 2014) was filled in by the attending nurse at the time of diagnosis.

The case investigation form (Appendix B) was used to gather various sets of information in four different sections as follows:
Section 1: date of admission/diagnosis, time of treatment, GPS coordinates of place of treatment (health facility/home), investigators name and rank.

Section 2: patient name and surname, age, gender, contact details, nationality, current occupation, village of current residence, name of the household head, name of the village headman.

Section 3: onset of fever, fever history, method of diagnosis, type of malaria infection, severity of infection, history of malaria infection, prescribed treatment, onset and type of symptoms prior to hospital visit.

Section 4: travel history, mode of travel, reason for travelling, means of protection from malaria

Section 5: net ownership, net usage, floor and wall material of sleeping structure, insecticidal spraying of sleeping structure, number of household residence.

The malaria positive patient was informed by the nurse about the study and that a malaria investigation team would be visiting their household to conduct an interview with them and the rest of the household members, with official consent to be confirmed upon arrival at the household from the head of household.

The case investigation forms were then forwarded to the Environmental Health Practitioners (EHP) at the Engela district hospital who proceeded to enter the information onto an electronic database before informing the study supervisor about the case via telephone or mobile text as shown in Figure 10.
Figure 10 Algorithm of the fever diagnosis procedure as it was performed on a patient by the attending staff at a health facility.
3.3.2 Reactive Case Detection (RACD)

All malaria index cases were traced within two weeks of the case being reported using the information provided on the case investigation from in order to conduct a detailed case investigation at their homes.

Index cases who provided a mobile number were first contacted in order to get helpful directions to the village and household. In instances where directions were unavailable, the index case households were located by asking village headmen, authorities at local schools or community members for directions to the index household or their village.

Once the index household was located, the researchers entered the household and introduced themselves before explaining the nature of the study to the household head and members of the household that were present at the time of the household visit.

3.3.3 Case Neighbourhood Investigation

In the case arm of the study, consent to proceed with the study was first sought from the head of the index household before any testing or inspections were done. Consent forms were provided either in English or Oshikwanyama, the common dialect of the district (Appendix C) and in the event that there were participants who could not read, the consent forms were read aloud in the preferred dialect. First, an interview was conducted with the household head to gather information relating to risk factors for malaria, such as, whether the house had been sprayed, bed net ownership and condition of nets (if any) and other socio-demographic information (Appendix D2-6,8 & 9).

Following this, basic demographic information, recent fever history, and travel history were recorded for each individual living in the household (Appendix D7).
Figure 11 A simplified example of the flow in which data collected during interviews was entered onto the tablets during household visits

All individuals were assigned a unique four letter barcode (Appendix E) and the consenting individuals were then screened for malaria via RDT (Appendix F). All individuals tested for malaria were informed of their results. Individuals who tested positive for malaria were immediately treated by the nurse and provided with the appropriate dosage of artemether-lumefantrine (Artefan®) while a case investigation form was filled out on them. All RDT samples were packed away into zip-lock bags
with a sachet of desiccant. The blood samples were stored in a cool dry location and periodically transferred to Windhoek where they were stored at -20°C until they were required for extraction of DNA.

Following the one-on-one interviews with the household members, sleeping rooms were inspected with assistance from an EHP to ascertain whether the rooms had been sprayed, to view whether sleeping nets were present or absent and to assess the condition of the nets, if present. In addition to the index household, individuals from up to four closest households (approximately 30 individuals) were also invited to participate in the study and this constituted a neighbourhood. The four surrounding households underwent the same interview and screening procedure as the index household did (Figure 12). Surrounding houses were only chosen if the head of the household was present and if they gave consent to participate in the study. If individuals were not present in any of the selected households, two return visits were made to complete data collection. If the third attempt was unsuccessful, the household was replaced by the nearest eligible neighbouring household.
Figure 12 Algorithm of the household visit procedure conducted by the malaria investigation team.
3.3.4 Control Neighbourhood Investigation

Each case household visited was matched with a control household in the control arm of the study. The control households were designated from randomly selected enumeration areas (EA) within the Engela Health District from the full census list provided by the Namibia Bureau of Statistics.

Control households underwent the same interview and screening procedure as described for the index households in the case neighbourhoods (Section 3.3.3). Four of the closest households surrounding the control household were also selected and recruited into the study.

Control households were only identifiable by a number, village name and GPS coordinates. No information on the residents of the particular household was provided to limit the influence of bias.

3.4 Study Population

The study population included all individuals living anywhere within the Engela Health District during the study period of, December 2012 to July 2014.

3.5 Study Sample

The study sample was selected by recruiting any individual or households that fulfilled a certain set of criteria.
Case individuals

Individuals were included in the study if they met both of the following criteria:

- the individual was a malaria case visiting any of the 17 chosen clinics testing positive by RDT/microscopy
- they were willing to participate in the study

Individuals were however excluded if they met any of the following criteria:

- the individual was a suspected malaria case visiting any of the 17 chosen clinics testing negative by RDT/microscopy
- they had an invalid test result when tested by RDT/microscopy
- they were unwilling to participate in the study

Control individuals

Controls were individuals living in the district who were only selected if they met all of the following criteria:

- they tested negative by RDT on recruitment
- they had not tested positive to malaria within 1 week prior to recruitment
- they were willing to participate in the study
A control was excluded if they met any of the following criteria:

- the individual tested positive by RDT on recruitment
- they tested positive by RDT one week prior to recruitment
- they were unwilling to participate in the study

Case and Control Households and Neighbourhoods

In addition to the index case/control households, individuals from up to four closest households were also invited into the study, this constituted a neighbourhood (Figure 13). Surrounding households were only chosen if they were within a 500m radius from the index case/control household and these distances were monitored using GPS devices. When a total of approximately 30 individuals were recruited from the surrounding households for each case/control neighbourhood before a total of four households were visited, addition of individuals was stopped.

Residents were only chosen if they were willing to participate in the study and were excluded from the study if they were unwilling to participate, if they did not normally sleep at the household or if the household head had not given consent for members of the household to be recruited into the study.
Figure 13 Diagram showing the selection overview of houses surrounding the index household in order to make up a neighbourhood. The red house signifies the index household which could either be the residence of the case individual or a randomly selected control household; the green houses signify the closest surrounding households selectable within the 500m radius of which only four are selected.

3.6 Field Work Procedure

3.6.1 Information Dissemination

Before beginning the study, local authorities and respective administrative units of chiefs in each of the 7 constituencies of the district were informed about the study and its activities. Information was shared with the localities of the study by the councillors’ through radio broadcasts. Consent and cooperation from the local authorities was sought in order to focus majority of the time available to household visits in order to
recruit the maximum number of individuals into the study from both case and control neighbourhoods.

Figure 14 A hierarchy diagram representing the order in which information about the study was disseminated and consent was sought from local authorities in the Engela Health District.

3.6.2 Interview Setup

Consent to recruit all individuals living within one household was sought from the head of household or guardian in charge. Household members were given the opportunity to read the consent form or have it read to them by one of the team members before any of the individuals were recruited into the study.
Questionnaire interviews with the household members were conducted by the study supervisor, while the nurse conducted the screening procedure. Any mentioned breeding sites in the surroundings were examined for the presence of mosquito larvae with assistance from the EHP.

CareStart™ malaria HRP-2/pLDH (Pf/pan) combo testing kits were used for the screening process. Safety lancets or lancets originally provided with the malaria testing kits were used to finger prick individuals. The ring finger of the less dominant hand was chosen for pricking. The area to be pricked was first cleaned with small pre-packaged pads soaked with 70% isopropyl alcohol or cotton balls moistened with methylated spirits. The finger was then pricked with a lancet, gently squeezed and the first drop of blood was wiped off with a fresh dry piece of cotton. Subsequent drops of blood squeezed out were then used in the RDT cassette. In the event where not enough blood was provided, another finger on the less dominant hand was pricked to try again. Malaria positive individuals presenting as secondary cases in households were immediately asked to present their hospital cards to the nurse for their fever history to be analysed. The nurse proceeded to record their temperature before prescribing anti-malarials to them and entering a follow-up appointment in their hospital card.

### 3.7 Data Management

Tablets with GPS capabilities were used to collect data from the interviews. A specially designed application for the questionnaire was installed on the tablets. The tablets were also GPS enabled to allow for the recording of geographic coordinates of
the households visited and sleeping structures viewed. The questionnaire was designed to automatically fill the coordinates into the required fields when prompted to (Appendix G).

A map application was also installed on the tablets that allowed the team to have a satellite view of the district and make it easier to follow routes and road markings in real time when first trying to locate index case households and index control households. The map application also came with a feature that allowed the team to record tracks to households in case return visits needed to be made. It also helped determine whether the index household had any surrounding houses within the 500m radius that could be included in the study to constitute a neighbourhood whenever visibility was hampered by vegetation.

Data on the tablet was backed up at the end of each day onto an online server (Appendix H). All the data collected was saved in csv file format and entries made on the questionnaire were automatically converted into and viewed as numbers in the downloaded csv spreadsheets. The data files were downloaded regularly and combined in order to double check and clean errors in the data such as double entries, skipped entries or incorrect entries using a legend as a guide. Errors that were discovered were then noted down and corrected on the tablets before they were backed up again. This allowed for any new corrections made to permanently reflect on the server unless changed again.
3.8 Data Analysis

All models were implemented in R statistical system using \textit{spatstat} package for spatial point pattern analysis and \textit{BayesX} for the structured additive regression model. An empirical Bayesian approach was used in implementing the STAR model.

Spatial analysis was implemented through three approaches. In the first approach, a spatial point pattern analysis was applied to establish whether cases cluster. Ripley’s K function was used to examine the extent of clustering by measuring the distance at which spatial clustering decayed. To assess whether hotspots of infection existed around confirmed cases, a density map based on the index cases was generated and then overlaid with the secondary cases.

In the second approach, cases and controls were used to produce a risk surface map. This was followed, as a third approach, by a flexible model using structured additive regression (STAR), which permitted simultaneous analysis of spatial, nonlinear, and fixed effects in a single model. The spatial effects accounted for both structured and unstructured effects captured at neighbourhood level. The nonlinear effects were modelled for longitude and latitude, while the fixed effects included both individual and housing characteristics: the individual covariates were (i) age of the respondent, (ii) gender (iii) whether the respondent slept under a net the previous night, and (iv) whether the respondent travelled in the past six weeks. The housing characteristics considered were (i) whether the sleeping structures were sprayed, (ii) whether the homesteads are closer to a breeding site, (iii) whether the sleeping structures have eaves, and (iv) whether a house has insecticide treated bed-net.
3.9 Research Ethics

There was ethical approval from the Ministry of Health and Social Services Biomedical Research Ethics committee, London School of Hygiene and Tropical Medicine (LSHTM), and the University of California, San Francisco (UCSF). This study was part of a larger study being conducted by LSHTM and UCSF. All study participants signed consent forms translated into the local language and consent for individuals under the age of 18 was provided by their parents or guardians. All clinical testing and treatment was conducted by a qualified nurse from the district clinic/hospital. Individuals participating in the study did not benefit financially from it. All records were kept confidential. Data was stored on a secure computer for up to ten years, with no personal identifiers of the study participants. Participants were identified primarily by their study number, personal identifiers for patients were not entered into the computerized database and no individual identities were used in any reports or publications resulting from the study.
CHAPTER FOUR: RESULTS

4.1 Distribution of Confirmed Malaria Cases

A total of 190 confirmed cases were reported from the Engela Health District during December 2012 – July 2014. Upon investigation, 70 (36.8%) of the cases were found to be of local individuals residing within the district, 47 (24.7%) of the cases were of Angolan nationals who do not reside within the district but only crossed the border seeking medical treatment, 65 (34.2%) of the cases were of untraceable individuals whose homesteads could not be located for investigation and 8 (4.2%) of the cases were asymptomatic cases that were discovered during reactive case detection (RACD) in the case and control neighbourhoods.

Table 3 Description and Distribution of confirmed malaria cases reported in the Engela Health District from December 2012 – July 2014.

<table>
<thead>
<tr>
<th>Description of Confirmed Cases</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local Residents</td>
<td>70</td>
<td>36.8</td>
</tr>
<tr>
<td>Angolan Nationals</td>
<td>47</td>
<td>24.7</td>
</tr>
<tr>
<td>Untraceable Cases</td>
<td>65</td>
<td>34.2</td>
</tr>
<tr>
<td>Asymptomatic Cases (detected through RACD)</td>
<td>8</td>
<td>4.2</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>190</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

From the total 190 confirmed malaria cases in Engela Health District, Engela District Hospital, Odibo Primary Health Clinic (PHC), Onamunama PHC (also known as
Hamukoto Wakapa PHC) and Ongenga Clinic were the top four health facilities in the district to report the most malaria cases. These facilities are among five of the health facilities located 3 to 5km away from the northern border shared with Angola (Figure 15). Three of them, Engela District Hospital, Odibo PHC and Onamunama PHC, making up a part of the 4 largest facilities in the district, the fifth being Ongha PHC further in land.

Figure 15 Location of the facilities where most of the confirmed malaria cases were reported
As can be seen in Figure 16, Engela District Hospital reported 96 cases, Odibo PHC reported 29 cases, Onamunama Clinic (Hamukoto Wakapa Clinic) and Ongenga Clinic both reported 10 cases. The first two clinics also had the highest number of Angolan cases and untraceable cases with Engela reporting 43 and 21 respectively and Odibo PHC reporting 16 and 2 respectively. Onamunama PHC and Ongenga Clinic reported 8 and 2 untraceable cases respectively. Local cases reported amongst these clinics were 31 from Engela District Hospital, 11 from Odibo PHC, 8 from Ongenga Clinic and 2 from Onamunama. The fifth clinic located close to the border is Edundja Clinic which reported 4 cases total with 3 being untraceable cases and 1 being an Angolan case.
Figure 16 Graph shows the number and classification of confirmed malaria cases reported at each clinic in the Engela Health District.
4.2 Reactive Case Detection (RACD)

RACD of the confirmed local cases was conducted in 132 neighbourhoods (Figure 17). A typical neighbourhood in the study was made of a maximum of five houses, that is, the index case or control household plus a maximum of four surrounding households within a 500m radius to the index household. A total of 66 neighbourhoods from the case arm and 66 neighbourhoods from the control arm were visited. Case neighbourhoods made up a total of 311 households and control neighbourhoods made up a total of 294 (Figure 17).

![Diagram showing the distribution of participants from RACD in the case and control arms of the study.](image)

**Figure 17** Diagram showing the distribution of participants from RACD in the case and control arms of the study.
From the case neighbourhoods, a total of 1981 (55.2%) individuals were recruited into the study. Household interviews were conducted successful with 1830 (92.4%) individuals of which 1728 (94.5%) consented to both an interview and screening for malaria while 102 (refusal rate of 5.1%) refused to be screened although they consented to being interviewed.

From the control neighbourhoods, a total of 1611 (44.8%) individuals were recruited into the study. Household interviews were successful on 1449 (89.9%) individuals of which 1299 (89.6%) consented to both an interview and screening for malaria while 150 (refusal rate of 9.3%) refused to be screened but only consented to being interviewed.

A total of 151 (7.6%) individuals from the case arm and 161 (10%) from the control arm of the study were not present for a full interview or screening of malaria at their households. However the same information as that collected from present individuals was still gathered on the absent individuals. The only information excluded was that on their fever history, after dark/late night activities and travel history.

### 4.3 Case Clustering and Risk Mapping (Spatial Analysis)

Cases and control households were geo-located during RACD and mapped to show their distribution in the district (Figure 18). Locations of secondary cases were included in the map to further distinguish where they were located. Clustering of cases
can be seen in the northern regions of the district with their location gradually becoming scattered southwards of the district.

Figure 18  Geolocation of cases, controls and asymptomatic cases in the Engela Health District.

Figure 19 is a surface risk map of malaria that was created to show the probability of risk of malaria infection in the district. The map shows how risk of infection changes throughout the district, taking into account the location of cases and controls. Probability of malaria infection was estimated at a mean of 2.2% (median of 1.1%), with a range between 0.04% and 28.3%.

The top of the key shows where there is elevated risk of infection around the mean and in turn where pockets of risk of infection are located. By observing the map,
concentrated risk of infection pockets can be seen spread out along the northern areas of the district with a gradual decrease in concentration moving south.

**Figure 19** Probability map of malaria risk of infection in the Engela health district

Graphs were plotted to test for clustering in order to draw a comparison between spatial change in risk by latitude and longitude. The graphs showed that increasing change in risk with latitude was observed as one moved from south to north (Figure 20a) with a significant increase as the graph line approaches zero. Decreasing change in malaria risk with longitude was observed as one moved from west to east (Figure 20b) although there was no significant reduction. This was consistent with the surface risk map in Figure 19, where most areas with increased risk of infection were observed to be present in the northern areas of the district and decreasing southwards of the district.
Figure 20 Malaria risk, given as log odds ratios, changing risk with (a) latitude and with (b) longitude.

4.4 Demographics

4.4.1 Gender Distribution

As can be seen Figure 21, from the case and control arms of the study more females (52% and 56%, respectively) participated in the study compared to males. However, most of the index cases investigated were male individuals (68%) compared to the females (32%). Statistically, male individuals were more likely to present with malaria infection and this implies an increased risk of malaria infection linked to male individuals (OR=2.09, 95% CI: 1.26-3.46, P=0.004).
4.4.2 Age Distribution

The age of study participants ranged from 1 day old to 102 years old. In the population described as index cases, the age group of 16 – 25 years had 29% of individuals, the case arm had 27% of individuals found to be between the ages 16 and 25 years and for the control arm where there was no malaria, 25% of individuals were found to be between 6 to 15 years (Figure 22).

The difference in age group shows that the control study arm has mostly young children while the case arm has mostly middle aged people. With the case neighbourhoods having more individuals between 16 and 25 years, these individuals may be at higher risk of malaria.
Figure 22 Age groups of all individuals from the index, case and control neighbourhoods

4.5 Breeding Sites

Of the total 605 households visited, 71% of case households were located near a potential breeding site while from the control arm they were 52% (Figure 23). A potential breeding site was regarded as any water body located near the households that was either natural or man-made that could stand as a habitat for the *Anopheles* mosquito to breed. Statistical analysis showed that there was a potential increase in risk of malaria infection for houses close to breeding sites (OR=1.08, 95%CI: 0.56-2.10, P=0.831).
Figure 23 Graph showing the percentage of households from the case and control neighbourhoods that are located near a potential breeding site.
Figure 24 The location of case households (red dot) and control households (blue dot) in relation to established water bodies of the Cuvelai drainage system in the district.

4.6 Mosquito Net Coverage

Net coverage (households owning a net) was 66% in case neighbourhoods with an average of 2.8 nets per household and an average of 6.4 people per household. Control neighbourhoods had a net coverage of 70% with an average of 2.6 nets per household and 5.5 people per household. Both ratios of nets per individuals met the recommend net distribution ratio of 1 net: 2 persons (Case 1:2.3, control 1:2.1).
Low net coverage is associated with increased risk of malaria infection (OR=0.89, 95% CI: 0.45-1.74, P=0.746). While, the likelihood of infection for those who sleep under a net is statistically lower, compared those who do not (OR=0.24, 95% CI: 0.11-0.50, p<0.001).

Among the households that own mosquito nets, 80% and more of them had their nets hanging up in the rooms (Figure 26). When net usage was observed, it was found to be similar across both study arms with 52% and 54.3 % of case and control neighbourhood net owners respectively, actually making use of their nets (Figure 27).
Figure 26 Graph showing percentage of the most commonly observed orientation of mosquito nets in sleeping structures

Figure 27 Graph showing percentage of net usage among net owners from case and control households
When asked for reasons as to why net usage was low, the most common reasons was due to nets not being enough in the household (Figure 29). In the case neighbourhoods, 40.8% of households gave this reason while 54.5% of control households had the same reason. Apart from nets not being enough, 21.1% of case households expressed that the weather being too hot to sleep comfortably under a net demotivated them from sleeping under a net. In control households 14.5% of households said lack of net usage was due to their nets not being hung up properly or at all in their sleeping structures. Other reasons expressed included nets causing itching, allergic reactions and an absence of mosquitoes in the area resulted in a lack of net usage among 14.5% of case households and 14.5% of control households.

**Figure 28 Correct usage of mosquito nets in sleeping structures**
Figure 29 Reasons for the lack of use of a mosquito net among net owners in case and control households.
4.7 IRS Coverage

Observation of sleeping structures revealed that more sleeping structures from case neighbourhoods (72.2%) compared to controls neighbourhoods (67.4%) were not sprayed with insecticide (Figure 31). The presence of eaves in a structure also presented an increased risk of malaria infection (OR=1.16, 95% CI: 0.77-1.73, P=0.482) with 70.5% of case neighbourhoods being exposed to mosquitoes easily entering the rooms compared to 64.2% of sleeping structures from control neighbourhoods (Figure 30).

**Figure 30** Graph showing the percentage of sleeping structures from both case and control households that have eaves.
Figure 31 Graph showing the percentage of sleeping rooms that are not covered by IRS

4.8 Travel History

Investigation into travel history revealed that travel occurred more among the index cases with 73% of them having travelled close to the time they reported malaria positive at a health facility (Figure 32). Looking at the travel history between case and control neighbourhoods, participants from the case neighbourhoods travelled 10% more compared to those from the control neighbourhoods (34.2% vs 24.1% respectively).
Breaking down travel history by gender revealed that travel was more common among the male individuals than the females (Figure 33). Majority of the index case males had travelled, representing almost half of the entire index case sample (45.7%). Among all participants from the case and control neighbourhoods, travel was more common among the males from the case neighbourhoods as opposed to the females (19.3% vs 14.8% respectively) while travel among the males and females from control neighbourhoods was fairly the same, varying only by 0.3%.

**Figure 32** Graph showing percentage of travel among combined male and female participants of the study
Among individuals who had travelled, Figure 34 shows that more case individuals (29.7%) travelled across the border to Angola than control individuals (8%). With unadjusted odds ratio for travel estimating at OR=5.95 (95% CI: 2.58-24.31, P=0.0018) and adjusted odds ratio estimating at 2.74 (95% CI: 1.65-4.57, P<0.001), travel was a significant associated risk factor of malaria infection in the region.
Figure 34 Graph showing individuals who travelled across the border to Angola.
CHAPTER FIVE: DISCUSSION

5.1 Passive and Reactive Case Detection (PCD and RACD)

With malaria incidence on the decline in Namibia, and as the country moves from the control phase to the elimination phase, it is important to understand the factors surrounding continued malaria transmission even in a low transmission setting. Relying on passive case detection (PCD) alone is unlikely to have the impact on the parasite population required to interrupt malaria transmission (Sturrock, Novotny, et al., 2013) as PCD focuses mainly on symptomatic patients reporting to a health facility for treatment (Gueye et al., 2013). However, it is well known that asymptomatic cases contribute to the continued transmission of malaria as gametocyte carriers providing parasite reservoirs in their communities (Zoghi et al., 2012). Information regarding these reservoirs is key knowledge for targeting foci of transmission (Sluydts et al., 2014). In order to identifying and treat all malaria infections, a strong surveillance system and appropriate response need to be implemented (Sturrock, Novotny, et al., 2013b). Reactive case detection (RACD) is such a surveillance tool, triggered when a case is identified by PCD (Davis et al., 2011) and involves visiting the household of the reported case and screening the household residents and the neighbours within a defined radius (Moonen, Cohen, Tatem, et al., 2010).

A RACD pilot study was implemented in the Engela Health District of the Ohangwena region focusing on all malaria positive cases presenting at any of the 17 health facilities spread out across the district. During PCD, data collected on cases included physical
address, information on heads of household and village headmen, patient demographic information and travel history via a standard questionnaire (Appendix A). This information was used to classify cases as either locally acquired or imported. By using the information provided, the patient’s household could be visited to determine whether they were truly a resident of the household and resided there at the time of infection onset or whether they had travelled from elsewhere seeking only temporary accommodation while receiving treatment at the nearest health facility.

Tracing cases reported from health facilities relies greatly on the timely provision of accurate and quality information of new cases (Kamanga, Moono, Stresman, Mharakurwa & Schiff, 2010). In order to successfully trace a malaria case to the household, timely provision of accurate malaria surveillance data is necessary (Moonen, Cohen, Snow, et al., 2010). In some events, incorrect or insufficient personal and residential details were provided on patients reporting malaria positive at a health facility. Looking at the 190 cases reported in district during the period of the study, 34.2% were untraceable due to this discrepancy. Upon enquiry from staff at health facilities as to why data was insufficient or lacking at times, various factors came to light. Some attending nurses spoke about not having enough time to collect the information from the patient due to patient overload and short staffing at particular health facilities, or the patient may have been a minor who wasn’t with a parent or guardian and lacked information such as contact details, correct name of household head or name of village headman to provide the nurse or the patient may have left the health facility prematurely during treatment without full information being acquired from them. Other recognised factors include confusion between Angolan villages and
Namibian villages that have the same name, resulting in nurses incorrectly assuming that patients live in Namibia or Angolan patients who purposefully provide false information in order to pay a lower hospital fee (Gueye et al., 2014). The factor of misleading information from Angolan nationals can lead to a distortion of the true burden of malaria in the country and also deplete resources during surveillance which could have been reserved for use on actual local cases. Additionally, the timeliness of nurses reporting cases to higher levels of the health system is often too slow for rapid action on index malaria cases. In some settings, failure of facilities to completely report cases at all results in accumulation of incomplete datasets and an under-reporting of malaria burden (Yukich et al., 2014).

5.2 Border Town Health Facilities

Looking at the health facilities in the district, Engela Hospital, Odibo PHC and Onamunama Clinic were the top three to report the most malaria cases. Engela Hospital and Odibo PHC are two out of three larger health facilities in the district with the ability and provisions to cater to more patients than most of the smaller facilities. The top three health facilities are also located in villages that are on average 5km away from the northern border of the country. This means that Angolan nationals who crossed the border in search of adequate healthcare may have found one of these three facilities to be easily accessible, closer to their village and better equipped to meet their medical needs than their own health facilities.
5.3 Asymptomatic Cases

Cases of symptomatic malaria detected at health facility level in areas of low transmission can help in the identification of hotspots, as additional asymptomatic cases can be found living in close proximity to the index case (Mosha et al., 2014). Screening of residents from households of confirmed malaria index cases presented a higher proportion of asymptomatic cases than was detected by screening residents from control households. The chances of finding an asymptomatic case was eight times more likely to occur in an index case household with only one asymptomatic case occurring from a household in the control neighbourhoods. Evidently this means that targeting index cases by RACD is a plausible method of identifying malaria hotspots of asymptomatic reservoirs in the district (Table 3). Geolocation of the asymptomatic cases onto the probability risk surface map (Figure 19) places them in areas identified as high risk areas.

5.4 Recruitment of Case and Control Individuals

More individuals were recruited in the case arm of the study (55.2%) compared to the control arm (44.8%). Individuals from control neighbourhoods were more likely to decline being a part of the study than case individuals. Reasons being that if no one was reported to have malaria in their area or if there are no mosquitoes in the area getting tested becomes less of a concern to them.
5.5 Risk Factors

Risk factor analysis performed for this study identified a number of variables that were associated with increased risk of malaria transmission. Such variables include: gender, age, IRS coverage and mosquito net coverage and travel history.

5.5.1 Gender

In low malaria transmission settings, malaria burden is usually known to shift from young children and pregnant women to men with occupational or behavioural factors that put them in contact with infectious mosquitoes (Littrell et al., 2013). The fact that 68% of the local confirmed malaria cases reported during the study were male corresponds with this fact. Females and children are more likely to stay indoors, travel less and use protective measures against exposure to malaria (Yeshiwondim, Gopal, Hailemariam, Dengela & Patel, 2009) while men are often known to engage in occupational or social activities that could increase their risk of malaria infection. Such activities could include travelling to areas of high endimicity whether for agricultural, employment or visitation purposes (K. Alemu, Worku, Berhane & Kumie, 2014) or spending extended periods of time at local watering holes in groups in the evening hours for leisure (Parks & Bryan, 2001)

5.5.2 Age Distribution

In high transmission areas, children under five and pregnant women are the most vulnerable groups. However, the highest age range in the study was found to be of individuals between the ages of 16 – 25 years and not the more commonly known risk population of children under five years old. With mosquito net distribution having
been prioritised to pregnant women and children less than five years, younger children were more inclined to sleep under a mosquito net compared to those aged five and up (Mawili-Mboumba et al., 2013; Winskill, Rowland, Mtove, Malima & Kirby, 2011) especially children who still sleep with their mother and are protected by her bed-net. This in turn may have gradually shifted risk of malaria infection to older children. Considering the fact that the case neighbourhood had the highest number of individuals within the 16 - 25 year age range, this identifies them as a potential malaria risk group. Since older children are more likely to work and play where the *Anopheles* vector is present, especially at dusk when *Anopheles* becomes active (Peterson et al., 2009) exposing them to increased risk of malaria infection.

5.5.3 Breeding Sites

The female *Anopheles* mosquito can lay her eggs in a wide range of locations. Potential breeding sites include fresh water or salt-water, vegetative or non-vegetative and shady or sunlit water bodies. These can include anything from ground pools, small streams, irrigated lands, freshwater marshes, forest pools, shallow water bodies in flood plains, and any other place with clean, slow-moving water. Some water bodies are present all year round while others only develop after heavy rainfall or as a result of drainage from other larger sources of water. In the study area breeding sites were considered to be naturally existing water bodies such as those of the Cuvelai drainage system, shallow waters within flood plains, lakes and small ponds that contained water all year round. Of the households from case neighbourhoods, 71% were found to be in close proximity to a potential breeding site.
Current interventions that target management of larval sources include checking the water bodies around households where cases were reported for larvae. Once *Anopholene* larvae have been identified to be breeding in the water body, the appropriate community authorities are informed of the intent to treat the water bodies with larvicides in order to eliminate the vector and decrease the chances of malaria transmission in that particular area. When water bodies that could prove to be potential breeding sites remain untreated, risk of malaria transmission increases. Holding into account that some individuals may be asymptomatic, being in contact with malaria transmitting mosquitoes puts the area at risk of high malaria transmission and thereby creating a hotspot of infection which if not managed could easily give rise to an epidemic.

### 5.5.4 Mosquito Net Coverage

Net coverage in Namibia has been set to 1 net per 2 people in a target population with NVDCP setting a goal to achieve 95% coverage of all at risk populations. Taking this into consideration, the case neighbourhoods visited should have a net coverage of 3.3 nets per person in a household, but actually have coverage of 2.8 nets present per household. According to the goal set by NVDCP, net coverage is 88%. While control households that should have 2.8 nets per person, actually have 2.6 nets per household, putting coverage at 93%, which is closer to the goal then the case neighbourhood coverage.

Case households were observed to have net coverage 4% lower than in control neighbourhoods. Considering if households are located close to breeding sites, being in possession of a mosquito net would decrease the likelihood of malaria transmission.
among individuals at night, especially in households where the cases originate from. Statistical analysis found that household members who actually made use of their nets could significantly reduce their risk of exposure to infection. Considering that 66% percent of case households are actually in possession of a mosquito net, it is of great concern when 52% of this proportion of net owners (which translates to 34% of the study sample from the case arm) does not make use of the mosquito nets they have. When asked for reasons why net use behaviour was so poor or lacking, the most common reason some household members claimed was that it was too hot to sleep under the nets, others claimed they have no way of hanging up the net in the sleeping structure or don’t know how to set the net up, while some others claimed that the nets caused “allergic reactions” or itching driving them to avoid using the net all together. Majority of individuals however claimed that although the household was in possession of mosquito nets, they were not enough to cover the number of people present in the household. Often adults would end up sacrificing their nets for the children to use. Some households had uneven gender and age distribution which ultimately meant that some individuals wouldn’t be able to sleep under a net. For example, in a four member household that only received two nets, two people would otherwise have to share a net. In the event that this particular household was made up of the grandmother, two girls (one under five and the other 20) and one boy of 17, one net would be shared between the grandmother and the youngest child, leaving one more net for the girl and boy to decide who uses it. In some households the only nets present were reserved for use by the elderly (grandparents) whose frail conditions considered them as at risk populations.
5.5.5 IRS Coverage

The increased risk of malaria in houses with walls that have holes can be due to increased access of mosquitoes to bite humans (Woyessa, Deressa, Ali & Lindtjørn, 2013). Statistical analysis revealed significance in the proportion of sleeping structures that were not sprayed. Over 70% of sleeping structures from case neighbourhoods had not been sprayed with any type of insecticide to ward of malaria transmitting mosquitoes. Coupled with the fact that the same percentage of sleeping structures had eaves which provide mosquitoes ease of access into the sleeping structures, highlights a major risk factor in malaria transmission.

5.5.6 Travel History

Looking at travel history, it was observed that majority of travelling took place amongst individuals from case neighbourhoods with 34.2% of case neighbourhood individuals compared to 24.1% from control neighbourhoods having travelled. Travel was also more common amongst the male individuals across each arm of the study, which corresponds with the high percentage of male cases that were reported in the district. Evidently males are engaged in frequent travel from home for harvesting, seasonal job seeking and other social affairs which puts them at an increased risk of malaria infection (K. Alemu et al., 2014). The most frequent destination amongst case individuals was Angola while control individuals mostly travelled to destinations within the Namibian borders. Patients who travelled to malaria endemic areas prior to the survey were more likely to be at risk of contracting malaria compared with those who did not travel (K. Alemu et al., 2014).
The Ohangwena border is very porous and can be crossed at any time with a border resident card that grants access to areas within 60km of the border without a passport to residents along the border in both countries (Gueye et al., 2014). Angolans nationals are believed to cross into Namibia to access better healthcare because of poorly equipped and staffed facilities in Angola, resulting from the long running civil war.

Most households have larger farms located across the border that need to be tended to from time to time, prompting the men in the household to travel anything from a few days to weeks at a time to these farms. Very often, they do not take any preventative measures with them to protect them from mosquitoes and end up being exposed to malaria infection which could easily result in their returning home as a reservoir of infection.

5.6 Limitations of the Study

During the execution of this study, some limitations were discovered that may have affected the data collected and therefore affected the quality of the results.

Completeness and timely reporting of cases greatly determined the ability to trace cases reported from any of the district health facilities. Missing information with regards to patient contact details and accurate detailing of the village the patient was from often times rendered some cases untraceable as they either couldn’t be called or the particular village was too big to determine a starting point considering the fact that time was not always available. Nurses were consistently reminded of the importance of complete and accurate data collection and of timely reporting of cases.
In order to conduct RACD and collect the necessary data needed for analysis, consistent transport was an integral part of the entire study. This unfortunately, was not always the case as the only vehicles available were on a shared schedule for different University programs. This results in days lapsing, active cased detection not being able to be done and possible asymptomatic cases being missed. Provisions for a vehicle specifically allocated to the study may have solved this issue.

In the event that transport was not an issue, availability of a nurse to perform RDTs and collect DBS was another limitation as only a qualified nurse was allowed to handle any blood work throughout the study. This limitation may have been avoidable through arrangements for a nurse who was fully dedicated to the study.

Often times, conducting the study questionnaire at some households was difficult due to the absence of a reliable adult or guardian who could accurately answer questions which were valuable in determining possible risk factors. At times the only adult present was a child of legal age who only recently returned home from boarding school and could not provide important details pertaining the household due to their time of absence. Parents were often reported to be at work only to arrive late at night or to have travelled and the minors left under the watch of a trusted neighbour.

While every effort was made to deal with the limitations encountered during the course of the study, they were unavoidable.
CHAPTER SIX: CONCLUSION

From this study we find that the risk factors linked to malaria infection in the Engela Health District are travel, low IRS coverage and low net coverage and net use. Population movement across the border to areas of high malaria transmission results in the importation of infections which easily contributes to the continued transmission of malaria in the district, while decreased IRS and net coverage also contribute to the maintenance of transmission pockets.

While RACD seems to be a feasible strategy in monitoring malaria transmission, improvements in the collection of quality information from patients need to be made. Collection of accurate and efficient details from patient is indirectly proportional to the time it takes to trace the patient to their household. The more the information is correct, the less time is spent on locating the patient’s household leaving more time available to trace other cases. Whereas when there is more incorrect information collected, more time and resources are spent and wasted on locating an otherwise non-existent house, decreasing the time available to trace more cases. This is especially important during malaria peak season when cases reported from health facilities become many. Asymptomatic cases were more likely to be discovered if RACD is conducted within a week of the index cases presenting at a health facility, highlighting the importance of time management and how quality patient data affects this process.

As the country moves from the control to the elimination phase, informing communities living in areas where there is still continued low malaria transmission of preventive measures and interventions is highly important in bringing transmission
down to zero. Some communities harbor a low risk perception and therefore develop poor treatment seeking behavior and limited interest or concern in the importance of prevention and treatment interventions. The fact that individuals know that malaria transmission is no longer high and that cases have decreased substantially means they are no longer concerned about protecting themselves from mosquitoes. Implementation of vector control strategies is met with new hesitation as household members no longer see the importance of IRS in their households either because ‘there are no mosquitoes in their area’ or because their sleeping structures are too full of furniture for them to remove. Incorrect use of mosquito nets is another factor adding to decreased net usage. Despite there being an average net coverage of 68% across the case and control neighbourhoods, it is suspected that some individuals might be using their nets as fishing nets, as fencing for their chicken pens or as soccer nets for the goal posts among other things, which would suggest a need for health education. By understanding and addressing the barriers to behavior change, the uptake of prevention services can be increased within the communities. However, bearing in mind the seasonal variation in mosquito prevalence, use of mosquito nets is also subject to seasonal changes. Data collected at different times of the year may not be comparable between populations as interviews during peak season give the impression of increased net usage while interviews during off-peak season give the impression of decreased net use.

Having carried out a pilot study of RACD in the Engela Health District, made it possible to determine the feasibility of such a surveillance tool and whether it yields helpful data and information which would otherwise aid the country in progressing
from malaria control to malaria elimination. Identification of the risk factors contributing to continued malaria transmission in the district provide a platform in determining whether existing preventative measures are under-utilized or need to be improved upon and also allow for the introduction of new ones that complement the goal to eliminate malaria in the country.
REFERENCES


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SARN. (2012). *Annual Trans-Kunene Malaria Initiative (TKMI) Stakeholders Meeting* (pp. 1–10). Retrieved from


APPENDIX

Appendix A: Table of WHO Approved Insecticides

WHO approved insecticides for malaria mosquito control. (David et al., 2013; WHOPES, 2014)

<table>
<thead>
<tr>
<th>Dates</th>
<th>Insecticide</th>
<th>Class &amp; formulation</th>
<th>Application</th>
<th>Dosage (g a.i./m²)</th>
<th>Mode of Action</th>
<th>Duration (months)</th>
<th>P450 Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1940-1945</td>
<td>DDT</td>
<td>OC WP</td>
<td>X</td>
<td>1-2</td>
<td>contact</td>
<td>&gt;6</td>
<td>R</td>
</tr>
<tr>
<td>1951-1955</td>
<td>Malathion</td>
<td>OP WP</td>
<td>X</td>
<td>2</td>
<td>Contact</td>
<td>2-3</td>
<td>R</td>
</tr>
<tr>
<td>1961-1965</td>
<td>Fenitrothion</td>
<td>OP WP</td>
<td>X</td>
<td>2</td>
<td>Contact &amp; airborne</td>
<td>3-6</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>-----</td>
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<td>-----</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Propoxur</td>
<td>Ca</td>
<td>WP</td>
<td>X</td>
<td>1-2</td>
<td></td>
<td>Contact &amp; airborne</td>
<td>3-6</td>
</tr>
<tr>
<td>Primiphos-methyl</td>
<td>OP</td>
<td>WP &amp; EC</td>
<td>X</td>
<td>1-2</td>
<td></td>
<td>Contact &amp; airborne</td>
<td>2-3</td>
</tr>
<tr>
<td>Bendiocarb</td>
<td>Ca</td>
<td>WP</td>
<td>X</td>
<td>0.1-0.4</td>
<td></td>
<td>Contact &amp; airborne</td>
<td>2-6</td>
</tr>
<tr>
<td>Permethrin</td>
<td>Pyr</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-cypermethrin</td>
<td>Pyr</td>
<td>WP &amp; SC</td>
<td>X</td>
<td>X</td>
<td>0.02-0.03</td>
<td>Contact</td>
<td>4-6</td>
</tr>
<tr>
<td>Alpha-cypermethrin</td>
<td>Pyr</td>
<td>WG-SB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cyfluthrin</td>
<td>Pyr</td>
<td>WP</td>
<td>X</td>
<td>X</td>
<td>0.02-0.05</td>
<td>Contact</td>
<td>3-6</td>
</tr>
<tr>
<td>Lambda-cyhalothrin</td>
<td>Pyr</td>
<td>WP, CS</td>
<td>X</td>
<td>X</td>
<td>0.02-0.03</td>
<td>Contact</td>
<td>3-6</td>
</tr>
<tr>
<td>Compound</td>
<td>Type</td>
<td>Formulation</td>
<td>Rate</td>
<td>Duration</td>
<td>Route</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>------</td>
<td>-------------</td>
<td>------</td>
<td>----------</td>
<td>-------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>Pyr</td>
<td>WP, WG</td>
<td>X</td>
<td>X</td>
<td>0.02-0.025</td>
<td>3-6</td>
<td>R</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>Pyr</td>
<td>WP</td>
<td>X</td>
<td>X</td>
<td>0.025-0.05</td>
<td>3-6</td>
<td>R</td>
</tr>
<tr>
<td>Etofenprox</td>
<td>Pyr</td>
<td>WP</td>
<td>X</td>
<td>X</td>
<td>0.1-0.3</td>
<td>3-6</td>
<td>R</td>
</tr>
</tbody>
</table>

1986-1990
Appendix B: Malaria Case Investigation Form

**MALARIA CASE INVESTIGATION FORM**
Namibia National Vector-Borne Disease Control Programme, MoHSS, Directorate of Special Programmes, Windhoek

<table>
<thead>
<tr>
<th>GPS coordinates of</th>
<th>health facility</th>
<th>home</th>
<th>Investigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| S                                                    | Date: ................................ |
| E                                                    | Time: ..................h..........min |

Investigation Conducted by: ____________________________ Rank: __________________

**PATIENT DETAILS**

<table>
<thead>
<tr>
<th>Patient Name</th>
<th>Age in completed years</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Home village</th>
<th>District</th>
<th>Pregnant</th>
<th>Yes (Trimester)</th>
<th>No</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Nationality</th>
<th>Namibia</th>
<th>Other, enter country name</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Current occupation</th>
<th>Unemployed</th>
<th>Farming/Agriculture</th>
<th>Other Manual Labour</th>
<th>Small-market sales or trade</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Nurse</th>
<th>Teacher</th>
<th>Professional</th>
<th>Other (specify)</th>
</tr>
</thead>
</table>

Location: Place of Occupation: ____________________________

**DIAGNOSIS**

<table>
<thead>
<tr>
<th>Presented with fever</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>History of fever</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
</table>

| Original diagnosis confirmed by | RDT | Microscopy (Specify, how many ___ parasites per microlitre (μl)) | P. Falciparum | Non-Falciparum | Mixed |
|--------------------------------|-----|---------------------------------------------------------------|...............|................|-------|

<table>
<thead>
<tr>
<th>Diagnosis Type</th>
<th>Uncomplicated</th>
<th>Severe</th>
<th>Unknown</th>
</tr>
</thead>
</table>

**PLEASE ASK THE FOLLOWING QUESTIONS:**

For how long were you experiencing signs & symptoms before you sought treatment at the health facility? ___ days

Has a family member been sick with fever, in the past week? | Yes | No | Don’t know |

Have you previously been diagnosed with malaria in the past 12 months? | Yes | No |

**TREATMENT**

<table>
<thead>
<tr>
<th>Treatment Prescribed</th>
<th>Artemether Lumefantrine</th>
<th>Oral Quinine</th>
<th>IM Quinine</th>
<th>IV Quinine</th>
<th>Other (specify)</th>
</tr>
</thead>
</table>

What malaria signs and symptoms were present?

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>Abdominal Pain</td>
<td>Headache</td>
<td>Chills</td>
</tr>
</tbody>
</table>
TRAVEL HISTORY

Have you spent a night anywhere else besides your town or community in the past 4 weeks? □ Yes  □ No

If yes, skip to Vector Control section
If no, please complete the table below.

<table>
<thead>
<tr>
<th>Village</th>
<th>District</th>
<th>Duration of stay</th>
<th>County Province Town</th>
<th>Duration of stay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Other Comments:

What was your reason for travel?
□ Business □ Visiting family or friends □ Shopping □ Holiday □ Other (specify ___________)

What was your means of travel?
□ Bus □ Car □ Bike □ Walked □ Airplane □ Other (specify ___________)

Did you use any of the following personal protection measures to prevent contracting malaria?
□ Mosquito net □ Mosquito repellent or coil □ None

VECTORS CONTROL & PERSONAL PROTECTION MEASURES

What are the exterior walls of the house primarily made of?
□ Sticks □ Grass □ Mud □ Corrugated iron sheets □ Bricks □ Other (specify ___________)

What is the roof primarily comprised of?
□ Sticks □ Grass □ Corrugated iron sheets □ Tin □ Asbestos □ Other (specify ___________)

Has this home been sprayed in the past year?
□ Yes □ No □ Don’t know

Do you own a bed net?
□ Yes □ No

How many bed nets do you have in the house?
□ Yes □ No

How many people live in the house? (Include all usual members)
□ Yes □ No

Is your bed net treated with insecticide?
□ Yes □ No

Did you sleep under your bed net anytime within the past week?
□ Yes □ No

If NO, why didn’t you sleep under your bed net within the past week?
□ Too hot □ Too cold □ Net not hung up □ Net worn out/poor condition □ Net was dirty

□ Other reason: ___________
Appendix C: Study Consent Form

Appendix C1: English

(English)

UNIVERSITY OF NAMIBIA / NATIONAL MALARIA CONTROL PROGRAMME

CONSENT TO BE IN RESEARCH

Study Title: Epidemiology of border malaria in Namibia

This is a medical research study, and you do not have to take part. The study coordinator from the University of Namibia/National Malaria Control Programme will explain this study to you. If you have any questions, you may ask the research coordinator.

You are being asked to take part in this study either because you have been diagnosed with malaria or you are a healthy individual with whom we can compare results. In this study, the researchers are collecting blood samples to learn more about the causes of malaria in this area. By looking at who does and does not have malaria, it is possible to find out why the disease is only occurring in some people. This information can then be used to better target control measures in those areas most at risk. About 7020 people
will give blood samples for this research. The Bill and Melinda Gates Foundation is paying for this research. The sponsors of this study cannot influence the results of this study.

**What will happen if I take part in this study?**

If you agree to be in this study, we will prick your finger to take a small amount of blood. This will take about one minute. The blood will be stored in laboratories in Windhoek and will be analyzed in Namibia and the USA for malaria parasites. We will also ask you a series of questions about your recent travel history, your household structure and other factors that might influence whether you are at risk of malaria.

**How long will I be in the study?**

Participation in the study will take a total of about 30 minutes.

**Are there risks?**

The finger prick may hurt a little. There is a small risk of bruising, and a rare risk of infection.

**Are there benefits?**

If you have a fever we can test you for malaria and refer you to a hospital if necessary. The results of this study may help the National Malaria Control Programme to develop
better strategies to prevent malaria in this area. There is no other direct benefit to you. The blood will be used only for research into malaria.

**Can I say “No”?**

Yes, you do not have to donate a blood sample for this study.

**Will my information be kept confidential?**

We will do our best to protect the information we collect from you and your medical record. Information which identifies you will be kept secure and restricted. However, your personal information may be given out if required by law. If information from this research is published or presented at scientific meetings, your name and other identifiers will not be used. Information which identifies you will be destroyed when this research is complete. The following organizations may look at information about you in your medical and research records: University of Namibia, University of California San Francisco, London School of Hygiene and Tropical Medicine.

**Are there any costs or payments?**

No. You will neither be paid nor charged to donate a blood sample.

**What if I get injured?**

Tell the study coordinator if you feel that you have been injured because of being in this research.
Treatment and Compensation for Injury:
If you are injured as a result of being in this study, treatment will be available. The costs of the treatment may be covered by the University of California depending on a number of factors. The University does not normally provide any other form of compensation for injury. For further information about this, you may call the office of the Committee on Human Research at +1 415-476-1814.

Who can answer my questions about the study?
You can talk to the study coordinator(s) about any questions, concerns, or complaints you have about this study. Contact the study coordinator Joyce Auala at 081 207 3473. If you wish to ask questions about the study or your rights as a research participant to someone other than the researchers or if you wish to voice any problems or concerns you may have about the study, please call the Office of the Committee on Human Research at +1 415-476-1814 or Chris Lourenco (Tel: 081 692 9940), the malaria elimination analyst at the Southern Africa Malaria Elimination Support Team or Stark Katokele (Tel: 081 292 8754), the National Malaria Control Program deputy manager in Namibia. You have been given copies of this consent form and the Experimental Subject's Bill of Rights to keep.

If you wish to be in this study, please sign or provide a thumb print below.

________________________  ___________________________
Date
Participant’s Signature/Thumb Print for Consent

Date
Person Obtaining Consent

Date
Witness signature (if participant does not speak/read English)

Appendix C2: Oshikwanayama

(Oshikwanyama)

OUNIVEESITI YANAMIBIA/OPROGRAMA YOPASHIWANA
YEKONDOLOLO LOMALARIA
UNIVERSITY OF NAMIBIA/NATIONAL MALARIA CONTROL PROGRAMME

EPITIKILO LOKUKALA MOMAPEKAPEKO
CONSENT TO BE IN RESEARCH

Oshipalanye shElihongo: Eshiivo leengaba domukifi woMalaria muNamibia

Study Title: Epidemiology of border malaria in Namibia


**Oshike tashi holoka po ngeenge nda kufa ombinga melihongo eli?**

Ngeenge owa dimina okukala melihongo eli, ohatu ku tu komunwe woye tu kufe mo eta lohonde. Eshi otashi pula konyala omunute umwe. Ohonde otai ka longifwa opo u konakonwe omalaria. Ohonde otai ka tuvikiilwa molabora yomOvenduka notai ka dongokununwa mokukonga mo oupuka womalaria omu moNamibia noko-USA. Ohatu ke ku pula yo oupulo vamwe kombainga yomalweendo oye mefimbo lapita,
omutungilo weumbo loye naikwao ya wedwa po oyo tai holola kutya onghalo yoye oli moshiponga komalaria.

**Oule u fike peni handi ka kala melihongo?**

Omukufimbinga melihongo ota kufa konyala oule wominute 30.

**Omu na omalixupulo eemwenyo?**

Ekufo lohonde komunwe ohali yehameke kanini. Opu na okalixupolumwenyo kanini ketunhilo nokalixupolumwenyo kanafangwa kekwato lombuto.

**Omu na omalikolo?**


**Ohai dulu okutya “Ahowe”?**

Heeno, oto dulu okukala inoo yandja omalolelo ohonde kelihongo eli.

**Omauyelele ange otaa dulu tuu okukalekwa meamen**

Ohatu ka fya noshisho okwaamena omauyelele, oo twa kufa kwoove nomavalululokolo oye opaunamiti. Omauyelele taa ku holola, otaa tulwa meameno nomengambeko. Nande ongaho, omauyelele opaumwene otaa dulu yoo okuyandjwa
ngeenge okwa pumbiwa paveta. Omauyelele aa omapekapeko ngeenge okwa nyanyangidwa ile a yelifwa moyoongalele yopaunongononi, Edina loye namadina aavo ve ku holola itaa ka longifwa. Omauyelele oo ta ku holola otaa ka pombolwa po konima eshi epekapeco eli la pwa. Omalutu taa shikula otaa dulu okukonga omauyelele momavalulohokololo oye opaunamiti nomomapekapeko: University of Namibia, University of California San Francisco, London School of Hygiene and Tropical Medicine.

Otapu futwa sha ile tapa yandjwa eefuto?
Ahowe. Ove ito dulu okufutwa ile u futifwe eshi to yandje omalolelo ohonde.

Ongahelipi ngeenge onda yehamekwa?
Shiivifila omuunganeki welihongo ngeenge ou udite wa yehamekwa momapekapeko aa.

Ouhaku neefuto kOmayehameko:
Ngeenge owa yehamekwa eshi u li melihongo eli, ouhaku otau monika. Eefuto douhaku otadi dulu okufutwa kOunivesiti yaCalifornia she likolelela komivalu doiningifi. Ounivesiti ihai yandje naanaa omikalo dimwe domafutilo komayehameko.

Omauyelele a wedwa po kombinga ei, dengela kombelewa yOkomiti i na sha noHuman Research, konomola: +1 415-476-1814.

Olye ta dulu okunyamukula omapulo ange kombinga yelihongo?
Oto dulu okupopya nomuunganeki (ovaunganeki) welihongo kombainga yepulo keshe, omalimbililo ile omanyenyeto oo u na kombainga yelihongo eli. Monafana nomuunganeki welihongo, Joyce Auala, konomola: 081 207 3473. Ngeenge ou na omapulo kombainga yelihongo ile wa hala okulongifa oufemba woye ongomukufimbinga momapekapeko okupula umwe e lili ehe shi ovaunganeki, ile ngeenge owa hala okuholola udjuu keshe ile omaliudo omalimbililo oo u na kombainga yelihongo, alikana dengela kongodi yomombelewa yOkomiti yoHuman Research, konomola: + 1 415-476-1814 ile kuChris Lourenc (Tel: 081 692 9940), omudongokononi nomuxulifipo womalaria mokangudu koSouthern Africa Malaria Elimination Support Team, ile kuStark Katokele (Tel: 081 292 8754), omuwiliki wopedu muNamibia wOprograma yoPashiwana yEkondololo loMalaria. Owa pewa eekopi dofooloma yepitikilo nosho yo okukala nofooloma yedina: Experimental Subject’s Bill of Rights.

Ngeenge owa hala okukala melihongo eli, alikana shaina ile tula po oshihako shomunwe wakula wokomake okuyuka pedu.

__________________________________________________
Efiku Eshainokasha lomukufimbinga / Oshihako shOmunwe wakula onga Epitikilo
Efiku Omunhu oo e na ofemba wEpitikilo

Efiku Eshainokasha lOmbangi (ngeenge omukufimbinga iha popi / lesha Oshiingilisa)
Appendix D: Questionnaire Format on Tablet

Appendix D1: Questionnaire Index Page

Welcome joyce!
Edit Account | Logout?

Tablet Number: 6000

Neighbourhoods
Legend
Test GPS
Troubleshooting
Backup Data*
Appendix D2: New neighbourhood Form

New Neighbourhood

Interviewer ID : 4

Village Name :

Case or Control?:

←please select→ ▼

Date: November 21, 2014

Any breeding sites found near by?

←please select→ ▼

Next Back Home

New Neighbourhood

Interviewer ID : 4

Village Name :

Case or Control?:

←please select→ ▼

Date: February 3, 2014

Any breeding sites found near by?

←please select→

case

case

case

control

control

control
Appendix D3: New Household Form

Showing all households listed under neighbourhood, select or add the one you wish to proceed with.

Please add a household

Add  Back Home

---

Add Household

Latitude:  Longitude:

Index Household?

`<-- please select--> ▼`

Name of Household Head:

Phone Number of Household Head:

What is the name of the nearest school?

Urban or Rural Area?

`<-- please select--> ▼`

Next  Back
Appendix D4: Additional household Forms

Showing all details for **household_id xxxx**. Select questionnaire options below.

**Jump** | **Push to Oruxmaps** | **Edit this household?**

*neighbourhood_id:*

*household_id:*

Index household?

Household Head Name:

Nearest School:

Household Head Number:

Area:

GPS Latitude:

GPS Longitude:

**Socio-Economic Indicators:** Open

**Mosquito Nets:** Open

**Individuals:** Open

**Net Ownership:** Open

**Sleeping Structure:** Open

Back
### Socio-Economic indicators

What is the main source of drinking water for members of your household?

**IF Piped water**
- Piped into dwelling
- Piped into yard/standpipe
- Public tap/standpipe
- Borehole

**IF Dug well**
- Protected well
- Unprotected well

**IF Water from spring**
- Protected Spring
- Unprotected Spring
- Rainwater
- Tanker truck

**IF Surface water**
- dam
- river
- lake
- pond
- stream
- canal/irrigation channel
- Bottle water

**IF Other Specify:**

What kind of toilet facilities does your household use?

**IF Other Specify:**

---
Does your household have **electricity**?
- Yes  - No

Does your household have a **radio**?
- Yes  - No

Does your household a **television**?
- Yes  - No

Does your household a **mobile phone**?
- Yes  - No

Does your household a **non-mobile phone**?
- Yes  - No

Does your household a **refrigerator**?
- Yes  - No

Does your household a **stove**?
- Yes  - No

What type of fuel does your household mainly use for cooking?

[<< please select>> ▼]

IF other Specify:

[ ]
Does any member of your household own a bicycle?

- Yes  - No

Does any member of your household own a motorcycle/motor scooter?

- Yes  - No

Does any member of your household own a car/truck?

- Yes  - No

Does any member of your household own a donkey?

- Yes  - No

Does any member of your household own a tractor?

- Yes  - No

Submit Home Back
Appendix D6: Mosquito Net Addition Form

Mosquito nets

Does your household have any mosquito nets used while sleeping?

Submit Back
Appendix D7: New Individual Investigation Form

Add Individual

Full Name: 

Individual ID: 

Index Case? 

Sex: 

Age (in years): 

Guest? 

Did (name) sleep here last night? 

Does (name) sleep under a mosquito net? 

Relationship to the household head: 

Occupation: 

If child under 18, read consent statement to parent/adult responsible for the child. 

Consent granted? 

Submit Back
<-- please select-->

- Head
- Wife/Husband
- Son/Daughter
- Son-inlaw / Daughtet-inlaw
- Grandchild
- Parent
- Parent-in-law
- Brother/Sister
- Other relatives
- Adopted/foster/stepchild
- Not related
- Don't know
Unemployed
Farming/Agriculture
Other manual labor
Small market sales or trade
Nurse/Teacher/Professional
Fisherman
Student
Other

Yes
No
Not present

Parent statement to parent/adult responsible for the child.
If child under 18, read consent statement to parent/adult responsible for the child.

Consent granted?
[Yes] ▼

Dry Blood Spot (DBS)
[← please select→] ▼

RDT Result
[← please select→] ▼

Confirm the patient ID on DBS and RDT
DBS:
[ ]
RDT:
[ ]

Reported fever in last 48 hours?
[← please select→] ▼

Did you sleep outside last night (10pm-6am)?
[← please select→] ▼

Were you outside for any other reason last night (10pm-6am)?
[← please select→] ▼

The last time you had a fever, did you seek treatment for it?
[← please select→] ▼

Have you been diagnosed with malaria in the past 2 weeks?
[← please select→] ▼

Have you taken any drugs for malaria in the last two weeks?
[← please select→] ▼

Have you spent any night outside this district in the last 6 weeks?
[← please select→] ▼

Submit Back
Appendix D8: Net Ownership and Usage Form

**Mosquito net ownership & usage**

<table>
<thead>
<tr>
<th>Question</th>
<th>Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net ID:</td>
<td></td>
</tr>
<tr>
<td>Net condition:</td>
<td></td>
</tr>
<tr>
<td>Ask respondent to show you the net(s) in the structure that they sleep in</td>
<td></td>
</tr>
<tr>
<td>How long ago did you obtain the mosquito net?</td>
<td></td>
</tr>
<tr>
<td>Where did you obtain the net?</td>
<td></td>
</tr>
<tr>
<td>Was the net purchased?</td>
<td></td>
</tr>
<tr>
<td>Brand of the mosquito net</td>
<td></td>
</tr>
<tr>
<td>When you got the net, was it already factory-treated with an insecticide to kill or repel mosquitoes?</td>
<td></td>
</tr>
<tr>
<td>Since you got the mosquito net, was it ever soaked or dipped in a liquid to repel mosquito or bugs?</td>
<td></td>
</tr>
<tr>
<td>How long ago was the net last soaked or dipped?</td>
<td></td>
</tr>
</tbody>
</table>

Submit Home Back
Net condition:
- Please select
- Good condition
- Average (a few small holes)
- Poor condition (2-3 torch size holes visible)
- NA

Ask respondent to show you the net(s) in the structure that they sleep in:
- Please select
- Not observed
- Net hanging
- Net used or still in bag
- Net taken during the day
How long ago did you obtain the mosquito net?

- Less than 6 months ago
- 6-12 months ago
- 1-3 years ago
- More than 3 years ago
- Don’t know

Where did you obtain the net?

- Government clinic/hospital
- Distribution at home
- Community healthy worker
- Retail shop/market
- Pharmacy
- Work place
- Don’t know
- Others
Since you got the mosquito net, was it ever soaked or dipped in a liquid to repel mosquito or bugs?

- Please select -
  - Yes
  - No
  - Not sure

Less than a month
More than 2 years ago
Never Soaked
Not sure
Appendix D9: Sleeping Structure Form

## Sleeping structure

### Get Current GPS Position

<table>
<thead>
<tr>
<th>Field</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household_id</td>
<td>6341</td>
</tr>
<tr>
<td>Neighbourhood_id</td>
<td>6081</td>
</tr>
<tr>
<td>User_id</td>
<td>4</td>
</tr>
<tr>
<td>StructureCode</td>
<td>N6081H6341S</td>
</tr>
<tr>
<td>Latitude</td>
<td></td>
</tr>
<tr>
<td>Longitude</td>
<td></td>
</tr>
</tbody>
</table>

### Main material of the floor

#### Natural floor
- Earth/sand
- Dung

#### Rudimentary floor
- Wood planks
- Palm/Bamboo

#### Finished floor
- Parquet/Polished wood
- Vinyl or Asphal strips
- Ceramic tiles
- Cement
- Carpet
- Other

#### Other

Specify Other floor: [Field]

Types of windows: [Field]

Specify Other windows: [Field]

Types of doorway: [Field]

Specify Other door: [Field]
Main material of the exterior wall

**Natural wall**
- Grass
- Cane/trucks/bamboo/reed

**Rudimentary wall**
- Bamboo/wood with mud
- Stone with mud
- Plywood
- Carton
- Reused wood

**Finished wall**
- Cement
- Stone with lime/cement
- Bricks
- Mud blocks
- Wood planks/shingles

Specify wall:

- When was the last time this structure was replastered or painted? [← please select→]
- Is there open space between the roof and the walls? [← please select→]
- Was this structure/room sprayed at any time in the last year? [← please select→]

[Submit]  [Back]
Appendix E: Example of Barcodes for RDT and Consent Form Use
Appendix F: CareStart Malaria RDT kit – Information Leaflet

CareStart™ Malaria HRP2/plDH (Pf/PAN) Combo Test

Rapid One Step Malaria HRP2/plDH Combo Test

A rapid test for the detection of HRP2 and parasite LDH in human blood.

Intended Use

For the rapid qualitative determination of Malaria HRP2 (Plasmodium vivax, P. falciparum and P. malariae) in human blood as an aid in the diagnosis of Malaria infection.

Summary

Malaria is a serious parasitic disease characterized by fever, chills, and anemia and is caused by the bite of infected Anopheles mosquitoes. There are four kinds of malaria that can infect humans: Plasmodium falciparum, P. vivax, P. malariae, and P. knowlesi. In humans, the parasites multiply asexually in the liver where they mature and release millions of the parasites. The disease is now estimated to be present in 100 countries worldwide, and it is estimated that there are over 500 million clinical cases and 2.7 million malaria-related deaths per year. At the present, malaria is diagnosed by looking for the parasites in a drop of blood. Blood will be put onto a microscope slide and stained so that the parasites will be visible under a microscope.

The CareStart™ Malaria HRP2/plDH combi Test contains a monoclonal strip, which is pre-coated with two monoclonal antibodies in two separate lines across a test strip. One monoclonal antibody (test line 1) is specific to human dehydrogenase (plDH) of the Plasmodium species P. falciparum, P. vivax, and P. malariae, and the other line (test line 2) is specific to human HRP2 of the Plasmodium species P. falciparum. The conjugate pad is dispensed with monoclonal antibodies, which are pan-specific to plDH and P. falciparum specific to HRP2.

So, the CareStart™ Malaria plDH/HRP2 Antigen Test is designed for the differential diagnosis between Plasmodium falciparum and the other Plasmodium species.

Materials Provided

- CareStart™ Malaria Antigen Test Kit contains following items to perform the assay:
  - Test Device
  - Package Insert
  - Assay Buffer
  - Sample Pipette (Optional)
  - Lancer (Optional)
  - Alcohol Swab (Optional)

Precautions

In order to obtain reproducible results, the following rules must be observed:

1) For in vitro diagnostic use only.
2) Use disposable gloves while handling potentially infectious material and performing the assay. Wash hands thoroughly afterwards.
3) Do not use beyond the expiration date.
4) Do not cut or stroke while handling specimen.
5) Clean up spills thoroughly using an appropriate disinfectant.

Specimen Collection and Storage

[Collection by venipuncture]

1) Collect the whole blood into the collection tube containing EDTA, citrate or heparin by venipuncture.
2) If specimens are not immediately tested, they should be refrigerated at 2–8°C. For storage periods greater than three days, freezing is recommended. They should be brought to room temperature prior to use. Using the specimens in the long-termkeeping more than three days can cause non-specific reaction.
3) When usage at 2–8°C, the whole blood sample should be used within three days.

[Collection using a lancet]

1) Clean the area to be pierced with an alcohol swab.
2) Squeeze the end of the lancet and pierce with a sterile lancet perpendicularly.
3) Wipe away the first drop of blood with sterile gauze or cotton.
4) Add a single pipette provided, and while gently squeezing the tube, point the open end in the blood and run the pipette on the guide release the pressure to draw blood into the sample pipette up to the black line.

1) Gently squeeze the tube 2) Immerse open end in blood 3) Gently release to draw blood

Test Procedure

1) Add 5 µl of whole blood into the Sample Well (test well).
2) Add two drops (60 µl) of assay buffer into the buffer well.
3) Read the test result in 20 min.
Interpretation of the test

<table>
<thead>
<tr>
<th>Negative (PAM specific)</th>
<th>Positive (P. falciparum)</th>
<th>Positive (P. falciparum)</th>
<th>Positive (P. falciparum or Mixed infection)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 2</td>
<td>C 2</td>
<td>C 2</td>
<td>C 2</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

1) Negative reaction
   - The presence of only one band in the Control Area within the result window indicates a negative result.

2) Invalid
   - The test is invalid if the line in the Control Area does not appear. If this occurs, the test should be repeated using a new strip.

3) P. vivax, P. Malariae, or P. Ovale Positive reaction
   - The presence of two color bands (one band in the Control Area and another band in the "2" area) indicates a positive result for P. vivax, P. malariae, or P. ovale. The pLDH present in the sample reacts with the anti-pLDH conjugate and moves through the test strip where the pLDH is captured by anti-specific anti-pLDH.

4) P. falciparum Positive reaction
   - The presence of three color bands (three bands in the Control, "2" and "1" areas) or two bands in one band in the Control Area indicates a positive result for P. falciparum.

5) Mixed infection of P. falciparum and other species Positive reaction

The presence of three color bands (three bands in the Control, "2" and "1" areas) indicates a positive result for P. falciparum or Mixed infection of P. falciparum and other species.

Limitations and Interferences

1) The test procedure, precautions and interpretation of results for this test must be followed when testing.
2) Anti-irritants such as heparin, EDTA, and citrate do not affect the test result.
3) The test requires a minimum of two tests.
4) The test is limited to the detection of antigen to Malaria. The test is very accurate in detecting malarial and P. falciparum, but insensitive in detecting false results can occur. Other clinically available tests are required if questionable results are obtained. As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

Performance Characteristics

The CareStart™ Malaria HRP2/pLDH combo kit has been tested with positive and negative clinical samples tested by microscopic examination of whole blood.

1) Malaria P. vivax results
   - Positive control: 96
   - Negative control: 4
   - 96/100 x 100% - 96%

2) Malaria P. falciparum results
   - Positive control: 96
   - Negative control: 2
   - 96/100 x 100% - 96%

3) Malaria-negative normal human specimen results
   - Positive control: 5
   - Negative control: 151
   - 95/200 x 100% - 97.5%

Within-run and between-run precisions have been determined by the testing 10 replicates of three specimens: a negative, a low-positive and a strong positive. The agreement between the test results and the expected results was 100%.

References


Access Bio, Inc.
2033 Route 130, Unit H
Montvale 07645, USA.
Tel: 1-732-297-2222
Fax: 1-732-297-3001
E-mail: info@accessbio.net
Web site: www.accessbio.com

G0131-C21
September 2009
Appendix G: Fetching and Insertion of GPS Coordinates into Form

Fetching current coordinates...

Household_id: 6341  
Neighbourhood_id: 6081  
User_id: 4  
StructureCode: N6081H6341S  
Latitude:   
Longitude:   

Get Current GPS Position

Household_id: 6341  
Neighbourhood_id: 6081  
User_id: 4  
StructureCode: N6081H6341S  
Latitude:   
Longitude:   

Get Current GPS Position

Household_id: 6341  
Neighbourhood_id: 6081  
User_id: 4  
StructureCode: N6081H6341S  
Latitude: -22.5731098  
Longitude: 17.0553325
Appendix H: Data Backup on Tablet and Server

Backup Data

Sends CSV backup files to designated FTP folder.

Backup to FTP

Click next after each page loads.

In this case "http://mrc.app-king.net/backups/6000"

Open phpMyAdmin

Open phpMyAdmin to view data and import/export tables.

Authentication Required

Index of /backup

<table>
<thead>
<tr>
<th>Name</th>
<th>Last modified</th>
<th>Size</th>
<th>Description</th>
</tr>
</thead>
<tbody>
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<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>27-Jan-2016 08:13</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2000/</td>
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<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>28-Mar-2015 01:17</td>
<td>-</td>
<td>-</td>
</tr>
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<td>-</td>
</tr>
</tbody>
</table>