A STUDY OF THE IMMUNITY TO MALARIA AMONG THE SAN PEOPLE IN THE TSUMEB AREA OF OSHIKOTO AND KAVANGO REGIONS OF NAMIBIA

A DISSERTATION SUBMITTED IN FULFILMENT

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Abstract

The San people are indigenous minorities in Namibia that are known for their hunting and gathering lifestyle. From personal observations, discussions, interviews, raw data from Tsintsabis clinic and results obtained from the research, one had to assume that the San people are immune to malaria. It is unclear how the disease does not affect them although they live in endemic areas. The objective of this study was to investigate how the San have survived without a major outbreak of malaria as well as the mechanism underlying this immunity. The sample consisted of two hundred participants that included the San and the other ethnic groups ("the other ethnic groups" in this study refers to tribes other than the San that lived in the same regions studied).

Questionnaires were administered and focus group discussions were conducted to both groups in Oshikoto and Kavango Regions to determine their knowledge of malaria. The presence of malaria parasites and the structure characteristics of the red blood cells in the blood samples of the San and other ethnic groups were examined microscopically by using thin and thick blood smears. Full blood cell count was measured and the role of nutrition played in the immunity boosting against malaria was investigated. The results were analyzed through descriptive and inferential statistics that included t-test and for indicator variables for the San and the other ethnic groups at 5% level of significance. Chi-square and t-test were used to evaluate differences between the shapes of red blood cell and presence of malaria parasites in the blood of the San and the other ethnic groups.

The results showed that the control groups had better knowledge of malaria (56%) compared to the San people who showed no knowledge of malaria. All San people

(100%) took traditional medicinal herbs but not specifically against malaria disease compared to 12% in the other ethnic groups. The chi square test indicated that the shapes of the red blood cells of the San (80%), displayed spikes and 2% showed mixed shape on the surface of the erythrocytes compared to the other ethnic groups of which 20% showed spikes and 1% showed a mixed shape. The t-test showed significant differences in the mean numbers of RBC, Hb, MCV, WBC, MON, BAS, LIC between the San and other ethnic groups. A t-test of the haemoglobin indicated that LAIcCHBbI ($p=0.003$) was significantly higher than the other Hb variants. The mean of the WBC of the San was significantly higher than that of the ethnic groups which suggested that the San are more likely immune to the malaria parasite. There were phenotypic variations in the San red blood cells which most likely as a result of genetic influences.

Food samples of the San analyzed showed the means of the following; 73.88% moisture; 0.83mg/100g Iron; 0.39mg/100g Zinc; 12.37mg/100g Vitamin C; 0.48mg/100g Antioxidants and 0.83mg/100g Flavonoids were all slightly higher than in the other ethnic groups of *Sorghum biocolor* and *Pennisetum glaucum*. *Pennisetum glaucum* had the highest content in Zinc 3.2mg/100g compared to that of the San food.

A study on the presence of fungi on the food of the San people was carried out and the following species were identified *R. stolonifer, S. cerevisiae, P. notatum, A. pezia, A. niger*. The fungi were possibly producing secondary metabolites that boost immunity against bacterial and some protozoan infections like malaria parasites.

The statistics above explain the immunity of the San, which have not been studied before and for which there is dearth of data in this regard. This knowledge could be useful in determining and developing interventions against transmission of malaria.

Among others, the significant contribution to the field of malariology is that the San people have a distinct morphology in haemoglobin C whereby 63.1% of the San people showed the presence of HbC and 36.9% in the other ethnic groups. Spiculated RBC, HbC and nutritional elements helped build immunity against malaria parasites in the San people. It is recommended that IgG of the San can be used to test for acquired immunity and development of vaccine in animal studies and therefore can be used to form basis for antimalarial vaccines and drugs.

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List of Abbreviations

ACT	Artemisinin-based Combination Theory
AIDS	Acquired Immuno Deficiency Syndrome
ALY	Alymphoplastic Cells
AOAC	Association of Analytical Chemists
ARV	Antiretroviral Drugs
AU	African Union
BAS	Basophils
BBC	British Broadcasting Corporation
CDR	Customized Dilution Ratio
CM	Cerebral Malaria
DCPP	Dichlorophenolindophenol
DDT	Dichlorodiphenyltrichloroethane
DNA	Dioxyribonucleic Acid
DPPH	Diphynly-1-Picrhydrazyl
DV ICP OES	Dual View Inductively Coupled Plasma Optical Emission Spectometry
EBA	Erythrocyte Binding Antigen

EDTA	Ethylenediaminetetracetic Acid
EOS	Eosinophils
EPA	Environmental Protection Agency
FBC	Full Blood Cell Count
G6PD	Glucose-6-Phosphate dehydrogenase
GAE	Garlic Acid Equivalent
GluNac	N-acetyl glucosamine
GMP	Global Malaria Programme
GPARC	Global Plan for Artemisinin Resistance Containment
Hb	Haemoglobin
HbA2	Haemoglobin A2
HbAlc	Glycohaemoglobin
HbAo	Haemoglobin Ao
HbC	Haemoglobin C
HbEE	Haemoglobin EE
HbF	Haemoglobin F
HbLAlc/CHb-1	Haemoglobin LAlc/CHb-1
HbSS	Sickle celled Haemoglobin
HCL	Hydrochloric Acid
HCT	Haematocrit
HIR	Health Information Report

HIS	Health Information Services
HIV	Human Immuno-deficiency Virus
HMIS	Health and Management Information System
IFN-y	Interferone-y
IgE	Immunoglobin E
IgG	Immunoglobin G
IgM	Immunoglobin M
ITNs	Insecticide Treated Nets
IVS	Intergrated Validation Station
KMRI	Kenya Medical Research Institute
KSCN	Potassium Thio Cynate
LIC	Large Immature Corpuscles
LLINs	Long Lasting Insecticidal Nets
LYM	Lymphocytes
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
MCV	Mean Cell Volume
MET	Ministry of Environment and Tourism
MHC	Major Histocompatibility Complex
MoHIS	Ministry of Health Information Services
MIS	Malaria Indicator Survey
MoHSS	Ministry of Health and Social Services
MON	Monocytes

MPA	Methiopropanine and Acetic Acid
MPV	Mean Platelet Volume
MRC	Multidisplinary Research Center
NCL	Nature Conservation Laws
NEPAD	New Partnership for Africa's Development
NEU	Neutrophils
NFPT	National Federation of Professional Trainer
NIAID	National Institute of Allergy and Infectious Diseases
NIP	Namibian Institute of Pathology
NK	Natural Killers
NMP	National Malaria Policy
NNP	Namibia National Policy
NO	Nitrogen Oxide
NSA	Namibian Statistics Agency
PCT	Platelet Crit
PDW	Platelet Distribution Width
PF4	Platelet Factor 4
PLDH	Parasite Lactate Dehydragenase
PLT	Platelets
RBC	Red Blood Cells
RDW	Red Cell Distribution Width
RPMSHP	Rational Pharmaceutical Management Sciences for Health Plus
SADC	Southern African Development Community

SAO	Southeast Asian Ovalocytosis
SPSS	Statistical Package for Social Sciences
SSI	Statens Serum Institute
TB	Tuberculosis
TNF	Tumor Necrosis Factor
UM	Uncomplicated Malaria
UNAM	University Of Namibia
UV light	UltraViolet Light
WBC	White Blood Cells
WHO	World Health Organisation
WIMSA	Working Group of Indigenous Minorities in Southern Africa
WIPO	World Intellectual Property Organisation
WMR	World Malaria Report

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Dedication

This dissertation is dedicated to the Almighty God who gave the wisdom and knowledge to undertake this study. The San people who were very forth coming in rendering their services to make this study successful and my family who were very supportive during the entire study. I Thank You Lord Jesus.

Declaration

I, C. Amoo, declare hereby 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.

Chipo Amoo

Date: 20th January 2014

CHAPTER ONE: INTRODUCTION

Malaria is a life threatening disease caused by parasites of *Plasmodium* species. Malaria is transmitted to people through the bites of infected *Anopheles* female mosquitoes. In 2010 the World Health Organization reported about 219 million cases of malaria with 660 000 deaths. It is has been reported that malaria mortality rates have fallen by more than 25% globally and by 33 % in Africa (WHO World Malaria report, 2012). In Namibia 199 deaths were reported in 2008 a reduction from 679 reported in 2000 (MOHSS, 2010). Efforts in Namibia are being made to eliminate malaria by 2015 (Feachem and The Malaria Elimination Group, 2009).

Every year, malaria affects an estimated 300 to 500 million people worldwide with over 1.8 to 3million deaths. Of these figures, about 1.5 to 3 million is mostly in children (WHO, 2009). More than 6 % of the deaths occur in India, Brazil, Sri Lanka, Afghanistan, Vietnam and Colombia, while over 90% of these cases occur in Sub-Saharan Africa. In Southern Africa, the malaria mortality during the year 2000 was estimated at 1, 144, 572 with countries like Zambia, Mozambique, Malawi and Angola reporting a high incidence of malaria (WHO, 2010). In Botswana, Swaziland, South Africa and Zimbabwe, transmission is seasonal and its intensity varies from hypo to medium-endemic levels in smaller parts of these countries (Demas, as cited in Kamwi, 2005). These factors depend on the type of parasite; *Plasmodium falciparum* causes severe illness amongst the four species that are *Plasmodium falciparum, P. vivax, P. ovale,* and *P.malariae.*

The vectors too play a very important role in the transmission of malaria; there are about 20 different species of *Anopheles* mosquitoes in the world (WHO, 2013). The mosquitoes breed in shallow waters of puddles, rice field, marshes and pools. The intensity of transmission is mostly on those places where the mosquito can complete its life cycle successfully. In Africa, the vector species have been thriving due mainly to climatic conditions that are favourable for the breeding of mosquitoes affecting the number and survival of mosquitoes, (such as rainfall, high temperature and humidity). Malaria disease is mostly during the rainy and after rainy season where people with no or little immunity are affected (WHO, 2013). In certain areas of Africa like those countries north of the Sahara, Libya, Tunisia and Morocco, which are, considered malaria free from transmission, only a few imported cases are reported (WHO, 2010).

Malaria in Namibia

Namibia has a surface area of 824 000 square km. It is located in the south-western part of the African continent. The climate is the driest in Africa with sunny, warm days and cooler nights, especially during the winter months. The population of Namibia stands as 2 165 828 (Namibia Statistics Agency, 2011).

In Namibia, malaria used to be a major public health problem with more than 600 000 cases recorded every year (MoHSS, 2005). Deaths due to malaria in Namibia affect all ages but are significantly higher in pregnant women and children. It was reported to be one of the leading causes of illness and deaths from 1999 to 2002

(MoHSS National Malaria Policy, 2005). This disease is prevalent in the northern part of the country where more than 65% of the population live. The inhabitants of Zambezi, Kavango, Kunene, Oshikoto, Ohangwena, Oshana, Otjozondjupa, Omaheke and Omusati regions constituting 65% of the entire Namibian population are at the highest risk of malaria in Namibia (MoHSS National Malaria Policy, 2005), although this is changing due to vector control measures undertaken by the government. This is a comprehensive programme to eliminate the transmission of malaria in Namibia (Feachem and The Malaria Elimination Group, 2009). From 2009 to 2011 the Kavango region has had the highest of both mortality and morbidity malaria cases compared to other regions (Ministry of Health Information Services, 2011). In the report published by HIS (2011) malaria cases of individuals18 years and older have been reported to be 5491, Zambezi having moderate transmission with 1061 cases and Oshikoto reported to have low transmission of 94 cases. Namibia became a signatory to the April 2000 Abuja Declaration on Roll back Malaria.

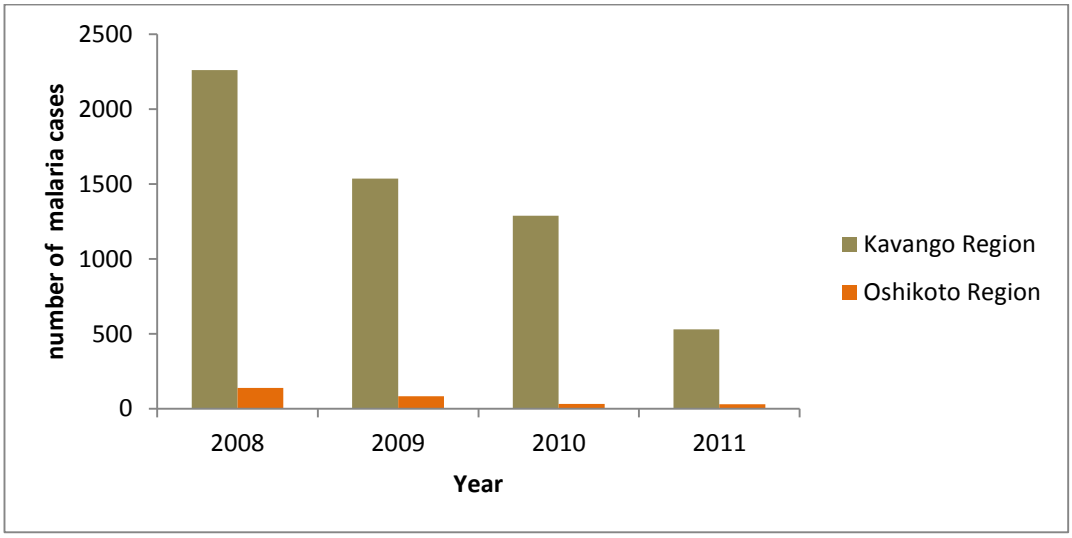


Figure 1.1 The number of inpatient malaria cases in Kavango and Oshikoto regions

The number of inpatient malaria cases in Kavango and Oshikoto regions (MoHIS, 2011). One can see that in Kavango and Oshikoto regions malaria cases have been on the decline. In 2008, the Kavango region was the highest with 2361 recorded cases while in 2011 it reduced to 531 recorded cases. This indicates a steep drop of the number of inpatient malaria cases. In Oshikoto region the number of malaria cases was much lower than Kavango region between 2008 and 2011. While Kavango had 2361 recorded cases, Oshikoto had 140 recorded cases during the same year in 2008. Similarly, in the years 2009, 2010 and 2011 Oshikoto had lower inpatient cases of malaria.

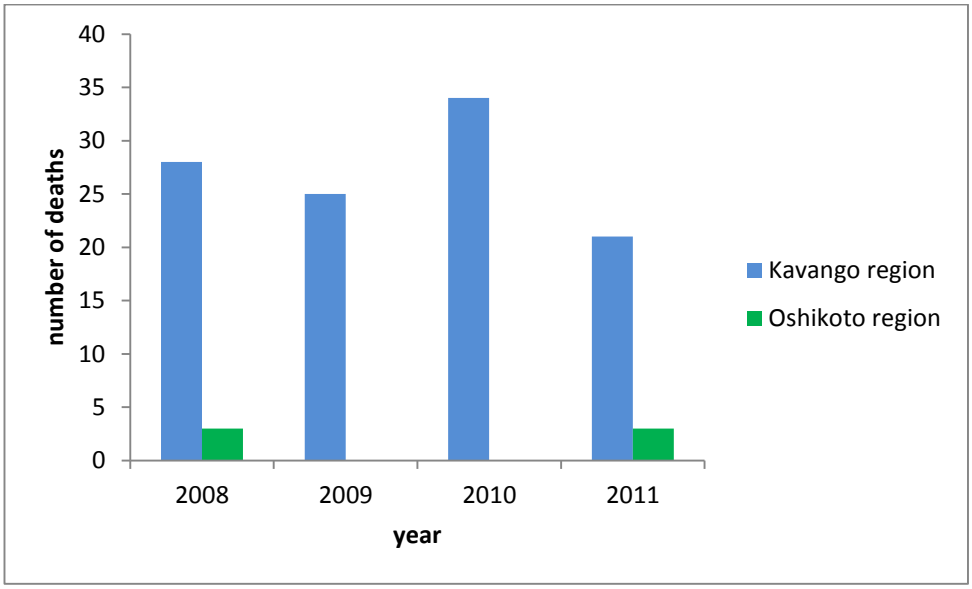


Figure 1.2 The number of inpatient malaria deaths in Kavango and Oshikoto regions

The figure 1.2 shows that there was a decline in the number of deaths in the Oshikoto regions and that there were no deaths recorded in 2009 and 2010. In 2010, the Kavango region recorded the highest number of deaths (35). Generally, there is a decline of deaths in the Kavango region even though the number of deaths is still higher compared to the recorded deaths in Oshikoto region.

The high rainfall patterns and warm climate in these regions favour the breeding and survival of the vector *Anopheles* mosquito. Mean annual rainfall in northern Namibia is generally high at 400-700 mm. see appendix 20. The two areas under study, Oshikoto and Kavango have a mean rainfall of between 500 to 600 mm per annum (MoHSS, 2005). The mean temperature ranges between 19^{o}C-34^{o}C. Due to the presence of perennial water bodies in the area, the north eastern regions have

relatively longer and more stable malaria transmission periods between November and June (MoHSS, 2005). *Plasmodium falciparum* is responsible for over 95% of malaria infections in Namibia (MoHSS, 2005; Malaria Indicator Survey, 2009).

The setbacks to malaria control include drug resistance in the parasite as well as the resistance of the mosquito vector to insecticides (WHO, 2009). Artemisinin was recommended by the WHO (2006) as the most effective medicine for treatment of drug resistant malaria parasites. The blood parasite is increasingly becoming resistant to Chroloquine, Sulphurdoxine-pyrimethamine and Mefloquine (WHO, 2007). In recent years, malaria parasites have been resistant to Artemisinin and this was first detected in Cambodia, Myanmar, Thailand and Vietnam and it is spreading to other nations. This will pose a health hazard to the public health of the Sub Saharan-Africa and other concerned nations (WHO, 2013).

The three major malaria vectors in Namibia are *Anopheles arabiensis, Anopheles gambie and Anopheles funestus. Anopheles arabiensis* is the commonest and has the widest distribution. It is the principal vector in the northern regions of Namibia (MoHSS National Malaria Policy, 2005). It breeds in the rain puddles (Oshikoto Region) and low–lying areas that collect water during the rainy season. Only the female mosquito transmits the disease. The vectors feed at night and therefore vector control measures such as in-door residual spraying of houses, use of insecticide-treated nets (ITNs) have been done successfully in many parts of the country. The eradication of the vector through insecticides has been successful in some endemic

areas, although in other areas the vector has been developing resistance to certain insecticides. WHO (2007) has recommended the use of pyrethroids such as Insectcidal Treated Nets (ITNs) *or* Long Lasting Insecticidal Nets (LLINs). In recent years, mosquitoes have developed resistance to the above insecticides and that has led to some countries to resort to the use of Dichlorodiphenyltrichloroethane (DDT) that had been banned due to its bad effects on the environment (WHO, 2013). Alternative insecticides are now being tried. Namibia switched back to using Dichlorophenolindophenol (DDT) since early days of the year two thousand due to outbreaks of malaria in the nation when alternative insecticides were used (Kamwi, Mfune, Kaaya & Jonazi, 2013).

Human immunity is a very important factor that controls both infection and development of the parasites in the body of human beings. Partial or complete immunity develops only after years of exposure to infection. A study needs to be done repeatedly to understand the mechanism of malaria infection and human response in order to develop effective drugs and vaccine. There is a lot of information on the life cycle of the parasite as well as its resistance to drugs of choice whether they are monotherapy drugs or synergetic ones. The *Plasmodium* parasites have managed to evade the effectiveness of drugs through mutation (Leoratti et al., 2008). The understanding of both the natural and acquired immunity in humans still needs a lot of research. Doolan, Dobano and Baird (2009) narrated that despite global eradication efforts, malaria has thrived and that it may decline for a period only to resurface after thirty to fourty years. The problem lies in the little

understanding in the resistance of the parasite to safe and affordable drugs especially in the affected areas of Africa and Asia where poverty is rampant. Lack of understanding in the mechanism of immune system of humans has led to the failure of developing vaccine against malaria parasites. Dodson (2013) reiterated the same sentiments by saying failure to control deaths caused by malaria parasites is due to unique evasion of the parasite from human immune system through mutation of its proteins. The parasite has a variety of these proteins on its surface, which keep changing at different stages of infection. This then leads to partial and short-lived immunity, which cannot protect individuals from reinfection (Kakkilaya, 2011: Duraisingh, 2012).

Young children and pregnant women are vulnerable because children would not have developed protective immunity. Langhorne, Ndungu, Sponaas and Marsh (2008) in their paper reported that children are at high risk of malaria infection due to absence of exposure to the parasite. Hence, a quarter of the world's children die from malaria and a million are from Sub- Saharan Africa. Pregnant women have weakened immunity that can result in miscarriage and maternal deaths (WHO, 2013). In Sub-Saharan Africa 25 million pregnant women are exposed to malaria every year (WHO, 2004) cited by (Ndama et al., 2006). People with HIV/AIDS are at risk of malaria due to weakened immunity. The planning and implementation of sustainable measures are achieved by encouraging research in relevant malaria fields.

The San people however, do not have such facilities as in-door residual spraying of houses, use of insecticides, or INTs that have been used successfully to kill the malaria parasites or to prevent transmission (focus group discussion by the researcher 2009). There is a dearth of information describing the immunity of the San people. Most of the traditional herbs they take for various elements have not been tested scientifically or proven effective against the diseases they claim to cure as there is no record of their efficacy. The few herbal plants that have had enormous publicity are the *Hoodia gordonii,* a stem succulent plant used by the San for years as an appetite suppressant and devil's claw, which have generated interest over their use in many nations.

The San are indigenous minorities that are found in small and scattered groups living in South Africa, Namibia, Botswana, Angola, Zambia and Zimbabwe. The San population is about 100 000 with 33 000 living in Namibia (Working Group of Indigenous Minorities in Southern Africa, 2010). Until recently, the San were known for their hunting and gathering lifestyle, which required them to move from one place to another in search of what the land can provide, in order to survive (Gunnestad, Larsen & Nguluka, 2010). The San people were usually found in small communities from ten to about thirty people around a water body where wild animals could come and drink water and the San would hunt and kill them. They had to move according to seasons and mobility was necessary for the regeneration of the environment. Lack of land rights and social pressures have led to many San communities becoming increasingly dependent on state welfare rather than their

tradition of hunting and gathering (Dieckmann, 2007). In Botswana, many San communities have been moved from their traditional ancestral territories to settlement areas created by the government (World Intellectual Property Organisation {WIPO}, 2005).

This study focuses on the high tolerance to malaria in the San people despite the harsh physical conditions they live in. Gunnestad, Larsen and Nguluka (2010) described the San as a minority group that lives in isolation from the rest of the other ethnic groups. Their social norms are different from other ethnic groups. They tend to keep to themselves and family is very important to them since they feel protected and safe as they live together. Anaya (2013) defined them as a disadvantaged people who live far from everyone and everything that has to do with modern day society such as education or health facilities. They are very satisfied with this kind of lifestyle if only they can be left alone to continue living this way. This gave the researcher an opportunity to carry out this study in their natural environment.

This group of the San that the researcher studied was of the !Kung clan who live in Tsintsabis and Bravo in Oshikoto and Kavango regions. They speak the !Kung language. Like the rest of the San, they live a hunting and gathering lifestyle, which is now very restricted by the government. The men went hunting with dogs and special kind of bows, poisoned arrows and spears. The women went foraging providing most of the food to the family. They could spend two to three days per

week searching for roots, nuts and berries. They depend on each other for survival and shared their wealth amongst themselves.

Study Area

The study was carried out in Tsintsabis and Bravo, which are 60 km and 90 km from Tsumeb and Rundu respectively in the Oshikoto and Kavango regions of Namibia. Tsintsabis has a surface area of 300-hectares. In 1993, it became a resettlement area of about 841 people of the San families (Dieckmann, 2007). Bordering it are semi-commercial farms owned by Oshiwambo and Kavango farmers. There were around 1500 people in 2010 (Ministry of Lands and Resettlement, 2010). About half of them were San. The population of San is difficult to numerate, as they never stay in one place. The census data is therefore speculative and incomplete. Currently the San, Kavangos, Oshiwambo, Damaras and Caprivians ethnic groups, bringing the total population to about 4000, also occupy the area (Dieckmann, 2007). The San's chief in 2013 confirmed by word of mouth that the population now stands at 5000 in Tsintsabis alone due to the new road that is being constructed and people from other towns who moved in looking for jobs.

The other place Bravo which is in Kavango region is about 90 km from Rundu and it is where the other San group lives. The population is made up mainly of indigenous !Kung-speaking communities. In Bravo, the chief of the San specified by word of mouth that the population of the San is approximately 800 people. Their lifestyle is more or less like that of the !Kung San in Tsintsabis. The majority of the San population are highly mobile in search of food, they have no rights to the land

(Suzman, 2001). What used to be their land was taken from them either to resettle other ethnic groups for farming purposes or to establish national parks (Etosha national park). The proposed new resettling scheme has put the San as a priority group but up to now, they have no land of their own except for those in Tsumkwe (Dieckmann, 2007). The two groups were chosen because they still, to some extent live the life of hunting and gathering in a malaria area of Northern part of Namibia (Suzman, 2001).

October to April is the rainy season and it coincides with malaria season (MoHSS, 2005). Despite the harsh conditions they face, they still live in the doom shaped huts, which are not properly constructed to protect them from mosquito bites.

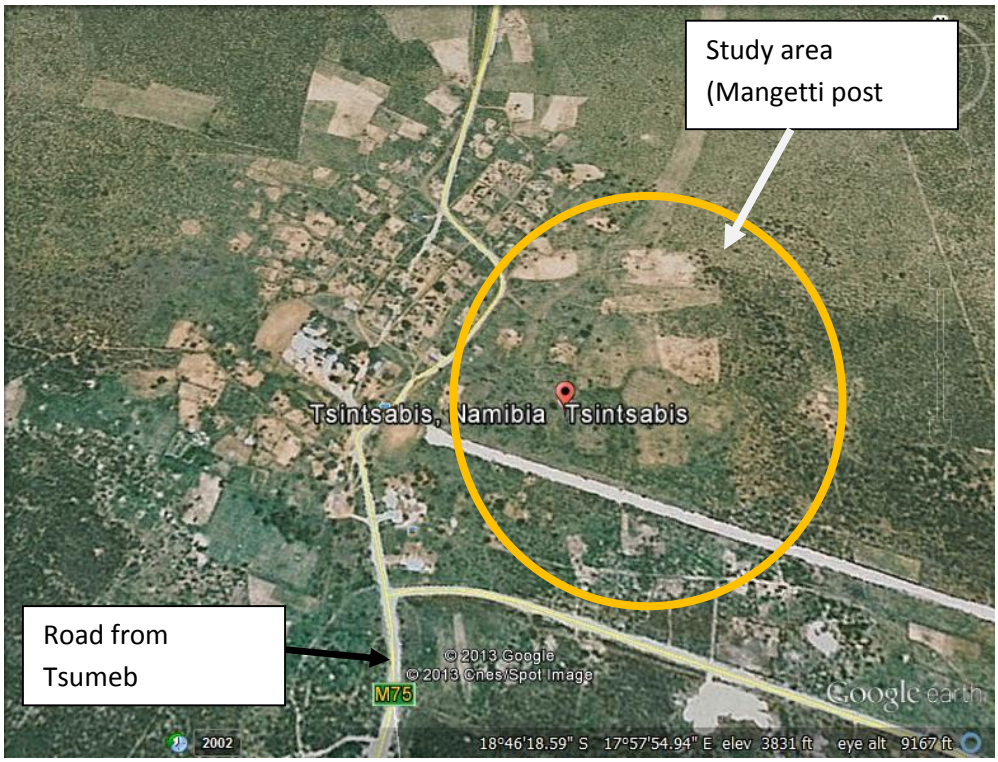


Figure 1.3 Map of Tsintsabis centre in Oshikoto Region Namibia.

This is where the clinic, school, community hall are situated (Adopted: Google Earth, 2013).

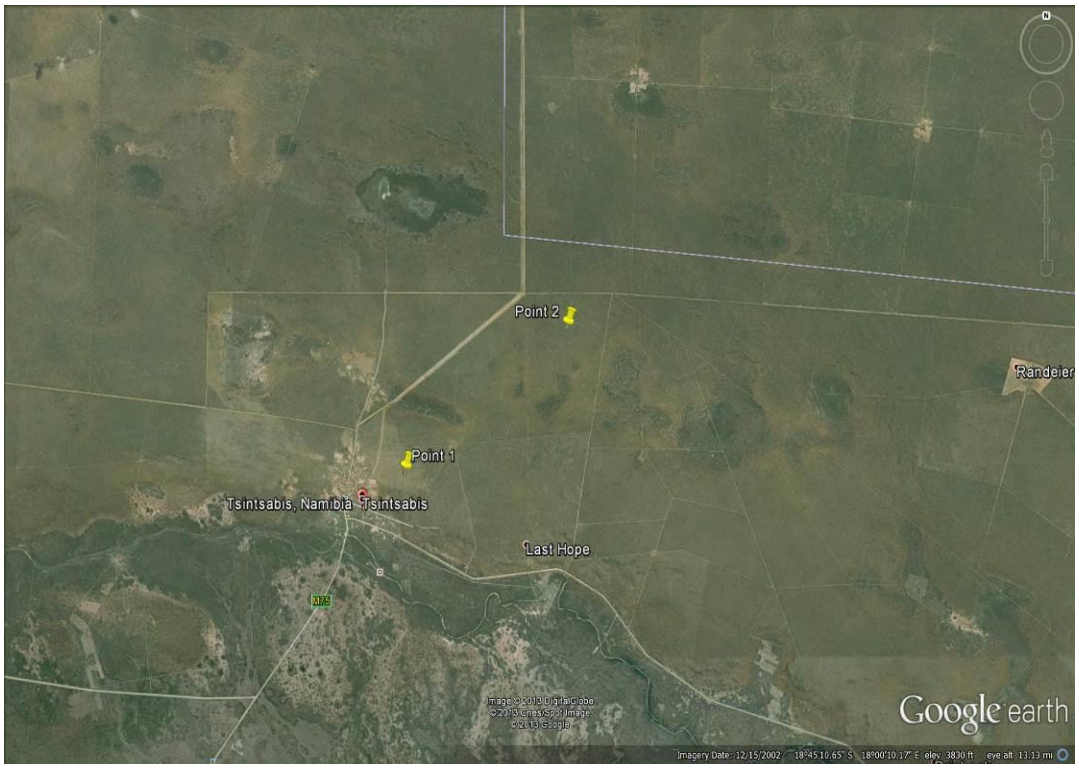


Figure 1.4 Map of the study area of Mangetti Post in Oshikoto region Namibia.

(Adopted: Google Earth 2013). The two places the San settled during the study were Point 1 which was slightly closer to Tsintsabis. It was the first area the study was carried out and point 2 is where they shifted to during the study in their nomadic style.

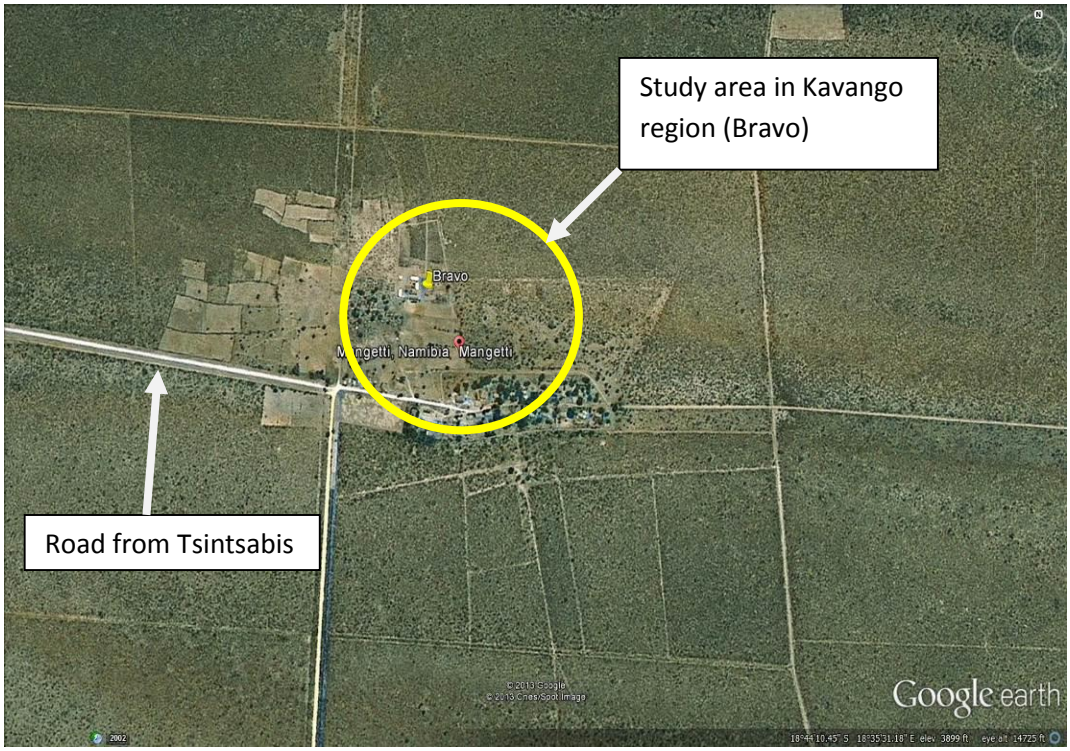


Figure 1.5 Map of the study area of Bravo in Kavango Region.

(adopted: Google Earth, 2013)

They depend on herbs for healing many ailments as well as communicating with the spirit world to cast out evil spirits from the sick person (focus group discussion, 2009). The government of Namibia is encouraging permanent settlements with modern homes. Some of the San people moved into these modern homes whilst others have resisted and are still living the nomadic life. Those in Bravo have made their community a permanent settlement and the government has put up permanent structures like schools and community hall. There are few other ethnic groups in the area like, the teachers at the school and an agriculture official who reside with the community. The agriculture official's main duty is to teach the San how to plough and grow crops like mahangu, sorghum and maize. These are crops grown by other

ethnic groups discouraging the San from hunting and gathering. The impact of such a change will only be noticed later, especially where their tolerance to malaria is concerned.

Figure 1.6 The San people taking *Hoodia spp* as an appetite suppressant for their hunting trips

Despite the San people being present in these malaria endemic areas and their vulnerability, they have survived the disease to date. These people are poorly protected from mosquito bites as their bodies are often half-naked (see Fig 1.5) and their dwellings are not protected from entry by mosquitoes as shown in Fig 1.4 the pictures of the typical San houses.

Figure 1.7 The different types of homes of the San people in Mangetti post and Bravo communities

The dressing code of the San as indicated below exposed them to Malaria infection.

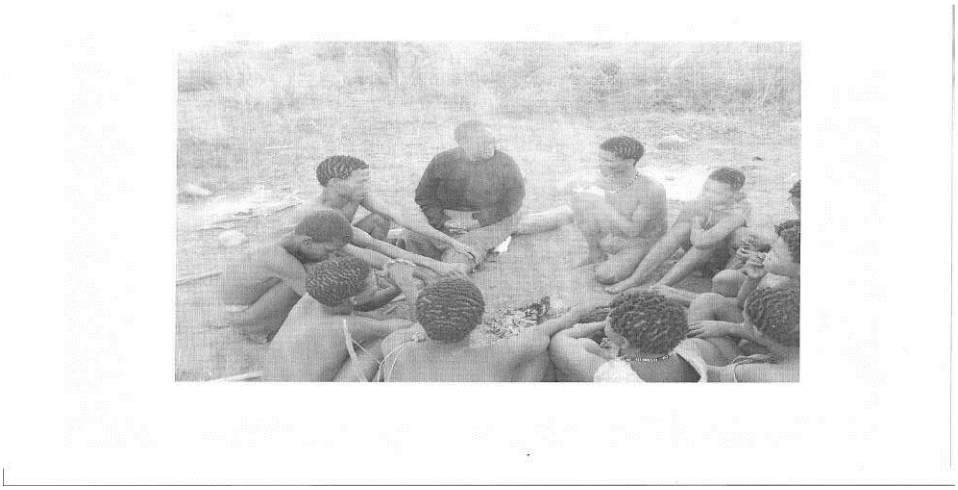


Figure 1.8 The researcher (C. Amoo) and the San people displaying their dress code during one of the many study visits

It is therefore crucial to understand what makes the San people resistant to malaria parasite and the extent to which they understand malaria disease, especially the ability to differentiate malarial symptoms from those rendered by flue, diarrhoea or HIV/AIDS. It is also important to investigate whether or not the San people are immune to malaria infection and if they seek treatment from public health care facilities when they fall ill.

1.1 Statement of the Problem

The problem that was investigated was why the San people have survived to this day without any clinical records or a study to show that they are victims of malaria despite living in endemic areas with other ethnic groups. The records in MoHSS (2010) National Malaria Monitoring and Evaluation Plan 2010-2016 gives figures that in 2008, there were 128 531 reported out patient malaria cases compared to 448265 cases in 2000. A total of 199 deaths were reported in 2008 compared to 679 deaths in 2000. From this information, the only people who visit the hospitals frequently are mostly the other ethnic groups who have suffered malaria epidemics especially during malaria season (MoHSS, 2009). With no literature available about the morbidity or mortality of the San from malaria disease, there was need to investigate if the San had any knowledge of malaria and whether they suffered from it. There is dearth of information concerning the possible theories that the immunity to malaria in the San people is due to the micronutrients found in their diet, or the traditional medicines they take, or the genetic makeup of their haemoglobin or the shape of the surface of the red blood cells, which restrict the entry of the parasite into the cells. What are the main mechanisms of immunity that have protected this group from the endemic of malaria disease? These are some of the questions that needed to be answered as one carried out investigations through research and experiments.

1.2 Objectives of the Study

The general objective is to study the immunity of the San people to malaria comparing it to the other ethnic groups.

The specific objectives are as follows:

1. To investigate the knowledge of malaria amongst the San people and the other ethnic groups.
2. To investigate the low incidence of the morbidity and mortality of malaria patients in the San people compared to the other ethnic groups (using questionnaires and focus group discussions).
3. To investigate immunity to malaria in the San people compared to the other ethnic groups.
4. To determine the presence and levels of microelements, antioxidants and antimalarial chemicals in the diet of the San people.
5. To detect the presence of malaria parasites in the blood sample of the San and the other ethnic group.
6. To investigate the shapes of the red blood cell surfaces and haemoglobin that might confer immunity of the San compared with the other ethnic groups.
7. To compare the size of the white blood cells, platelets and quantities of IgG between the San and the other ethnic groups in Oshikoto and Kavango regions.
8. To identify the fungi that commonly grows on most foods (staple food) of the San.

1.3 Hypotheses

The low incidence of malaria in the San people is due to various indicators. The hypotheses below were used in determining the immunity against malaria parasites in the San people.

1. H_o: The San people have no knowledge of malaria compared to the other ethnic groups.

   H_a: The San people have knowledge of malaria compared to the other ethnic groups.

2. H_o: The San people are not immune to malaria disease compared to the other ethnic groups.

   H_a: The San people are immune to malaria disease compared to the other ethnic groups.

3. H_o: The low incidence of the morbidity and mortality of malaria cases in the San people is not equal to that of the other ethnic groups.

   H_a: The low incidence of the morbidity and mortality of malaria cases in the San people is equal to that of the other ethnic groups.

4. H_o: There are no malaria parasites in the blood samples of the San people compared to that of the ethnic groups.

   H_a: There are malaria parasites in the blood samples of the San people compared to that of the ethnic groups.

5. H_o: The diet of the San has no medicinal properties that confer immunity against malaria parasite.

   H_a: The diet of the San has medicinal properties that confer immunity against malaria parasites.

6. H_o: The shapes of the red blood cells surfaces of the San people do not confer immunity against the malaria parasites.

   H_a: The shapes of the red blood cells surfaces of the San People do confer immunity against the malaria parasites.

7. H_o: The genetic deformation of HbC in the red blood cells of the San people does not confer their immunity against malaria parasites.

   $H_{a;}$ The genetic deformation of HbC in the red blood cells of the San people does confer their immunity against malaria parasites.

8. H_o: There is no difference in size of white blood cells, platelets and quantities of IgG between the San and other ethnic groups

   H_a: There is difference in size of white blood cells, platelets and quantities of IgG between the San and other ethnic groups

9. H_o: The fungi identified on the food of the San people have no effect on their immunity.

   H_a: The fungi identified in the food of the San people have an effect on their immunity.

CHAPTER TWO: LITERATURE REVIEW

Malaria is a life threatening disease caused by the parasite of the genus *Plasmodium.* An infected female Anopheles mosquito transmits it. Malaria was reported to be one cause of the poor economic performances and persistent poverty of many African

nations (WHO, 2010). Although it is preventable and curable, the resources to control this disease in many African countries are limited (WHO World Malaria Report, 2012). Malaria morbidity and mortality rates are high in countries like Zambia, Malawi, Cote d' Ivoire, Nigeria and Angola (WHO World Malaria Report, 2012).

Malaria causes about a million deaths in sub Saharan states (WHO, 2000). The latest figures reported by WHO are assessed as 660 000 (six hundred and sixty thousand) deaths, a reduced number compared to year 2000 (WHO, 2013). The vast majority of cases occur in children under 5 years old and pregnant women (Brundtland as cited in Kamwi, 2005). He stated that malaria was the single largest disease in Africa and a primary cause of poverty, citing that three thousand children die from malaria every day. Africa has the highest morbidity and mortality rate about 90% of the world's malaria deaths (WHO, 2013).

In Namibia, the malaria burden is on the decline (MoHSS, 2010), although it used to be a major cause of morbidity and mortality (MoHSS, 2002). The trend of malaria was on the increase slightly each year with 21% in 2002 to 2003, 22% in 2004 to 2005 and 23% in 2004 to 2005 of all admissions (Ministry of Health Information Services, 2002/2003 – 2004/2005). Data collected from Health and Management Information Systems (HMIS) showed a reduction in the prevalence of malaria cases in Namibia. In 2008 there were 128 531 reported out patients cases compared to 448

265 in 2000. Death indices reported were 199 in 2008 and 679 in 2000, showing a percentage death decline at 70.6 % (MoHSS HIS, 2010).

There has been a cooperated effort by the Governments of Botswana, Namibia, South Africa and Swaziland to eliminate malaria from the region by the year 2015 (Feachem and The Malaria Elimination Group, 2009). Malaria border transmission will remain a problem if the other bordering countries will not cooperate in implementing this programme. On 25 April 2000 heads of states or representatives of 44 African countries assembled in Abuja Nigeria to approve a plan action for controlling malaria. These nations endorsed the use of insecticide-treated mosquito nets (WHO, 2000).

According to Kenya's National Malaria Strategy paper, malaria in Africa kills 26 000 (twenty six thousand children) per year (WHO, 2002). The other major difficulty faced by many African states is the delivery of treatment in remote areas where the highest burden of the disease is prevalent. Poor roads, wars, financial constraints are some of the prohibiting factors. Global funding preparedness to finance and maintain the eradication of malaria has been dwindling most probably due to the decline of the world economy (Feachem and The Malaria Elimination Group, 2009). African countries are encouraged to set up Trust funds and introduce special taxes on lucrative goods. This would go a long way in maintaining malaria activities.

There have not been any known malaria outbreaks amongst the San people whose estimated population is 100 000 all living in Southern Africa. 7 000 live in Angola, 7 000 in South Africa, 1 000 Zambia, 1 000 in Zimbabwe, 33 000 in Namibia and 46 000 in Botswana (Africa, 2010). They have no formal employment; instead, they are hunter-gatherers and 60% are non-literate (Africa, 2010). Malaria outbreaks are unknown amongst them, but one cannot rule out any outbreaks in malaria in the future since the government of Namibia is now resettling the San people in more modern homes and changing their diet to more of the staple food of other ethnic groups leading to a total change of their lifestyle (Africa, 2010).

The San people have never adapted to agricultural practices throughout their cultural history and their association with other cultural groups will increase the danger of being integrated with the other ethnic groups (Le Roux & White, 2004). This may be the reason why many San groups have been afraid to send their children to public schools. A follow up of this transition is encouraged in the form of research both scientifically and socially. The World Intellectual Property Organization (WIPO) seminar stated that the San people of Southern Africa are known as the oldest gene holders known to man. The San generally live in harsh conditions without any modern machines to survive (WIPO, 2005).

According to Schuster et al., (2010), the human vector was probably the most dangerous agent of the spread of infectious diseases among hunter-gatherers. Schuster et al., (2010) identified several genetic adaptations in the San people that

make them susceptible to certain diseases of an agricultural lifestyle that include high-fat diet as well as HIV/AIDS.

Arama et al., (2010) carried a similar study in Mali between two ethnic groups, the Fulani and the Dogon who live in a community where malaria is endemic. Yet the Fulani tribe is more immune to malaria than the Dogon tribe (Arama et al., 2010). Traditionally the Fulani are nomadic, pastoralist and traders of the animal products (Tarig, 2011). This tribe is found in most African countries especially in West Africa though a few are found in East Africa and Central Africa. In all these nations, they live in seclusion from the local agriculture populations (Tarig, 2011).

2.1 The role of micronutrients in the diet of the San people in response to their immunity

The body of a human being needs two major types of nutrients to keep them healthy and these are micronutrients and macronutrients. Micronutrients are needed in small amounts for the body to function properly. Most of the diseases are due to deficiency of micronutrients. Rath Institute (2010) states that micro nutrients taken prophylactically could help in strengthening the immune system of the body thereby reducing many diseases that are caused when one's immune system is weak.

Micronutrients are needed only in small amounts, their deficiencies lead to critical health problems. In fact, most of the diseases and conditions that people face today are due to deficiency of one or more micronutrients. WHO (2009) said that

elimination of micronutrient deficiencies will increase labour efficiency multi-fold. Nutrients such as iron, zinc, Vitamin C, antioxidants, flavonoids are some of the substances that play a very important role in boosting immunity towards malaria disease.

The diet of the San people is composed mainly of roots, fruits, seeds, leaves and tubers, most of which are eaten raw. Thomas (2007) in her book The Old Way stated that the !Kung's food is plentiful and nutritious because it is eaten raw but to other people this would be less edible and would not be suitable to be a staple food. In addition, the quantity will be limited especially if it is to be eaten raw by a large number of people. Most of these foods like berries, truffles and spinach like leaves are seasonal pending on the amount of rainfall. If there is drought, the other ethnic groups are provided with drought relief food which the government provides yet the San's diet is not provided as a package either to the San or to the other ethnic groups (Jenkins, 2006; Thomas, 2007).

To confirm the literature from other authors, Jenkins (2006) stated that the San's basic diet was mainly melons, seeds, nuts, roots and antelope being the staple food which include 25 species of bulbs, rhizomes, corms and tubers. Roots were used as a source of water.

Thomas (2007) stated that meat did not play any role in as far as immunity is concerned but was important in uniting the San people. This was confirmed by Lee (2003) who reiterated that 70% of the San's caloric intake came from various berries, roots and nuts and only 30% from meat intake.

Jean-louis (1990) stated that cooking dissolves toxic substances from the particles that have side effects on many individuals due to toxemia. Jean-louis (1990) further noted that cooked food contains less mineral salts and other vitamins especially vitamin C and A that boost up the immune system of humans.

2.1.1 Zinc

National Federation of Professional Trainers (2010), as cited by Ortega (2010), states that zinc is the most effective boost on immune system compared to other micronutrients and vitamins. It is believed to be responsible for the manufacturing and maintenance of T cells in acquired immune system (Kelly, Jauret, Jensen & Chan, 2007). A deficiency of this mineral ion will reduce the production of the T cells, which will result in weaker acquired immunity the body needs to fight the parasite *P. falciparum.* Zinc combined with vitamin A plays a very important role in reducing infection caused by *Plasmodium falciparum* (Zeba et al., 2008).

2.1.2 Iron

Iron exists in two forms, as an essential component of functional proteins, examples being haemoglobin which can combine with transportation of gases or act as storage proteins like in transferrin and lactoferrin which are adapted to prevent iron from causing tissue damage to the body (Prentice, 2008). Iron is a mineral that plays an important role in human metabolism as it carries oxygen around the body (WHO, 2006). Free iron in the plasma can be toxic to tissues and freely available to the pathogens as well causing them to multiply so the administration of iron is very

important (Kohgo, Ikuta, Ohtake, Torimoto & Kato, 2008). Spanierman (2013) further added that excessive iron absorption is lethal to the body. Individuals show signs of GI toxicity if they ingest more than 20mg/kg of iron except for pregnant women. Moderate intoxication is above 40mg/kg and severe toxicity is when intake exceeds 60mg/kg. Severe overdose causes oxidative phosphorylation and mitochondrial dysfunction. This results in cellular death. Therefore, one has to stick to the recommended daily allowance.

Iron plays a central role in immune system towards malaria. There is an interaction between the host and the parasite at the intra- erythrocyte phase of infection where the high level of iron reduces morbidity (WHO, 2006). This interaction is not well understood. However, iron is an abundant element but it is insoluble at body pH and therefore cannot easily be used by the body (Prentice, 2008). MedicineNet (2014) states that adverse effects from iron-deficiency are anaemia and other diseases. This can be lethal as the body will not be able to carry sufficient oxygen molecules for oxidation of glucose. In children, the deficiency will further have adverse effects on their health and development is affected. Therefore the way iron is administered is of utmost importance. A study carried out in Tanzania and Zanzibar led to recommendation by WHO (2006) on the safety of administering iron to infants and young children in the malaria endemic areas as there was an increased rate of morbidity and mortality after administration of iron –folic acid supplements to the children.

2.1.3. Antioxidants

Antioxidants boost the immune system in the body by fighting off free radicals in the body of human beings and other harmful foreign bodies by neutralizing them (Sudhanshu, Sandhya & Ekta , 2010). Free radicals cause many ailments in humans including malaria (Sudhanshu et al., 2010). The free radicals released into the environment through pollution, radiation, chemicals, physical stress and change in gene expression like in malaria parasites can mutate to evade human immune system (Pourmorad & Hosseinimerhr, 2006). The body is capable of producing these antioxidants if one is healthy. There are many antioxidants found in plants especially medicinal plants, (Sudhanshu et al., 2010). Reis et al., (2010) found out that treating mice with chloroquine and two antioxidants agents, (desferoxamine and N-acetylcysteine) was effective in eliminating cerebral malaria. A combination of artesunate with antioxidants showed similar results, in that it was very effective in treating cerebral malaria.

Flavanoids are very powerful antioxidants and active compounds found in many African malaria therapies that reduce the severity of malaria infections and help to clear the parasite by enhancing immune response (Maranz, 2011). There is a correlation between acquired immunity and diet especially with flavonoids. Maranz & Deitsch (2010) explained that flavonoids taken orally during initial infection boosted immune response on reinfection after treatment has been stopped. Trape, Pison, Spiegel, Enel & Rogier (2002) carried out a study in Senegal where antimalarial substances containing antioxidants were given to patients who had been

infected with the malaria parasite, and the substances wiped out the parasite that had killed many people.

Garlic has antioxidant properties that protects against oxidation, caused by parasite toxins (Formular 256 Ingredients, 2011). Garlic and onion have compounds that can treat malaria. Feng et al., (2012) reported that allicin (diallyl thiosulfinate), a major active compound found in garlic, has properties that act against *Plasmodium yoelii*. It attacks the sporozoite and erythrocytic stages of the parasite by inhibiting cysteine proteases. Feng et al., (2012) discovered that allicin injected in a mice reduced the number of parasites and prolonged the life of the host.

The immune response was noted in allicin treated mice by the presence of interferon y *(*IFN-y*)* and tumor necrosis factor (TNF), $CD4^+$ T-cells and macrophages and were found in higher numbers (Feng et al., 2012). Coppi, Cabbinian, Milrelman & Sinnins, (2006) though they agreed with Feng that allicin attacks the sporozoite and merozoites stages but they discovered that neither stage was completely wiped out. The metabolites were not very active against *Plasmodium* parasites they concluded. A question that still stands out is how much of garlic does one have to eat to acquire the immunity that is required to protect oneself against *Plasmodium* parasites.

2.1.4 Vitamin C

Vitamin C strengthens the immune system and activates the production of some antibodies and it is an antioxidant, which neutralizes free radicals and toxins. The

rind of a lemon has some oil that encourages the production of white blood cells that fight malaria disease whose seeds help in reducing fevers (Formula 256 Ingredients, 2011). The body needs iron for its daily functions and it combines with Vitamin C for iron absorption which is required in the formation of red blood cells (McCarty, 2011).

2.2 Immunity

The San have immunity to malaria whose mechanism is not known. Schuster et al., (2010) carried out a study on Khoisan (San) and Bantu genomes from Southern Africa where they looked at the genetic makeup of the mitochondria of the two groups. Their results showed that the two groups had different genomes from each other as well as the individual San genomes. They further concluded that due to this difference, the San were more immune to many diseases as compared to the Bantu.

This study mainly focused on two kinds of immunity, the natural and the acquired immune systems. The Natural Immunity is an inherited immunity that prevents the establishment of the infection. This kind of immunity does not depend on infection by the parasite. It is naturally found in the blood like alterations in the structure of both the haemoglobin (Hb) and the cell membrane of the red blood cells (Kakkilaya, 2011). Duffy antigen proteins are normally found on the surface of the red blood cells. They act as receptors to *P.vivax* parasite; some people do not have these Duffy antigens on the surface of the red blood cells and are therefore resistant to the infection of this parasite (Robert, 2013) (Fortin, Stevenson and Gros, 2002).

Fortin et al., (2002) carried out a comparative study on three ethnic groups, Fulani, Mossi, and Rimaibe who live in an endemic area of Burkina Faso. The investigation was on the presence of genetic factors that determine the interethnic influences on the malaria parasite infection. In the case of the San this phenomena (Duffy antigen) was not carried out because *P.vivax* is not a very common parasite in the area studied. Therefore, one cannot speculate whether Duffy antigens protect the San against malaria parasites.

Another form of inherited natural immune system in humans is where the glycophorin is found on the surface of the red blood cells. *Plasmodium falciparum* has at least two receptors each of which binds to glycophorin A or B and if due to mutation one lacks any of the different types of glycophorin onto which the parasite is supposed to bind itself onto the surface of the red blood cells, the person will be protected from the invasion by the parasite (Robert, 2013). Hadley, Erkmen and Kaufman (1986) had earlier on discovered that glycophorin A and amino sugar N-acetyl glucosamine (GluNAc) could inhibit the multiplication of malaria parasite of *Plasmodium falciparum* in vitro and not necessarily on the invasion of the red blood cell. Hadley, Erkmen and Kaufman (1986) later on, coated to human blood cells mouse monoclonal antibodies against glycophorin A and the results showed that the red blood cells resisted the invasion by either *P. falciparum* or *P. knowlesi*. This then supported the theory that these parasites could not invade cells that lacked glycophorin A, because of the decreased deformity of the cell membrane, they lacked the binding site onto which the parasite could bind itself. Hadley et al., (1986) also

mentioned that, deficiency of glycophorin C and band 4.1 inhibited invasions by both *P. falciparum* and *P. knowlesi*. This indicates the role of red blood cell membrane in the invasion of the parasites into the erythrocytes. These deformities help individuals to build natural immunity in humans, although this deficiency was not observed in this study. Otherwise, one would speculate that this could have played an important role on the protection of the San against malaria parasite infection if the study of glycophorin was carried out. Further research would help in understanding whether the San people can be protected from the parasite by the lack of these antigens binding sites and glycophorin.

2.2.1 Natural Immunity

The inherited disorder of haemoglobin plays a very important role in protection against malaria parasite (Fortin, Stevenson, and Gros, 2002). Haemoglobin is composed of four globin chains (amino acids) and haemoglobin component. The most common form of haemoglobin is HbA_O, which constitutes 90% in adults, consists of 2α and 2β globin chains, HbA2 has 2α and 2β globin chains and HbF has 2α and γ globin chains found in babies up to 6 months of age (Rhea, Robert, and Molinaro, 2012).

Mutations or replacement of amino acids at position 6 of either the Alpha or Beta globin chains lead to haemoglobin variants (abnormal haemoglobin's) (Fortin et al., 2002). There are four most common haemoglobins variants HbS, C, E, α and β Thalassemia. The homozygous Sickle cell HbSS is when HbA valine is replaced by

glutamine at position 6 of the β chain. This causes deformity in the shape of red blood cells. The affected cell will take the shape of a sickle, which will lead to sickle cell anaemia.

Haemoglobin C position 6 of lysine is replaced by glutamine and if the offspring inherits mutated gene from both parents the homozygous genotype HbC will cause haemolytic anaemia and enlargement of the spleen. The people who have HbC live a very healthy clinical life (Rhea et al., 2012). Haemoglobin E lysine is replaced at position 26 by glutamine and the homozygous genotype for HbEE causes mild haemolytic anaemia and mild enlargement of the spleen (Fortin et al., 2002). Thalassemia is a disease that is caused when the α or β globin chains produce more amino acids than the other chain, bringing about an imbalance between the chains. This has an effect on the red blood cells that causes them to mature early leading to their destruction in the bone marrow (Bailey and Gwinnutt, 2007). The heterozygous of the above haemoglobin variants do not cause any diseases if the genetic combination of genes is non-variant.

Both types of haemoglobin whether homozygous or heterozygous play a very important role in providing protection against malaria (Fortin et al., 2002). According to Tan et al., (2011), HbAS and HbAC facilitate the production of IgG in response to P. falciparum. Tan et al., (2011) further mentioned that the mechanism where the variants haemoglobin deters the development of the malaria parasite in the red blood cells is not clear. On the San people such studies have not been done

before, and therefore further studies will enhance and confirm the immunity brought about by any of the abnormal haemoglobins. This is the first time that such a study has been undertaken.

The glycohaemoglobin in this state (Haemoglobin LA1c/CHb-1) is unstable though it will take a stable form later to HbA1c (Hinzmann, Schlaeger and Tran, 2012). Glycosylation is a non-enzymatic reaction in which glucose is bound to the ®-chains of the haemoglobin. The site is at position 6 of valine at the N terminal amino group of the β chain of globin. (Hinzmann et al., 2012). Other sites for glycosylation are 66 and 17 of the β chain. It is interesting to note that this position six (6) valine of the amino group is where mutation of the other haemoglobin variants takes place, the mechanism is not clear (Rhea et al., 2012).

The glycated haemoglobin is used as a marker for the glucose status in people with diabetes and it is used in the therapy of the disease (Hinzmann et al., 2012). Rhea et al., (2012) stated that the Hispanic African Americans have both diabetes and either HbC or HbS trait. Rhea et al., (2012) further explained that this combination is not only confined to America but is spread worldwide. In Thailand, the Tharu people are immune to malaria because of β thalassemia haemoglobin which is affected by HbE and diabetes (Rhea et al., 2012).

The red blood cells are biconcave discs measuring about 7.5µm in diameter and the thick outer rim measures approximately 2.5µm (Bailey & Gwinnut, 2007). The shape

creates a large surface area to accommodate haemoglobin molecule. Not all red blood cells have this shape; some have lost the shape as an adaptive mechanism for survival during adverse condition (Robert, 2013). Malaria has been a number one killer disease especially in Africa, south of the Sahara. The genetic disorders of the shape of the red blood cells provides protection against malaria disease and the parasite either on the deformity of the cell membrane of the red blood cells or mutation on the receptors found on the cell surface will not infect the mutilated cells (Robert, 2013).

The different shapes of the red blood cell membrane such as Sickle cell, Southern Asian Ovalocytosis (oval cells), Echinocytes (with spikes on the surfaces of the red blood cells) and the HbC cells provide immunity against malaria infection. A better understanding of the natural defence mechanism against the killer disease and the *Plasmodium* parasite will help a lot in the eradication of malaria without spending the meagre resources that Africa has (Modiano et al., 2001).

Other erythrocyte mutations discovered the absence of the enzyme Glucose-6-phosphate dehydrogenase (G6PD) in some people. The functions of this enzyme are to catalyse the metabolism of glucose and maintain the balance of glutathione, which is oxidant (Fortin et al., 2002). G6PD deficiency affects about 400 million people, some of whom are protected against malaria infection. In the case of the San, there is no empirical evidence to show that they do not have G6PD enzyme so one cannot speculate that their immunity against malaria parasites is due to lack of G6PD. Given

an opportunity, a follow up study on the absence or presence of G6PD would be recommended.

2.2.2 Acquired Immunity

People who live in malaria endemic areas acquire immunity through multiple exposures to malaria parasites. In the acquired immunity the human response is either humoral or cell mediated immunity.

Kakkilaya (2011) reported that acquired immunity develops after acute infection. Yet Staten's Serum Institute (1996) defined it as immunity that develops progressively over several years of exposure to immunity. Nogaro et al., (2011) reiterated by defining it as an accelerated response after repeated exposure of the same pathogen to reduce the disease and or eliminate the parasite so that re-infection will not occur. Wipasa et al., (2010) disagreed by saying, although acquired immunity occurred after repeated infections over a number of years this will not bring sterility to re-infection regardless of asymptomatic infections that may exist throughout one's life.

In the sub Saharan Africa malaria is rampant even with development of new drugs and eradication attempts of the parasite. These exercises are futile due to mutation that occurs on the parasite surfaces (Robert, 2013). Nevertheless, Doolan, Dobano and Baird (2009) argue that even if the disease is in high endemic areas most people rarely experience serious disease because of the acquired immunity achieved by continuous exposure to the parasite. Doolan et al., (2010) further reported that people

will feel healthy despite a population of parasites in their blood. With these findings, one can conceptualize that this can be applicable to the status of the San's acquired immunity. This is due to several years of exposure that could have brought about sterility to the infection of the parasite. Only repeated surveys and research work can confirm this.

Acquired Immune system attacks the parasite at various stages which are pre-erythrocytes sporozoites, asexual merozoites and sexual stages gametocytes. The response of the body is to stimulate the bone marrow to produce B cells that are involved in humoral immunity. The specific immunoglobulin are produced like antibodies that act on malaria in the form IgG, IgE and IgM or the T cells that are involved in the cell mediated immunity to produce the phagocytes that engulf the pathogens (Kakkilaya, 2011). These antibodies are specific to the antigens by binding themselves to the surface of the antigens rendering them ineffective. The antibody-antigens complex can be digested by the phagocytes or ferried to the spleen where the parasites are destroyed.

Cell-mediated immune response is induced by the presence of *Plasmodium spp* and its protection depends on the number of infections one had. These cells include T cells, Natural Killer (NK), $CD4^+$ cells, $CD8^+$ cells and phagocytes divided into two granulocytes and agranulocytes. Granulocytes were composed of eosinophils, neutrophils and basophils and agranulocytes are monocytes and macrophages all these were to be investigated. The above- mentioned cells are non-specific in their

attack to the antigens except for the B cells. They attack the asexual blood stage of the parasite and they are regarded as anti-disease immunity or anti-parasite immunity or interestingly a sterilizing immunity that protects one against new or further infections but keeps a small quantity of asymptomatic parasites to perpetuate the acquired immunity for life (Kakkilaya, 2011).

The humoral response to *Plasmodium spp* are short lived in naturally acquired immunity, meaning that malaria infections fail to keep antibodies and B or T memory cells for a durable time (Wipasa et al., 2010). Chandele, Mukerjee, Gobardhan, Ahamed & Chauhan (2011) reported that that the T- cells die after the clearance of the disease but some of the cells survive longer. Memory T-cells are long-lived and they confer protection against the disease for a long time. Dorfaman et al., (2005) reported that if a person is repeatedly exposed to malaria parasites the presence of antibody levels decline rapidly after infection delaying the acquisition of immunity. The durability and maintenance of memory cells in the blood plasma after infection still need to be investigated since there is limited knowledge on this mechanism.

Crotty and Ahmed (2004) cited by Dorfaman et al., (2005) indicated that memory B cells are required to maintain lifelong antibody immunity in human beings. Migot et al., cited by Bolad et al., (2005) specified that surface IgM B lymphocytes had a long lasting memory that can prevail long after malaria infection. Chandele et al., (2011) suggested that for memory T cells to offer maximum protection possible they must possess the following; (i) long lasting, (ii) high efficacy and (iii) rapid response to

antigens infection. Wipasa et al., (2010) conducted a study, on malaria-specific on memory B cells in adults from a low transmission set up in Thailand and found out that memory B cells lasted for more than seven years in the absence of repeated infection.

2.3 Platelets

Platelets are defined as not true cells but are made from fragments of large bone marrow cells and are called megakaryocytes (Zaki, 2011). Conglei et al., (2012) call them small anucleate cells that circulate in the blood. These small diskettes measure about 3µm in diameter and about 300 000 cells per µl of the blood (Zaki, 2011).

Platelets have always been known to play a role in blood clotting, yet of late, it has been discovered that platelets have other important functions among others in the immune response to malaria infections. Platelets have been discovered to stop the growth of malaria parasites by attaching themselves to the infected red blood cells and destroy the parasite inside. It is more active on *P. falciparum* parasites (Aprile, 2013). Dzik et al., (2010) cited in Campbell and Ryder (2012) explained that monocytes and phagocytes in conjunction with platelets attack the *P.vivax* pathogenesis of malaria parasite by binding themselves to the parasitized red blood cells and thereby kill the parasite. Zaki (2011) indicated that platelets compete with *P.vivax* for Duffy antigens receptors on the erythrocytes required for invasion by *Plasmodium vivax* and thereby kill the parasite.

The granules released by the platelets do kill the parasite. Further studies in the underlying mechanism of the role of platelets in immune response are encouraged Flaumenhaft (2013).

2.4 Vaccines

Despite the tremendous strides in biotechnology during the past 5 decades and the application to malaria, the many breakthroughs in the molecular biology, genetics, immunology and vaccinology have not brought about useful results as far as vaccination against malaria is concerned (Doolan, Dobano & Baired, 2009). There are currently no licensed vaccines against malaria even though a trial of Mosquirix (RTS,S/ASO1) is being carried out in seven (7) African countries (WHO, 2013).

Vaccination against malaria infection has proved to be very difficult due to a number of reasons. The parasite can easily change its membrane morphology by expressing different proteins that make the immune system of the host less effective (Kakkilaya, 2011). Kakkilaya (2011) indicated that the malaria parasite during its life cycle could show a great variety of proteins at different stages of infection. This is why naturally acquired immunity after infection is short lived due to the complexity of this change of the proteins in the parasites. This has hindered the development of an effective vaccination. The malaria parasite evades the human immune system by changing its surface protein to enter into new erythrocytes where it can switch on and off at high speed (Duraisingh, 2012). This is to avoid being detected by the immune system and managed to identify a gene located on the surface of the parasite that has the ability

to mutate and enter the red blood cell. The parasite returns to its normal structure when inside the red blood cell (Duraisingh, 2012). Probably with this new discovery, the development of an effective vaccine against malaria parasites is closer than expected. Stirnadel, Al-Yaman, Genton, Alpers & Smith (2000) mentioned that the development and testing of vaccine is a problem due to interaction of host antibodies response to specific malaria antigens, that is the identification of antigens. In their study, Wipasa et al., (2010) reiterated that B cell responses to malaria parasite could be sustained for a long period after infection.

The complexity of memory cells reactions with the antigens needs to be looked into especially the quantity and quality of the immune responses against the parasite infection at the merozoites stage if the development of a vaccine has to succeed (Chandele et al., 2010). The malaria parasite does not only evade the immune system by the factors mentioned above but can also affect the immune system by suppression of immune response (Kakkilaya, 2011). Chandele et al., (2010) explained that the infected red blood cell with haemozoin could stop the maturation of dendritic cells that are a product of T cell that are involved in the immunity against malaria parasite.

2.5 Malaria Treatment and Drug Resistance

Treatment of malaria has emerged as a great challenge due to drug resistance by the parasite especially *P. falciparum* (WHO, 2013). New drug resistant strain of the parasite have been identified by a number of researchers in western Cambodia which are genetically different from others. Miotto et al., (2013) cited by British

Broadcasting Corporation states that, every new drug on the market has been rendered useless as the parasites mutates after a period of the drug use. Resistance of *Plasmodium falciparum* to Chroloquine, one of the cheapest and the most used drug in Africa, the parasite have developed resistance to it as well. In 1993 Malawi was the first African country to replace Chroloquine with sulfadoxine-pyrimethamine due to the parasite resistance to the drug (Kublin et al., 2003).

Artemisinin is currently the drug of choice, but it is now reported that the malaria parasite are becoming resistant to it. The drug had been doing very well in the treatment of malaria world-wide especially where it was used with other drugs but until recently reports have emerged that *P. falciparum* has developed resistance to Artemisinin on the Cambodia-Thailand border (Dondorp et al., 2010). In 2011, the Global Plan for Artemisinin Resistance Containment (GPARC) met to discuss the emergency and spread of resistance to Artemisinin (WHO, 2012). The situation in Africa where Artemisinin is being monitored, there have been no reports of resistance of the parasite during treatment with Artemisinin (WHO, 2012).

2.6 Herbal treatment of malaria

One would assume that the medicinal herbs that the San people use are being used for prophylaxis and more research is required to test if they cure the malaria disease. In the article written by Dan, Mchombu & Mosimane (2010) one of the options was to dissolve a herb called tima in boiling water and inhale the steam. Various herbal remedies are used for many ailments, including chewing the roots of Aruba called !gomaba (!gomaba is the name of the root, not the plant) or by cooking them and

drinking the infusion (Dan, Mchombu & Mosimane, 2010). Dan, Mchombu & Mosimane (2010), suggested in their article that one of the options of treating malaria was to dissolve a herb called tima in boiling water and inhale the steam but there is no evidence to show that tima treats malaria or other ailments. The roots of the edada or #aroba bush shrubs can also be boiled and the infusion taken orally. Naruba, //gam//gambe and tima herbs are used to treat headaches by boiling the roots which can be taken orally (Dan, Mchombu & Mosimane, 2010). Whether these herbs treated malarial symptoms or malaria disease is yet to be confirmed. There is no empirical evidence towards the treatment of malaria disease amongst the San people.

Amoo, Bosl, Du Pisani, Horn & Harring (2010) in their article "Intellectual property under the Namibian Constitution," reported that there is a unique interaction between the San people and the land in the use of plants and animals for survival. Some of the plants like the *Hoodia gordonii*, which is a succulent plant that the San eat to suppress both hunger and thirst during their hunting and foraging trips was very popular. This plant has become a very valuable medicinal asset to many international pharmaceutical companies like Phytopharm in the United Kingdom and Pfizer in the United States of America. Devil's claw (*Harpagophytum procumbens* and *H zeyheri*) is another plant found in the Kalahari desert that is not used only locally as a medicinal plant but is also used for research as well as medicine on international markets. All remedies are extracted either, from the leaves, roots, seeds or stems of the plants. They are then boiled, roasted, or chewed raw (Leffers, 2008). The examined bitter plants reported to have traditionally antimalarial properties usually

contain bitter compounds such as alkaloids, limonoids or quassionoids (Titanji, Zofou & Ngemenya, 2008).

The increasing prevalence of malaria in the Sub Saharan region is due to the re-emergence of the parasite and spread of drug resistant parasites. Efforts are now being directed towards the discovery and development of the new antimalarial agents. Clarkson et al., (2004) carried out a study, which was tested in vitro for *Plasmodium falciparum* strain using the parasite lactate dehydrogenase (pLDH) enzyme.

In Malagasy malaria is a treated using traditional practice of decoctions or infusions extracted from bitter plants, (Randrianarivelojosia et al., 2003). The use of traditional herbs for the treatment of malaria and other fevers is common in Cameroon. Plant medicines play an important role in their daily health care especially in the rural areas. Local medicines are preferred to modern medicines in ethnic groups of Paka Pygmies in South Eastern Cameroon (Titanji, Zofou and Ngwenya, 2008). Traditional medicines are commonly sold in markets and public places or administered by healers in traditional clinics.

Most of the research into antimalarial activities is focused on finding an indigenous drug that is effective and affordable especially among the poor who are living in endemic areas (Adebayo & Kretti, 2011). Most of these drugs are administered as a single ingredient orally without proper prescription. There have been disagreements

among scholars about the efficacy of these traditional medicines since they have been no evidence produced to attribute to their eradication of malaria disease. (Maranz, 2011). On the contrary, Rukunga et al., (2009) discovered some antimalarial substance in the plants they studied in Kenya. It is yet to be proven how effective and at which stage of malarial parasite life cycle these substances are active. The major problem is either the traditional therapists or the elders of the communities hold the secrecy of these remedies. This makes it more complicated in that it cannot be tested and be used globally. Although today many of these drugs have been tested, it has only been done in vitro (WHO, 2006). Maranz (2011) states further saying that Africa, despite its rich medicinal plant tradition, there has been a clear failure to eradicate the disease. Verma, Kumar and Bussmann (2007) feel that there is now an unsustainable use of ecosystem services without replenishing the plants.

2.7 Importance of Fungi on the treatment of Malaria

Fungi play vital roles in the biosphere. They have been known to be essential for the recycling of nutrients in all terrestrial habitats since fungi and bacteria are the dominant saprophytes that decompose the complex components of plant and animal debris, such as cellulose and lignin in plants and animal tissues. As opportunistic heterotrophs, they have mycelium, which penetrates into solid substrates, and spores of long-range dispersal (Reece et al., 2011). Some of the fungi are used as medicines in the form of antibiotics like *Pennicilum spp* kills different forms of bacteria (Rivera & Seifert, 2011).

Scientists have come up with new ideas of using fungi as a new insecticide called "bio-pesticide" due to increasing incidences of many insecticide resistances in the vector *Anopheles spp* mosquitoes (Chandler et al., 2011). The fungi *Metarhizium anisopliae* and *Beauveria bassiana* have shown that these two species are able to infect and kill the *Anopheles* mosquitoes within 14 days (Blanford et al., 2005) given the right conditions like pH, humidity, temperature and the right substrate. The fungal spore can infect the *Anopheles* mosquito by contact onto the exoskeleton where the spore will germinate into the body of the vector using enzymes to penetrate into the host haemocoel. It then feeds on the haemocoel cells that will then kill the insect (Clarkson and Charnley, 1996 cited by Farenhorst et al., 2009). This mode of action is called entomopathogenic fungi (Farenhorst et al., 2009).

In recent study carried out by Fang et al., (2011) it was discovered that a genetically engineered fungus carrying a human and a scorpion toxin had a lethal effect on the *Anopheles spp* mosquitoes. They used *Metarhizium anisopliae* a fungus that has a natural affinity for infecting mosquitoes. The combination of the human antibody and scorpion toxin fungus infected the mosquito targeting the parasite in the mosquito before it attacks man (Fang et al., 2011). Fang et al., (2011) investigated the effects of transgenic fungus by spraying mosquitoes all heavily infected with malaria parasite divided into three groups. The first group of mosquitoes was sprayed with transgenic fungus, the second group with natural strain of *Metarhizium anisopliae* and the last group was not sprayed with anything.

The results indicated that those mosquitoes sprayed with transgenic fungus had 25% of the parasite found in the salivary glands of the mosquito compared to 87% in those sprayed with natural strain of the fungus and 94% in those that were not sprayed (Fang et al., 2011). Transgenic fungus (*Metarhizium* product) has proved to kill the parasites (sporozoites) in the mosquito; this exercise can now be tested in Africa for its efficacy (Fang et al., 2011). (Fang et al., 2011) further reported that they wanted to test other combinations to make sure that they find the most effective insecticide to combat the disease.

(Fang et al., 2011) came up with a new method where instead of killing the *Anopheles* mosquito, they would kill the parasite by manipulating the fungus to produce a protein, which is anti-malarial. He further stated that, the fungus acted like a hypodermic syringe in the blood of the insect that produces anti-malaria protein, and within a few days it would basically have cured the mosquito of malaria. Ferguson, Mackinnon, Chan & Read (2007) argued that the malaria parasite like in the past can still develop some resistance no matter what is done to the fungus but he suggested that the anti-malarial proteins should always be modified to kill the parasite.

The climate encourages the growth of fungi especially during rainy seasons when malaria cases are high. (Blanford et al., 2011) reported that there was no need to use the transgenic fungi if the natural fungi can kill the mosquito. (Blanford et al., 2011)

further published another paper where they stated that non-transgenic fungi killed resistant mosquitos and the application of spores in the field helped to kill the vector. Yong (2011) argues that *Manisopliae* fungi is non-specific and can infect other insects that are beneficial to humankind and the environment. Blanford et al., (2011) share the same sentiments saying that there is very little research done on use of natural strains to study their effectiveness on malaria parasite or the mosquito. However, Blanford et al., (2011) does not rule out the use of transgenic fungi. Their biggest problem is to convince the funders of the viability of this method. The release of transgenic fungi into the environment may meet lots of resistance from other members of the society, who may feel that the impact of this method on the environment is detrimental to the environment in the end.

Some fungal species produce mycotoxins from the moulds. Fungi are used in the production of several drugs. Notably, the *Penicillium chrysogenum* mould is used for the production of the antibiotic Penicillin. Similarly, *Aspergillus terreus* is used to reduce cholesterol (Borkar, 2012).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Sensitisation

Letters of permission to carry out the research in these areas was obtained from the Ministry of Health and Social Services (MoHSS), NIP, Lancet laboratories, Ministry of Environment and Tourism, Otjikoto Secondary school and the local chiefs of Tsintsabis as well as that of Bravo granted verbal permission. Meetings were organised with all the elders in the two areas and the study was fully explained to the two groups in both in Bravo and Mangetti Posts (See Appendices 14-20).

3.2 Research Design

The study was both qualitative and quantitative. One needed to do a survey by carrying out a study to get to know the participants (from the two groups) and their background knowledge of malaria. The two groups studied were the San and the other ethnic groups. The San could not read or write hence a focus group discussion was carried out. Questionnaires were handed out to the study participants of the other ethnic groups in Tsumeb district and Rundu district in the Oshikoto and Kavango regions respectively. The questions in the questionnaire were both of qualitative and quantitative nature. The measurements of blood and food samples were done quantitatively.

Convenience Sampling was used because this study is quite sensitive in the sense that blood collection was involved therefore there was need to have participants who were willing (BusinessDictionary, 2014). In reference to the San, two groups were

identified, those who lived in resettlement areas provided for by the government and a group of those who still lived in the bush. The group that lived in the bush is the one the researcher concentrated on. In reference to the other ethnic groups I took those who looked healthy and were willing to participate especially the youth. This study was a case-control study with the San being the cases and the other ethnic groups ("the other ethnic groups" in this study refers to tribes other than the San that lived in the same regions studied) the control group. The blood samples that were analyzed were of equal quantity as per standard methods (4ml in each vacutainer). The parameters that were experimented on were full blood cell count, microscopic blood observations, analysis of micronutrients in plants and fungi. A focus group discussion was carried out with the San people and recorded on tape. Questionnaires were administered to the other ethnic groups. For analysis purposes, data was collected, recorded, coded and analyzed and the outputs were interpreted as shown in the results.

3.2.1 Qualitative Study

Focus group discussion with the San people

A focus group discussion was set up to get the view of the participants on their knowledge of malaria. It was a mixed group of men and women. This group of the San are the ones that have been resettled in Tsintsabis from the bush. Not everyone moved to Tsintsabis Township, a number of them remained in the bush at Mangetti Post approximately 10 Kilometres from Tsintsabis. The exact mileage could not be

determined as they were scattered in the thick bushes of Mangetti area. Their lifestyle did not change much, they continued to hunt and gather fruits, roots, herbs and animals living in dome huts. The study focused on this group not necessarily on those who resettled in the Tsintsabis centre. They still lived a nomadic life during the entire period of study. The location of the consecutive focus group discussions were at Mangetti Post and at Bravo Centre in Kavango region about 90 kilometres north-east of Rundu.

The tools used in the focus group discussion were a tape recorder, camera and a notebook. The last two were used in qualitative and quantitative data analysis. The interpreter Mr Karl Damaseb came from Tsumeb town. The meeting was pre-planned and informal. In Tsintsabis center the first meeting/focus group discussion was held in the community hall with about 30 participants. Nine of the participants came from Mangetti Post but the rest were the San who had settled in Tsintsabis center from the surrounding areas. Therefore, the rest of the discussion was conducted in Bravo with 25 participants at a homestead and in Mangetti post with 28 participants also at homestead. The population in both places was mainly made of women with fewer men.

The samples were conveniently selected which reflected a true representative of the San community studied. The discussion was of a structured nature, in that there were pre-planned questions with open answers on the knowledge of malaria. The recommended number of a focus group discussion is between 8-12 participants but in

this case, the number of participants was too large because the chief had already invited the participants and we could not send them away. There was one facilitator, who was the researcher and hence it was not possible to subdivide the group into smaller numbers. The discussion lasted two hours. A similar set up was carried out at both Bravo and Mangetti Post.

With the other ethnic group which was the control group, questionnaires were handed out in Tsumeb and a group of Kavango Catholic church members who had come to Tsumeb for a church camp meeting for a week. One hundred and twenty questionnaires were handed out, sixty to those from Tsumeb and the other sixty to those from Kavango region.

3.2.2 Quantitative Study

The data collected was analysed statistically using descriptive methods on the SPSS Version 20 and Microsoft excel. This was done on the parameter variables and in situations where gender and tribe were converted into numerical figures, the San for example, would be placed as 1 and other ethnic groups as 2. This was done so that, the data could be entered into Statistical Package for Social Sciences (SPSS) Version 20 software and analysed. The non-parametric data were noted and were not statistically analysed. For example, a symptom of malaria, herbs taken when one was feeling sick and the staple foods. The Questionnaire and discussion notes are in the appendix.

3.3 Collection of plants for nutritional analysis

In order to investigate the type of botanical species, which the San people use for treatment of various diseases and ailments, as well as food, various species were collected from the nearby bushes. This was done with the help of local female field guides. Sometimes, this involved walking long distance of up to 10 km. Different types of species were collected and identified using local dialect. The species were numbered according to species and were each put in separate plastic bags for further identification and chemical analysis of moisture, micronutrients such as vitamins c, zinc, iron, phenols and antioxidants.

Table 3.1 The different plant species collected for analysis of micronutrients, phenols, antioxidants and moisture content

Scientific Name	Part of plant collected
Pentarrhinum insipidum	Leaves
Asparagus sp. (a)	Berries
Hyphaene petersiana	Leaves
Amaranthus petersiana	Leaves
Vangueria infausta	Fruit
Fockea angustifolia	Tuber
Maeru schinzii	Tuber
Cf. vangueira sp.	fruit
Sclerocarya birrea	Flesh

Grewia bicolor juss var. bicolor	Fruit
Bauhinia macrantha	seeds
Corallocarpus triangularis	Roots
Citrillus sp.	Fruit
Citiullus Ianutus	Fruit
Pennisetum glaucum	Seeds
Lapeirousia coeculea	tuber
Lapeirousia bainesii	tuber
Baikeiaea plurijuga	seeds
Ceropegia tentaculata	tuber
Ceropegia leucotaenia	tuber
Chaemacrista absus	Fruit/seeds
Grewia flavescens	fruit
Cucumis anguria var. longaculeatus	fruit
Ocimum sp.	Leaves
Rhus tenuinervis	Seeds
Sorghum bicolor	Seeds

3.3.1 Chemical analysis of the samples

Chemical analyses of the samples were carried out to determine the presence and frequencies of micronutrients, antioxidants, phenols in the food and herbs the San people eat as well as the staple food of the other ethnic groups. The analysis was carried out to find the micronutrients in the San people's food as it is one of the

attributes of their immune system. The quantity of the micronutrients that boosts the immune system of individuals was also measured. The samples were analysed at two different laboratories. One set was done at the University of Namibia Chemistry and Biochemistry Laboratory and the other set was done at Analytical Laboratories 5 km south of Windhoek.

3.3.1.1 Determination of moisture in samples

The Official Methods of Analysis of Association of Analytical communities (2014) were used to determine the moisture content of each of the 15 samples provided. Each sample was weighed on Adam balance and the results recorded. The samples were then oven dried at 100ºC for a period until the weight was constant. This dry mass of the samples was recorded.

3.3.1.2 Determination of Zinc

The Official Methods of Analysis of Association of Analytical communities (2014) were used in analysis of zinc by dry ashing. This method uses a muffle furnace capable of maintaining temperatures of 500-600ºC. Water and volatiles were vaporised and organic substances burned in the presence of oxygen in air to carbon dioxide. Ash content represented the total mineral content in food for nutritional evaluation.

The dried samples were weighed in a crucible using the Adam balance and were burnt to ashes using Heraeus muffle furnace at 500ºC. The ash was dissolved in

hydrochloric acid on the sand bath. The elements were determined by using Perkin Elmer optima 7000 DV ICP-OES and samples were then read on the spectrophotometer at 458 nm. The results were reported as mg/100g wet weight basis.

3.3.1.3 Determination of vitamin C

In order to determine the quantity of Vitamin C found in the samples of fruits, tubers and leaves collected, The Lebensmittal Analytik Grundzüge-Methoden-Anwendung by Reinhard (1992) was used. The fresh wet samples were weighed on Adam balance and readings noted down. The samples were macerated with oxalic acid and Vitamin C was determined on the oxalic acid extract using the redox titration with 2,6-dichlorophenolindophenol (DCPP). Results were recorded in mg/100g wet weight basis.

3.3.2 Chemical Analysis of samples from the Biochemistry Laboratory (University of Namibia)

The following samples were analysed:

S_1: *Sorghum bicolor*

S_2: *Pennisetum glaucum*

S_3: *Bauhinia macrantha*

S_4: *Rhus tenuinervis*

S_5: *Vangueria infausta*

S_6: *Amaranthus petersiana*

S_7: *Citiullus Ianutus*

S_8: *Corallocarpus triangularis*

S_9: *Cuccumis metuliferus*

S_{10}: *Cucumis anguria var.longaculeatus*

S_{11}: *Grewia bicolor var. bicolor*

S_{12}: *Lapeirousia coeculea*

S_{13}: *Citrillus sp*

S_{14}: *Sclerocarya birrea*

S_{15}: *Fockea angustifolia*

S_{16}: *Pentarrtinum insipidum*

S_{17}: *Hypaene petersiana*

S_{18}: *Maeru schinzii*

S_{19}: *Ceropegia tentaculata*

All the above samples were analysed at both the Unam biochemistry laboratory and the Analytical chemical laboratory.

3.3.2.1 Iron content.

Dry ashing method was used to determine the iron content like in the previous experiment with zinc (Official Methods of Analysis of Association of Analytical communities, 2014). A 2.5 g of each sample was weighed on a triple balance and placed in a crucible and was placed in a muffle furnace at 600ºC for 20 minutes. The crucible was cooled and the ash transferred to a small beaker 100 ml, and added 10 ml of 2.0 M HCI which was stirred for 1 minute. Then 10ml of distilled water was

further added and stirred for 2 minutes. The mixture was filtered and the filtrate was collected where 2.5 ml of 0.1 M of Potassium thio cyanate (KSCN) was added to the filtrate and mixed well. The contents were placed in an atomic absorption spectrophotometer and read at 458 nm.

3.3.2.2 Vitamin C (Ascorbic acid) content

Vitamin C analysis: 10 g of fresh samples were weighed and homogenised with 100 ml of extracting solution containing 0.3 M methiopropanine (MPA) and acetic acid (1.4 M). The mixture was poured into a conical flask and stirred for 15 minutes at room temperature. The mixture was filtered to obtain a clear extract. Standard sample of 50 mg of ascorbic acid was weighed and diluted to 100 ml with MPA (0.3 M) and acetic acid (1.4 M). The mixture was poured into a conical flask and stirred for 15 minutes at room temperature. The mixture was filtered when there was a precipitation formed. Three replicates each of standard and samples were titrated with 2,6-dichlorophenolindophenol (DCPP) solution to a pink endpoint lasting ten seconds.

3.3.2.3 Antioxidant content

Analysis of the samples was carried out in the laboratories using the Official Methods of Analysis of Association of Analytical communities (2014). A gram of freshly washed sample was weighed, homogenised for 3 minutes and extracted with 10 ml of ethanol (70% w/v concentration in distilled water.). The extract was filtered using a clean muslin cloth. The extract was then centrifuged at 10000rpm for 15 minutes. An aliquot of 25µm of samples extracted with 3 ml of 2,2 diphynly-1-

picryhydrazyl (DPPH) 25mM ethanolic solution. The mixture was left in the dark at room temperature for 20 minutes and thereafter absorbance was measured at 515 nm against a blank of ethanol without DPPH radical. The results were expressed as percentage of inhibition of the DPPH radical calculated as follows: Percentage inhibition of DPPH = {1 – (Abs sample/abs control)} x 100, where Abs control is the absorbance of DPPH solution without extract.

3.2.2.4 Total phenolic content

In order to investigate the phenolic content in each sample the following procedure was carried out. Official Methods of Analysis of Association of Analytical communities (2014) were used. Extracts of the samples 300µl were added to 2.25 ml of 1; 10 Folin Ciocaltea's reagent and incubated at room temperature for 5 minutes. 2.25 ml (60 g/l) of sodium carbonate solution was added and incubated for 90 min at room temperature. Absorbance was measured at 725 nm. The total phenolic content of the fruits were expressed as Garlic acid equivalents (GAE) mg/100g of edible of the fruits. Flavonoids results were also noted down from the samples. The SPSS Version 20 software was used to analyse the results and this was done by measuring the means which was descriptive statistics of measuring the variables, contingent tables and graphs were drawn.

3.4 Collection of blood samples

In order to investigate any differences in the blood composition of the two groups the quantitative assessment and genetic variance of the haemoglobin, shape of red blood cells and quantity of immunoglobin antibodies (IgG) were carried out. Full blood cell

count which included red and white blood cell count, haemoglobin, haematocrit, mean cell volume, mean corpuscular haemoglobin, platelets, malaria blood parasites and many other variables which were not of significance to this study like mean corpuscular haemoglobin concentration and Red cell distribution width that could be noted in the blood samples were performed. Initially 200 blood samples were to be collected from all participants but due to technical problems both at collection points and in the analysis of the blood in the laboratory, a total 147 blood samples were read and analysed in both Tsumeb and Windhoek Namibian Institute of Pathology Laboratories (NIP). Blood samples were collected from the San and the other ethnic groups in the Oshikoto and Kavango regions of Namibia. The population sample of the San for blood collection was 100 participants, 50 were from Tsintsabis in Oshikoto region and the other 50 from Bravo in Kavango region. From the other ethnic groups, blood was collected from 50 participants from Tsumeb district at Otjikoto Secondary school. In Kavango the collection of blood samples from other ethnic groups was not easy. Since human blood is always considered a taboo to be given to anyone who is not a hospital official only 10 participants came forward but then the researcher had to go back and collect 60 more blood samples of the other ethnic groups for microscopic observations. In Tsintsabis the participants gathered at the San school and in Bravo the blood collection was done at Bravo school. The blood samples for abnormal haemoglobins was analysed at Lancet Laboratories in South Africa.

3.4.1 Equipment for sample drawing

For drawing blood, the following equipment was used: Vacutainer Needles, size 20G to 22g, Vacutainer glass tubes with yellow and purple tops of 4ml. The one with yellow top had Ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. A tourniquet was used to tie the arm so that the veins would be visible, methylated spirit with disinfection swabs, micropore tape, dental rolls, adhesive dressing, rubber gloves, pillow to support the arm of the blood donor and a disposal box were employed.

3.4.2 Blood collection

Before collection of blood, both parents and school authorities at Otjikoto Secondary School were sensitized and permission was granted. Participants of ages between 14 and 19 of both sexes were called in one by one. Two of 4 ml tubes were used, one was to collect whole blood with EDTA as an anticoagulant and had a purple top and the other was for serum without any anticoagulant with a yellow top. The blood serum with EDTA was mixed thoroughly by inverting the mixture towards the stopper 6 times.

All the tubes were labelled with identification code, which had all ID information written on. At Otjikoto school fifty blood samples were collected and the place was cleared of all items used. The blood samples were properly packed in the cooler boxes that had ice and were taken to NIP laboratories in Tsumeb for measurement of various blood components mentioned above. The same procedure of blood collection

was followed at the Tsintsabis and Bravo communities. The age group was between 5 – 70 years of age.

3.5 Laboratory Procedures

The blood samples were divided into three sets, one was for full blood count, the other for thick and thin smears and the one with a yellow top was for IgG analysis to be done in Windhoek laboratories. The blood samples were taken to Tsumeb Namibia Institute of Pathology (NIP) for the following parameters to be measured, full blood count using a Pentra XL80 machine that does the measurement of the blood components especially the blood cells haemoglobin and haematocrit (HORIBA medical, 2014). The blood smears and staining of both thick and thin were done at Tsumeb NIP. IgG count was done at Windhoek NIP. The one in Windhoek just gave a general output of IgG and not the various components of IgG or any other antibody. Since IgG plays an important role in Immunity against *Plasmodium falciparum* this analysis had to suffice for this study. There were no laboratories that could measure either the DNA or the haemoglobin variances (abnormalities) in Namibia. Measurement of haemoglobin abnormalities was done at Lancet Laboratories in South Africa.

3.5.1 Thick and thin smears

Thick and thin smears were made simultaneously on both ends of the slide. These smears were air-dried and then the thin smear was fixed in 100% alcohol but the thick smear was not fixed. The smears were then stained by Giemsa stain (10%) and observed under fluorescent microscope that uses UV light (Olympus U-TV 0.5 XC-

3) to observe the presence of the malaria parasite and the shape of the red blood cells (Olympus, 2014). Photos of the slides were taken for further studies, if there was any necessity for that. Contingency table and the Chi square test for the shapes of the red blood cells were drawn to see the association of the variables between the San and the other ethnic groups.

3.5.2 Full blood count using Pentra XL80

Pentra XL80 is an automatic validation machine that runs samples according to user's setting, like micro sampling, customized dilution ration, automated sample location, single screen viewing for the bar coded data and sample identification. It measures and calculates the percentages of Full Blood Cell Count (FBC), Red Blood Cell (RBC), Platelets (PLT), Haemoglobin (HB), Haematocrit (HCT), Red Cell Distribution Width (RDW) and calculated their percentages. It also calculated the quantity of Mean Cell Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC). Platelet Crit (it is the % volume of blood occupied by platelets (PCT), Platelet Distribution Width (PDW), White Blood Cells (WBC) in percentages and its differential count, Neutrophils (NEU), Lymphocytes (LYM), Monocytes (MON), Eosinophils (EOS), Basophils (BAS), Alympho Plastic Cells) (ALY) and Large Immature Corpuscles (LIC) were also analysed. Automatic sample re-run was performed immediately to see if there was an error in the readings or calculations (HORIBA medical, 2014). The samples that were bar coded were put in Pentra XL80 according to the arrangements on the racks and results were printed and collected for Descriptive statistical analysis that

measures the means, confidence of interval at 95%, and contingent tables and graphs were drawn. With respect to inferential statistical, student T- test was carried out.

3.5.3 IgG Measurements

The samples had been refrigerated at 4°C at NIP Tsumeb before they were transported to Windhoek NIP the following day. The machine Beckman Coulter Synchron LX System was used to measure the blood samples. Measurements of Immunoglobulin G in this case were used in the diagnosis of immune deficiencies and the concentration of IgG in the blood samples by turbid metric method that is the reaction of IgG combining with specific antibody to form insoluble antigen-antibody complexes.

The Synchron System dilutes the samples and dispenses sample and reagent volumes into a cuvette. It then monitors the change in absorbance at 340 nanometres. This change is proportional to the concentration of immunoglobulin in the samples, which were calculated by the system. The results were printed and collected form NIP for Descriptive statistical analysis that measures the means and confidence of interval at 95%, and contingent tables and graphs were drawn. With respect to inferential statistical, student T- test was carried out.

3.5.4 Measurement of Abnormal Haemoglobin using Biorad D-10 Instrument

New set of blood samples were analysed using the Bio-Rad D-10 instrument (BIO-RAD, 2013). Haemoglobin was measured on the High Performance Liquid Chromatography using Electrophoresis process. This was able to separate the haemoglobin variances when the machine was switched on and blood samples injected into a well with a gel. Since haemoglobin types have different electrical charges the components could easily be separated in this electrical charged field during electrophoresis. During this process an electrical current was passed through the haemoglobin of the blood samples and caused the haemoglobin types to separate at different rates and formed bands or graphs. By comparing the pattern formed with that of a normal blood sample one can see the types and the quantities of haemoglobin present in the blood samples. The results were printed and collected for Descriptive statistical analysis that measures the means and confidence of interval at 95%, contingent tables and graphs were drawn. With respect to inferential statistical, student T- test was carried out and Chi square tests were carried to check if the association was significant.

For those that are negatively charged the retention time is shorter than those that are positively charged. This way the instrument is able to diagnose and identify the abnormal haemoglobin components in the blood samples. Abnormal haemoglobin such as sickle cell haemoglobin, thalassemia haemoglobin, haemoglobin, C, D, E, M and many more rare ones can be identified by using Bio-rad D-10 automated machine.

3.6 Collection of plants for fungi growth and identification

Collection of the plants

The women volunteered to go to the bush with the researcher to collect different samples of the plants. They gave some plants that had been dried for consumption. Leaves, tubers, roots, fruits, seeds, bark and stems were identified and put in labelled plastic bags. They were taken to the laboratory for analysis of endophytic fungi, which could give some health benefits to the San and not the other ethnic groups.

3.6.1 Growth of Fungi on the staple food of both the San and other ethnic groups

Samples of plants collected were tested for the presence of fungi in the Microbiology laboratory at the University of Namibia. The food collected were mainly of the two groups, the San and the other ethnic groups staple foods. The food items collected included *Corallocarpus triangularis, Sclerocarya birrea, Citiullus Ianutus, Cucumis angora var. longaculeatus, Corallocarpus triangularis, sorghum bicolor* and *Amaranthus petersiana*. *Corallocarpus triangularis* and *Sorghum bicolor* are mainly staple food for the other ethnic groups but are being introduced to the San as part of their diet. The food samples analysed for fungi were mainly in the groups of tubers, roots, leaves, seeds and fruits that are either eaten fresh or sun dried in the open or cooked in the case of mahangu, sorghum and spinach.

3.6.2 Isolation of endophytic fungi

The samples were cut into small pieces and soaked in sterile distilled water for 15 - 20 minutes to activate the growth of fungi since some seeds and bark were too dry

(Thermo Scientific, 2014). The fungi were cultured on Potato dextrose agar (2%) which was dissolved and allowed to cool and set in petri dishes. The samples were transferred onto the agar using sterilized forceps for 15 minutes and then removed leaving the inocula behind. The inocula were spread out using a clean glass rod (cleaned in alcohol) on the agar plate. The agar plates were incubated at 37°C for 24-48 hours. A subculture was carried out for proper identification in some overgrown species to get a pure culture.

3.6.3 Microscopic observations

Photos of the pure cultures of grown fungi were taken, with slides cleaned with alcohol. Using sterilised inoculating loops the researcher transferred fungi from the plate onto a drop of water put on the slide. The slide was air dried and stained in Chrystal Violet for 5 minutes. The stain was rinsed, dried and a drop of oil was put on the slide to observe the slide at objective lens X100 using an Olympus light microscope. Different types of fungi were identified morphologically. Photos of the slides were taken for further studies.

3.7 Summary of Data analysis (Statistical analysis)

Data was recorded and analysed on IBM SPSS Version 20 software and Microsoft Excel. Presence or absence of malaria was determined among Oshiwambo, Kavango and San speaking people. Descriptive summary statistics in the form of frequency tables, charts and graphs, and cross tabulations were compiled. On quantitative variables, measures of centrality and dispersion were computed. Percentage counts

were calculated, and compared using the Pearson Chi-square statistic, one sample T-tests was carried out to the haemoglobin, IgG and red blood cells, white blood cells, iron, zinc, Vitamin C, flavourants, antioxidants contents against normal standards. Two independent sample t-tests were used to test the differences between group means for indicator variable of the San and the other groups were used to compare mean numbers of those with malaria across the sampling sites.

3.7.1 Collection of data from the hospital and Health Information Services

Nation-wide data on malaria was collected from the district hospitals, Tsintsabis Clinic and HIS (Health Information Services, Windhoek). The data was on mortality, morbidity, in-patients, out patients and hospitalised patients. In the hospitals and clinics, patients were not admitted on tribal lines and this made the research a bit difficult, as one could not tell from the numbers who were the San or the other ethnic groups. Therefore, data supplied was for patients who suffered or died from malaria.

CHAPTER 4: RESULTS

4.1 Overview

This chapter provides the descriptive data on the San people and other ethnic groups on the tolerance to malaria. The results include both the qualitative and quantitative studies carried out. The survey included conducting focus group discussions and distribution of questionnaires on the knowledge of malaria, the symptoms, the staple food of both groups, visits to the hospitals if any, the various traditional medicines taken when ill, visits by Ministry of Health officials to spray their homes with insecticides and distribution of mosquito nets. Data from Ministry of Health Information Services of in and out patients including deaths from malaria during various years in Namibia was also collected for comparison and confirmation with results obtained during the survey.

Blood sample analysis included haematological, parasitology parameters and the different shapes of the red blood cells were also investigated. Extra study was carried out on the fungal infection on the herbs and food the San eat. A qualitative survey of 200 respondents comprising of 83 San people and 117 of the other ethnic groups was carried out in the two regions (Oshikoto and Kavango). The quantitative sample was 147 for full blood cell count and IgG and 200 for microscopic observations.

4.2 Results from the survey of the Questionnaire

4.2.1 Analysis output of coded questions of other ethnic groups

The sample size of the other ethnic group participants was 117 people. This included men and women between the ages of 11 to 60 years made out of, Thimbukushu, Oshiwambo, Damara and a few Hereros who lived in these two regions.

The composition of the respondents in the other ethnic groups

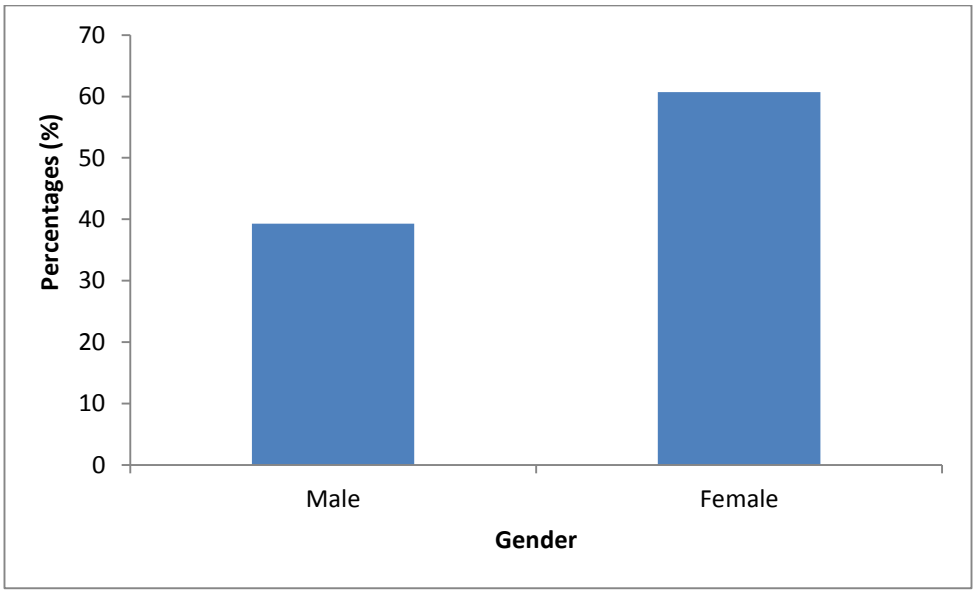


Figure 4.1 Composition of the respondents in the other ethnic groups

In the study group 60.7 % of the respondents were female and 39.3 % were male out of a total 117 participants. There were more female participants who took part in the survey compared to the males in both regions studied.

4.2.2 Respondents from the other ethnic group according to age

The survey indicated that the youngest respondent was 11 years old whereas the oldest was 60 years old, with an average age of 29. The wide range of age in this group of study will reflect the knowledge of malaria disease transmission in various groups studied including children of school going age. The older people if they are knowledgeable will educate the younger population on the disease or the school going children would make illiterate parents understand more of the disease.

4.2.3 General knowledge of malaria amongst the other ethnic groups.

The number of respondents that answered the question on the knowledge of malaria are indicated on the table 4.1 below. The coded results from the questionnaires of the other ethnic groups showed that 24.8% said malaria was caused by a female *Anopheles* mosquito and 7.7 % said it was caused by a female mosquito, 23.1 % said it was caused by mosquito, 41.0% did not know the cause or vector of malaria disease and 3.4% did not respond to the question. From the survey there was a total of 55.6% participants who had some knowledge of malaria disease compared to 44.4% who did not have the knowledge of the disease. Since the questionnaires were conducted in urban areas it was expected that urban residents should be more knowledgeable on malaria disease considering the facilities and resources about malaria available to those communities as compared to rural people. About 24.8% were able to mention the genus scientific name *Anopheles* mosquito which is the female mosquito vector that carries the malaria parasites. From the survey there was

no one who knew about the parasite (*Plasmodium falciparum*) that caused the malaria disease.

Table 4.1 Responses of the knowledge of malaria disease in the other ethnic groups

Respondent Answers	Frequency	Percentage (%)
Female mosquito	9	7.7
Anopheles female mosquito	29	24.8
Mosquito	27	23.1
Did not know	48	41.0
Did not answer the question	4	3.4

4.2.4 Symptoms of malaria by the respondents of the other ethnic groups.

From the MoHSS National Malaria Policy of Namibia [NMP], (2005), a patient with a positive laboratory test for malaria parasites usually showed these symptoms and signs of fever, rigors and chills, loss of appetite, vomiting, headache, general body and muscle pain and diarrhea. A patient with severe or complicated malaria has the following symptoms and signs, excessive drowsiness, multiple convulsions, jaundice, passing of dark urine, passing of little or no urine, and difficulty in breathing and bleeding tendencies (MoHSS NMP, 2005).

In the aabsence of laboratory facilities, the diagnosis of malaria is based on the symptoms and signs, which can be confirmed by rapid test at the clinic. In this study

the following results were obtained using the Questionnaire that was completed by respondents of the other ethnic groups.

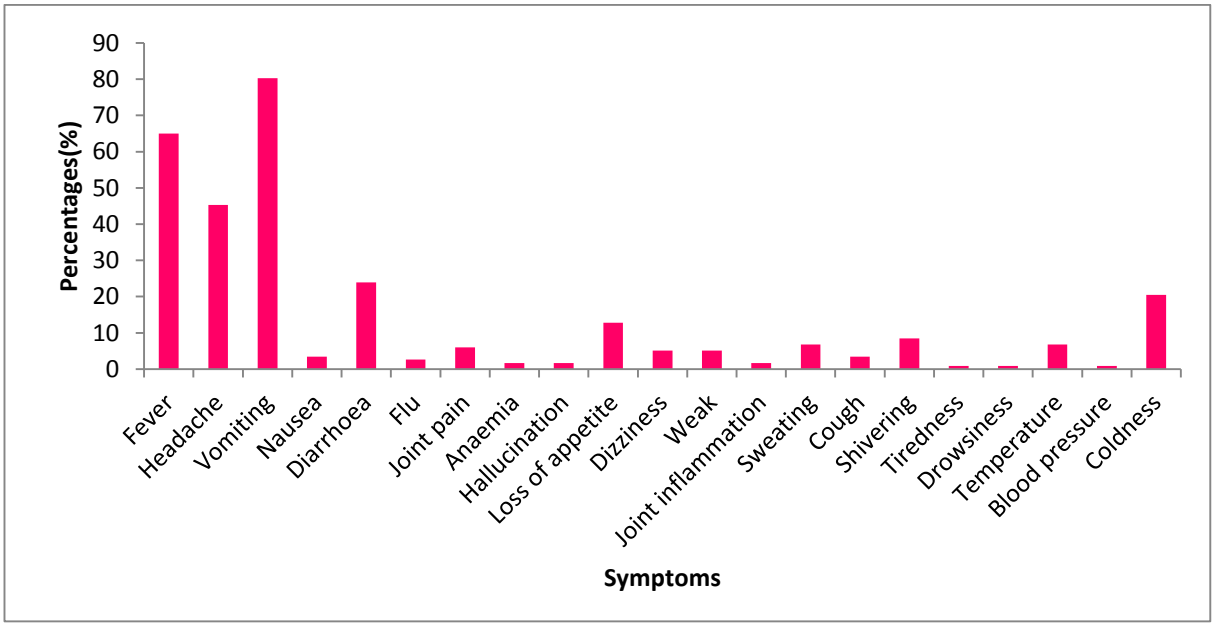


Figure 4.2 The knowledge of the malaria symptoms by the respondents of the other ethnic groups

The symptoms of malaria mentioned during the study by the other ethnic groups were 80% recorded vomiting whilst 20% did not. Fever had 65% respondents who knew the symptom compared to 35 % who did not. Only 45% recorded headache as one of the symptoms with 55% who did not. Diarrhoea and coldness were recorded by 24% and 22% respondents respectively. (The rest of the figures are in the tables in the appendix number 2). The most common symptoms indicated by the graph were mainly vomiting, fever, headache and diarrhoea. The rest of the symptoms did not

feature a lot from many respondents. This was an indication that the other ethnic groups had knowledge of the symptoms of malaria disease.

4.2.5 The number of people that suffered from malaria amongst the other ethnic groups.

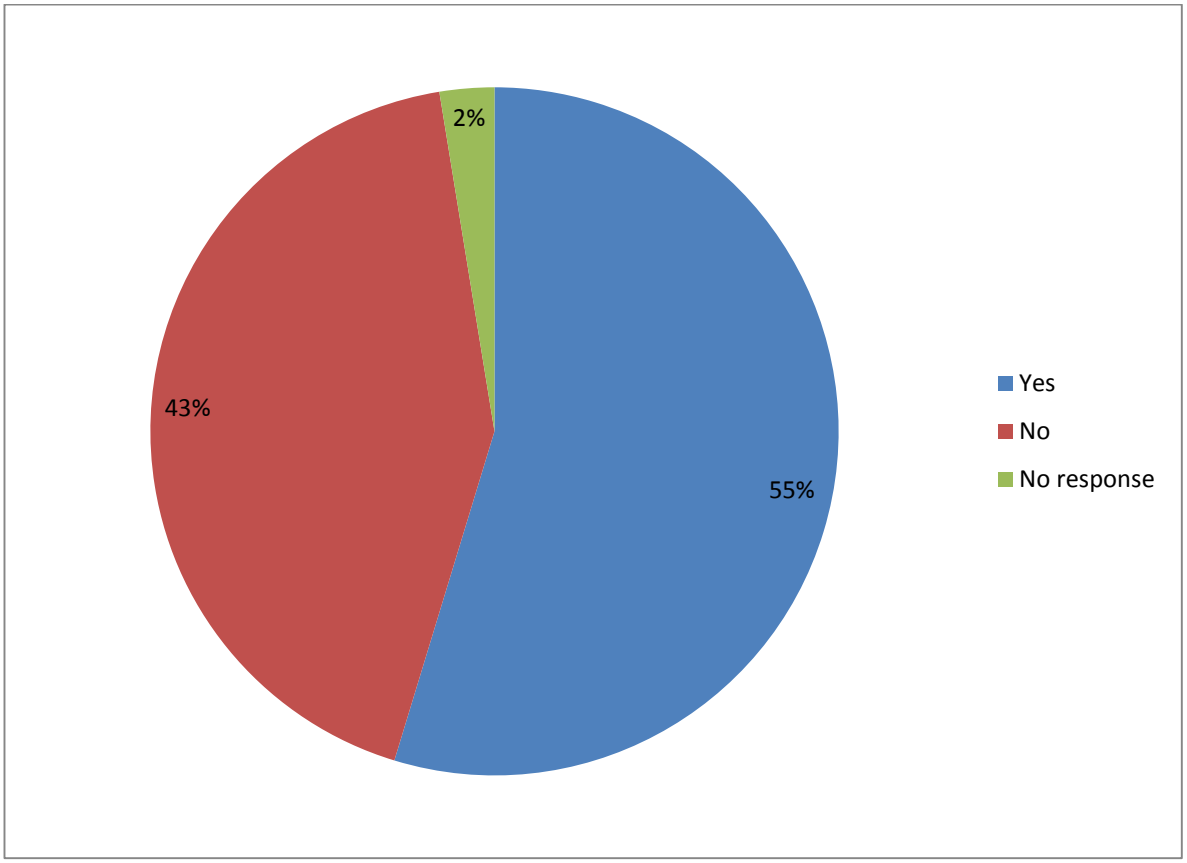


Figure 4.3 Number of respondents that suffered from malaria amongst the other ethnic group

It is seen that 55 % of the respondents suffered from malaria with 43 % who did not suffer from malaria whilst the rest did not respond to the question. The two percentages from the survey indicate that there is still more monitoring of the disease that is required on the transmission of malaria parasites.

4.2.6 The total number of respondents of the other ethnic groups that visited the hospital or clinic.

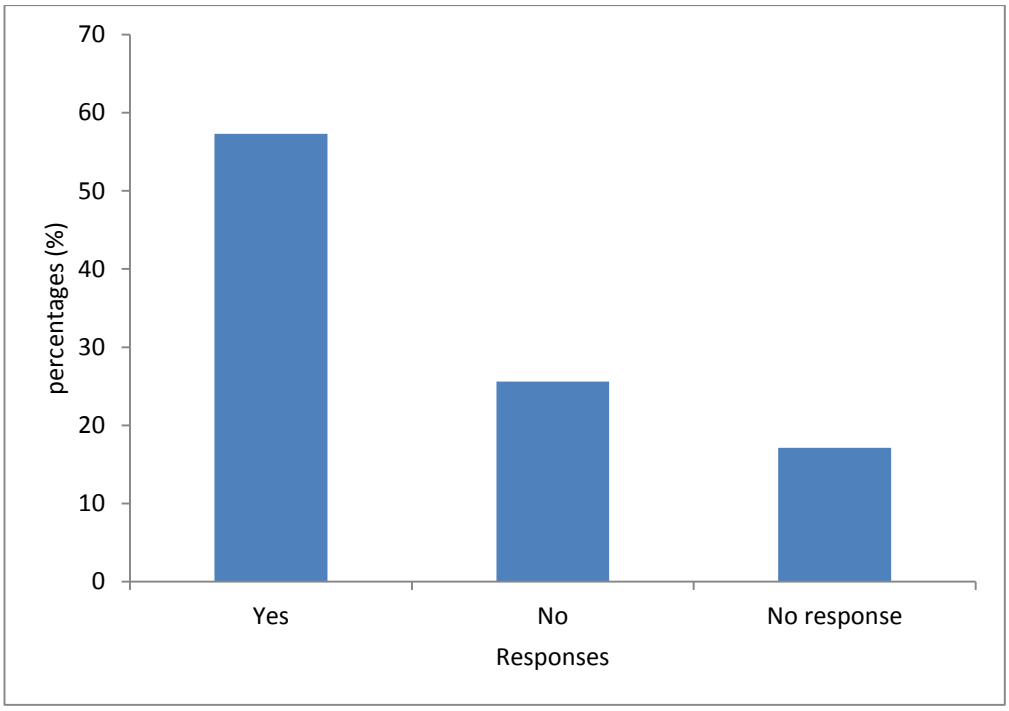


Figure 4.4 The total number of respondents that visited the hospital or clinic from the other ethnic groups

Figure 4.4 shows that 57 % of the respondents went to the hospital or clinic and 25 % did not visit the hospital or clinic and the rest did not attempt to answer. One can tell from the outpatient data that many people went to the hospital after the symptoms of malaria were found to be present. (MoHSS, 2011) see annex 1.

4.2.7 Members of the family of other ethnic groups who suffered from malaria

Majority of the respondents had 2 family members who suffered from malaria as shown in the table 4.2 78.6% of the other ethnic groups' family members suffered

from malaria whilst 21.4% did not have any member of the family that had suffered from malaria.

Table 4.2 Members of the family of the other ethnic groups who suffered from Malaria

Family Members who suffered from Malaria	Frequency	Percent
1	12	10.3
2	38	32.5
3	13	11.1
4	6	5.1
5	9	7.7
6	2	1.7
7	5	4.3
10	4	3.4
23	2	1.7
25	1	0.9
Yes	92	78.6
No	25	21.4
Total	117	100.0

4.2.8 Visit to the hospital or clinic of the members of the families of the other ethnic groups who went for malaria treatment.

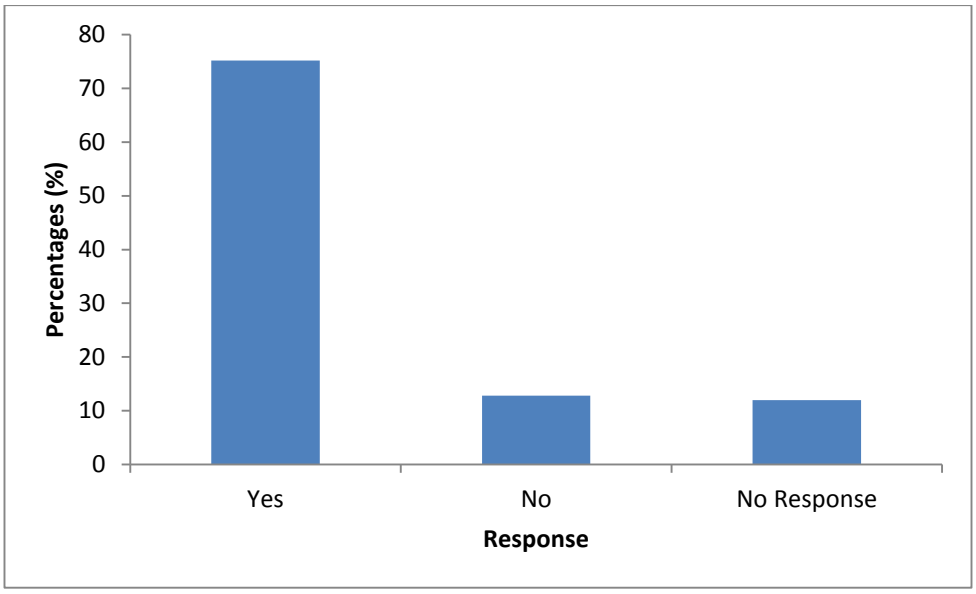


Figure 4.5 The percentages of respondents that visited the clinic for malaria treatment from the other ethnic groups

Of these respondents 75 % went either to a hospital or clinic for treatment of malaria, 13 % did not visit either the clinic or hospital and 12 % did not respond to the question. The majority from the other ethnic groups went to the hospital.

4.2.9 Relatives who died from malaria from the other ethnic groups

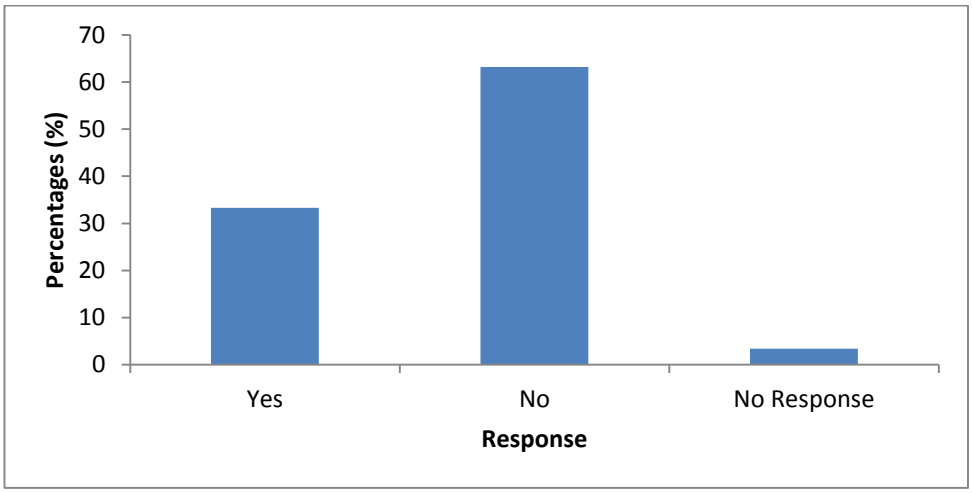


Figure 4.6 Relatives who died from malaria from the other ethnic groups

The results show that 33% of the respondent's relatives had died of malaria and 63 % did not die from the disease. The remaining 3% did not respond to the question. The majority of the participants did not have relatives that died from malaria.

4.2.10 The other ethnic group members that took traditional medicine for the treatment of malaria

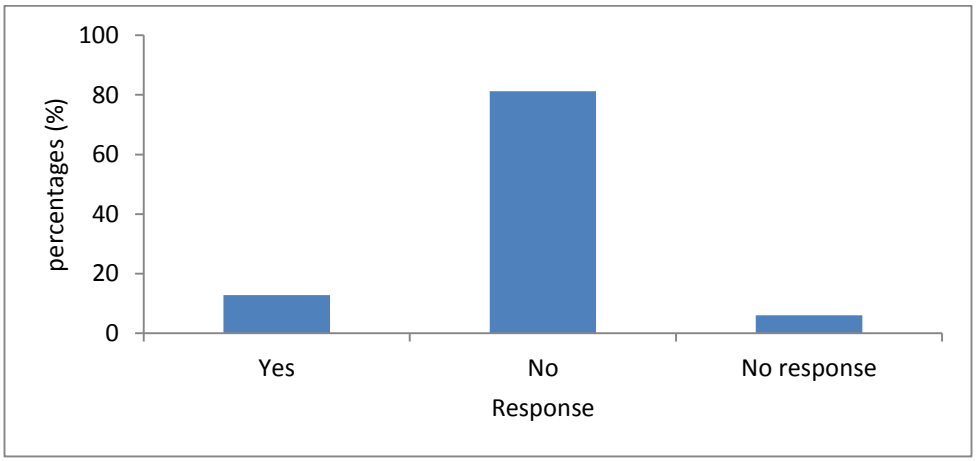


Figure 4.7 Other ethnic groups members that took traditional medicine to cure malaria

The results reflected only 12 % of the other ethnic groups that took traditional medicine, 81 % claimed to have never used traditional medicine to cure malaria and 6% did not respond to the question. Many of the respondents reported that they did not believe in traditional medicine because malaria could only be cured at the hospitals and yet others said they were Christians and will not take any traditional medicine. One would assume that the campaign from MoHSS government officials has been successful in bringing malaria awareness to the nation and the drugs of choice were being administered effectively.

4.2.11 The different types of traditional medicines taken to cure malaria.

The respondents of the other ethnic groups rarely took traditional medicines as a means of curing the malaria disease due to improvement of medical technology which has taken the globe by storm. The few that have remained loyal to their cultural roots used medicines such as Iidimba, Elephant dung, Eucalyptus leaves, Omumborobanga leaves, Bitter-bush leaves, Roots of a Chichirica flower, Aloe Vera, Tukulula and Sangani.

4.2.12 The different traditional medicines that were effective

From the few respondents that used the traditional medicine two of the three respondents that used Elephant dung and Eucalyptus leaves confirmed that it was effective whilst the one indicated that they were not effective. The respondent that

used Omungundi leaves indicated that these leaves were not effective. However the respondents that used Bitter-bush leaves, Aloe vera, Sangani, Tukulula and Roots of the Chichirica flower confirmed that these medicines were very effective, with medicines such as the Sangani curing respondents from severe headaches. Within the fifteen respondents five did not comment on the effectiveness of traditional medicines.

4.2.13 Malaria disease in the communities of the other ethnic groups.

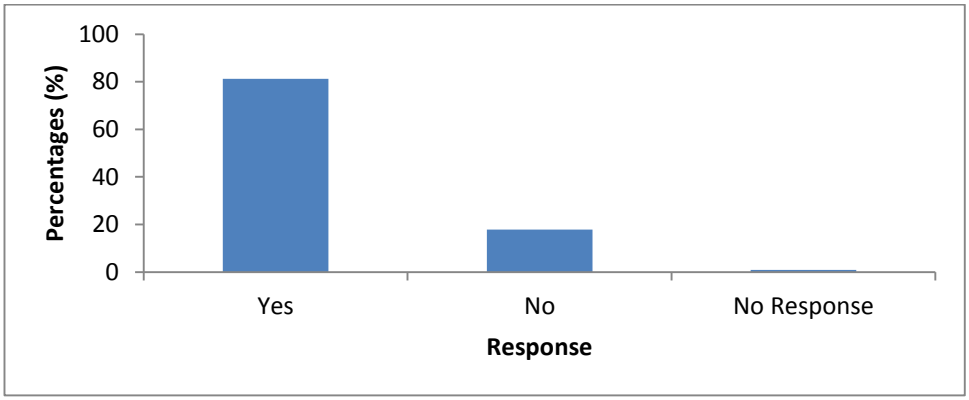


Figure 4.8 The percentage of the malaria disease in the communities of the other ethnic groups

The majority of the respondents (81%) showed that malaria was common in their community, while only 18% said malaria was not common in the area. About 2% of the respondents gave no response.

4.2.14 Homes of the other ethnic groups sprayed with insecticides by government officials

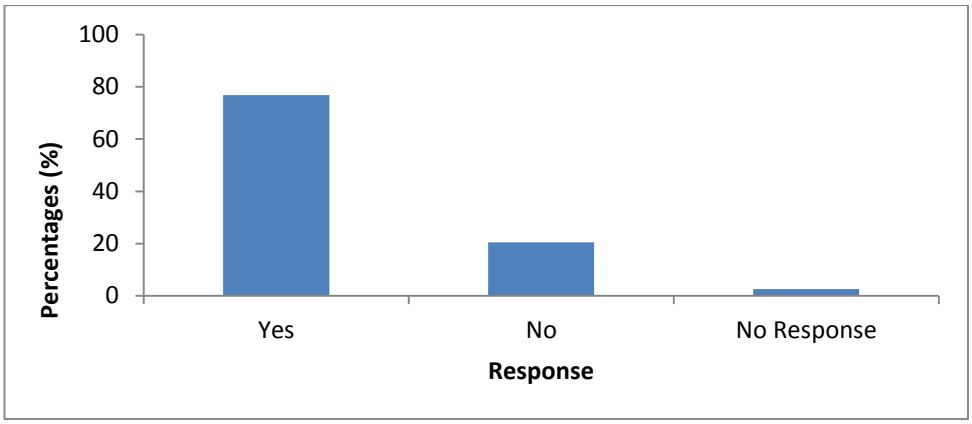


Figure 4.9 The percentage of homes of the other ethnic groups sprayed with insecticides by government officials

The respondents that reported that their homes were sprayed with insecticides by the government officials were 75%, those whose homes were not sprayed were 17 % and about 8% did not respond to this question.

4.2.15 The number of times the government official spray the homes of the other ethnic groups

The number of visits by government officials to spray their homes varied between one to twelve times in a year. The number of times the government officials visit an area varies between 1or 2 times in a year depending on the prevalence of the disease (MoHSS, 2009).

Table 4.2 indicates the number of times the government official sprayed the homes of the other ethnic groups

Number of times sprayed	Frequency	Percent
1	75	64.1
2	18	15.4
3	4	3.4
4	1	0.9
7	1	0.9
12	1	0.9
Total Yes	100	85.5
Total No	17	14.5
Overall Total	117	100.0

4.2.16 The percentage of respondents of the other ethnic groups provided with mosquito nets.

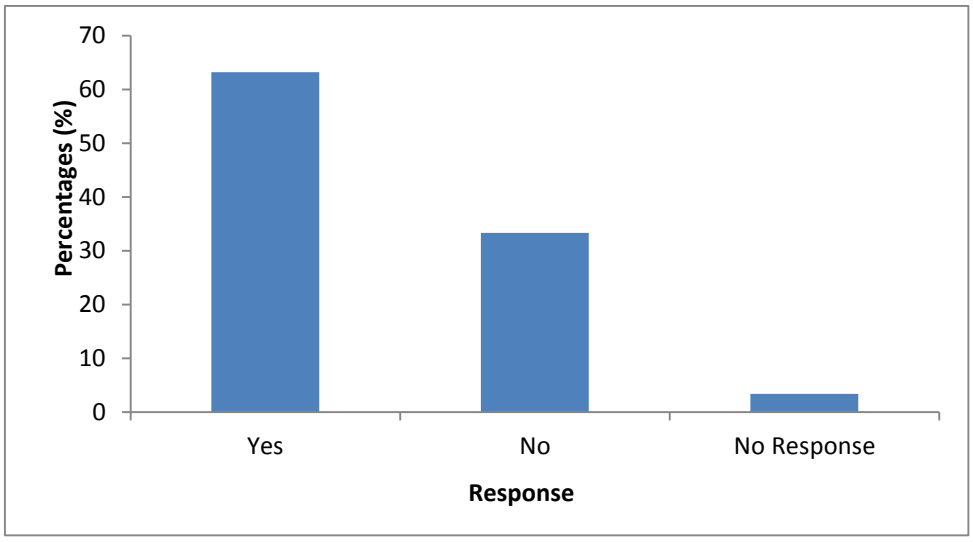


Figure 4.10 The percentage of the respondents of the other ethnic groups provided with mosquito nets

As shown in figure 4.10, 63.2 % of the respondents indicated that they had been provided with mosquito nets by the government whereas 33.3% said they had not. The remaining 3.4% did not give a response. The higher percentage of the respondents that received mosquito nets is a good sign that Namibia is in the forefront in the elimination of malaria disease in the nation.

4.2.17 Efforts being made to clear away breeding ground for mosquitoes

The respondents efforts made in the community to clear away the breeding place for mosquitos was to keep their surroundings clean by draining stagnant water pools, cutting of tall grass, weeding and organizing cleaning campaigns. Settling far from water points to avoid mosquito bites was number one key measure in malaria prevention.

4.2.18 The type of staple food in the community

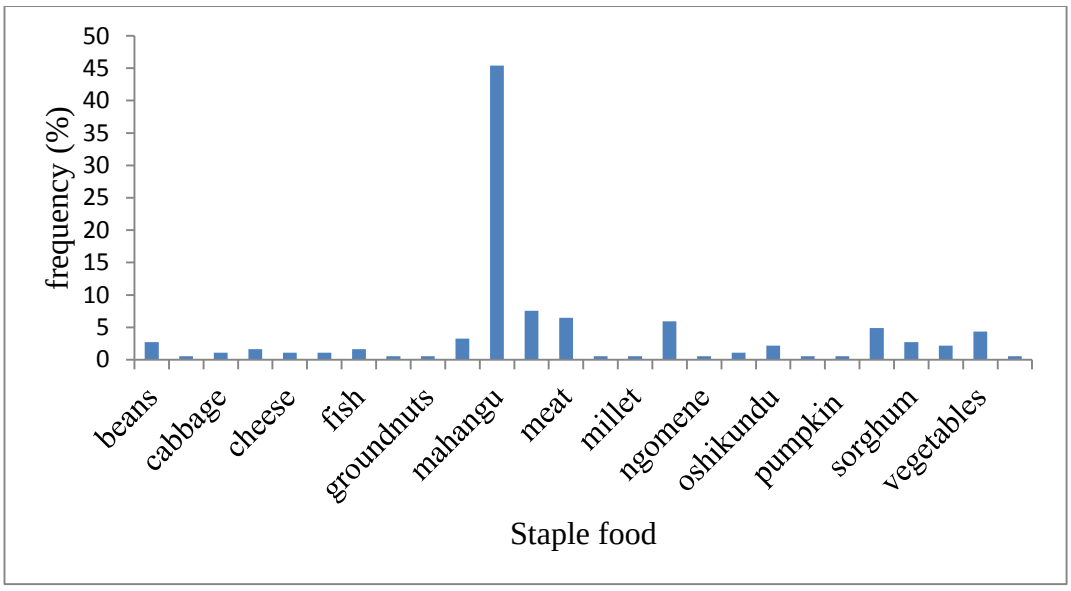


Figure 4.11 The type of staple food in the community

The majority of the respondents ate Mahangu as their staple food followed by maize meal and meat. The staple food mentioned, ranged from porridge with vegetables, Mahangu, porridge with mutete, maize, beans, fish, cabbage, meat, pasta, fruits, bread, chicken, Mopani worms, spinach, oshikundu, ngomene, rice, groundnuts, wild spinach, milk, sorghum, ontaku, omaere, shima porridge, with pumpkin leaves, millet and bush food.

4.3 Focus group discussion carried out with the San

A focus group conducted amongst the San both at Tsintsabis and Bravo indicated that the San had no knowledge of what malaria was. The discussion participants were between the ages of 30 to 80 years old.

The San's responses during the discussions concerning the malaria symptoms were vague despite the interpreter trying to give leading answers to fever and headache but the symptoms reported could have been those of AIDS, TB or Flu. Majority of the San (98%) used traditional medicines as a form of general therapy. The San do not really think western medicine is more effective than their own diverse medicinal practices. The names of traditional medicine cited were elephant dung, eucalyptus leaves, omumborombega leaves, omughudi leaves, chichirica flower root, aloe vera, takula sangana root, loro leaves, habub roots, labu, cayenne, hardkool tree leaves, Abubt/abs and ≠unixa/ushu.

The efforts of the San to clear the breeding ground of the mosquitoes are at a minimal because these people have no knowledge of the malaria disease or how it comes about. As shown from the figures below, the San are nomadic and still living in old types of settlements that have no use for mosquito nets and spraying. The group the researcher studied at Bravo and Mangetti Post are still living in huts made of sticks and some with grass and plastic as shown in the Figures 1.6 and 1.7. The group from Mangetti post has moved away from Tsintsabis as they are resisting change in their lifestyle. Since 2009 when the study was started the same group had moved three times and the last time in 2013 they had moved to a different area due to a death of an elderly man in one of the families.

It was discovered that the San first treated their patient with traditional medicine before they took the patient to the clinic if the illness was persistent like TB and AIDS which are now on the rise amongst the San people. Those in Bravo no longer take their patients to the nearest hospital or clinic which is Nkurunkure 90km and Tsintsabis 30km away respectively because of lack of transport. The San relied on traditional medicines to treat their patients but many of their patients are dying of TB and HIV/ AIDS (Jager, Prinsloo & Joubert, 2010). Out of 83 San respondents all had not suffered from malaria though they had symptoms of fever and headaches. These symptoms were not confirmed clinically to have been associated with the malaria disease.

The San people eat leaves, tubers, fruits and roots of the following plants; *Pentarrtinum insipidum, Ceropegia leucoteania, Hyphaene petersiana, Amaranthus*

petersiana, Vangueria infausta, Fokea angustifolia, Maeru schinzii, Lapeirousia coeculea, Sclerocarya birrea, Grewia bicolor var. bicolor, Bauhina macrantha, Corallocarpus triangularis, Citrillus sp, Citiullus Ianutus and *Pennisetum glaucum.* In addition, they eat game meat which they hunt. The San people do not take water for several days but this is compensated by their foods that have high moisture content.

4.4 San food nutrient Analysis

Interest was in the micro nutrients found in the plant food of the San. The results of the following components zinc, iron, vitamin C, antioxidants, flavonoids and moisture have been illustrated below showing their mean contents in the various foods analyzed.

The assessment of different micro elements in the San people's food was done at the Analytical laboratory in Windhoek and the University of Namibia Biochemistry laboratory.

Table 4.3 Recommended Daily Allowance

Extracted from internationaldrugmart.com (2003)

Men

Age	11- 14	15 - 18	19- 24	25- 50	+51
Vitamin C	50	60	60	60	60
Iron	12	12	10	10	10

Zinc	15	15- 18	15	15	15

Women

Age	11-14	15-18	19-24	25-50	+51	Pregnant	Lactating (first 6 months)	Lactating (second 6 months)
Vitamin C	50	60	60	60	60	70	95	90
Iron	15	15	15	15	10	30	15	15
Zinc	12	12	12	12	12	15	19	16

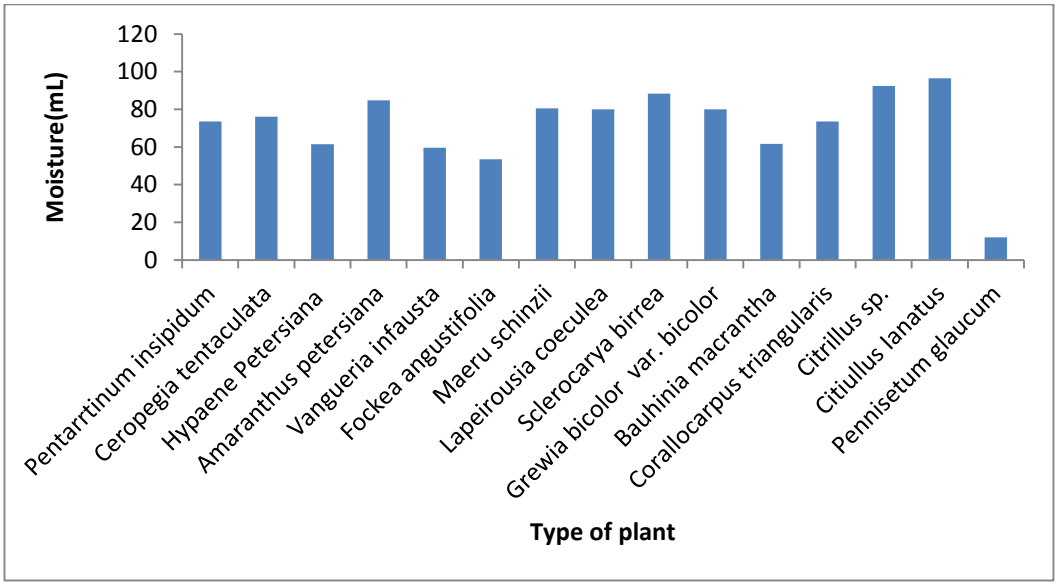


Figure 4.12 Moisture content in the foods

In Figure 4.12. With an exception of *Pennisetum glaucum*, all the San peoples food analyzed had moisture content over 50% with *Citiullus Ianutus* having the highest content at about 96% and *Citrillus sp.* at about 92%.

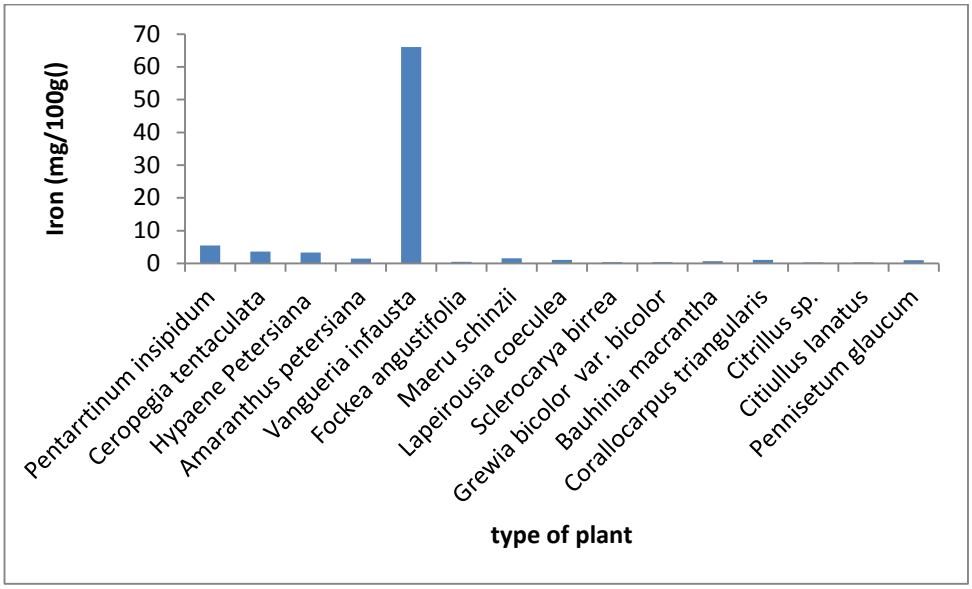


Figure 4.13 Iron content in the nutrients

The iron content was highest in *Vangueria infausta* with 66 mg/100g. The rest of the food components had less than 10mg/100g.

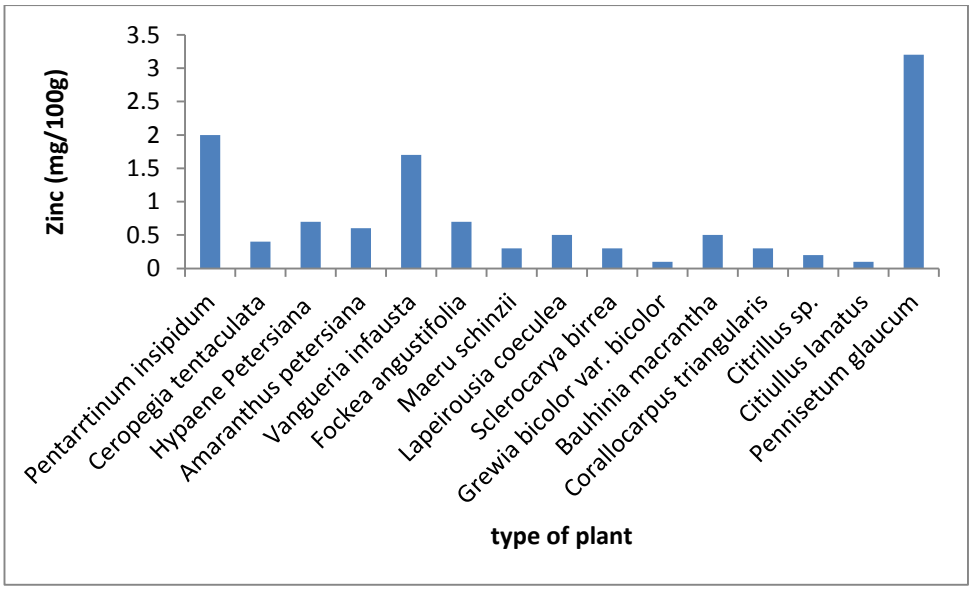


Figure 4.14 Zinc content in the nutrients

Figure 4.14 indicates that zinc is found in very minute concentrations in most food samples analysed. The highest content was found in *Pennisetum glaucum* with 3.2mg/100g, followed by *Pentarrtinum insipidum* which had 2mg/100g and *Vangueria infausta* with 1.7mg/100g and the majority of the food samples had a concentration of less than 1 mg/100g.

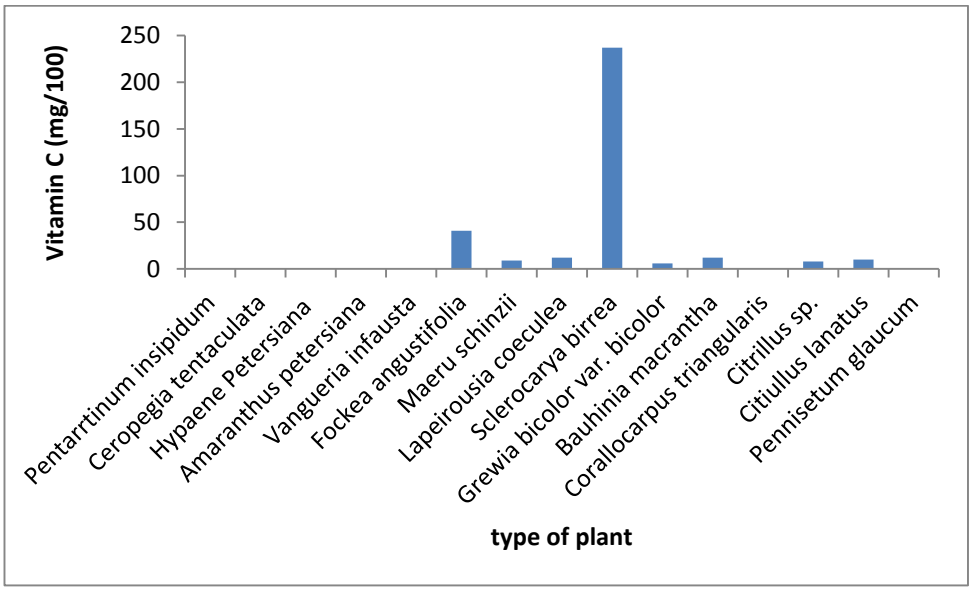


Figure 4.15 Vitamin C in the nutrients

Vitamin C content was found to be highest in *Sclerocarya birrea* fruit which had 237mg/100g, followed by *Maeru schinzii* with 41mg/100g. The other food samples had very little vitamin C in them and in a few samples, vitamin C could not be read. The vitamin C content found in *Sclerocarya birrea* is higher than the normal requirement given by Internationaldrugmart (2003) values. The San peoples food mean value is slightly higher indicating a good level of the presence of vitamin C needed for their immune system.

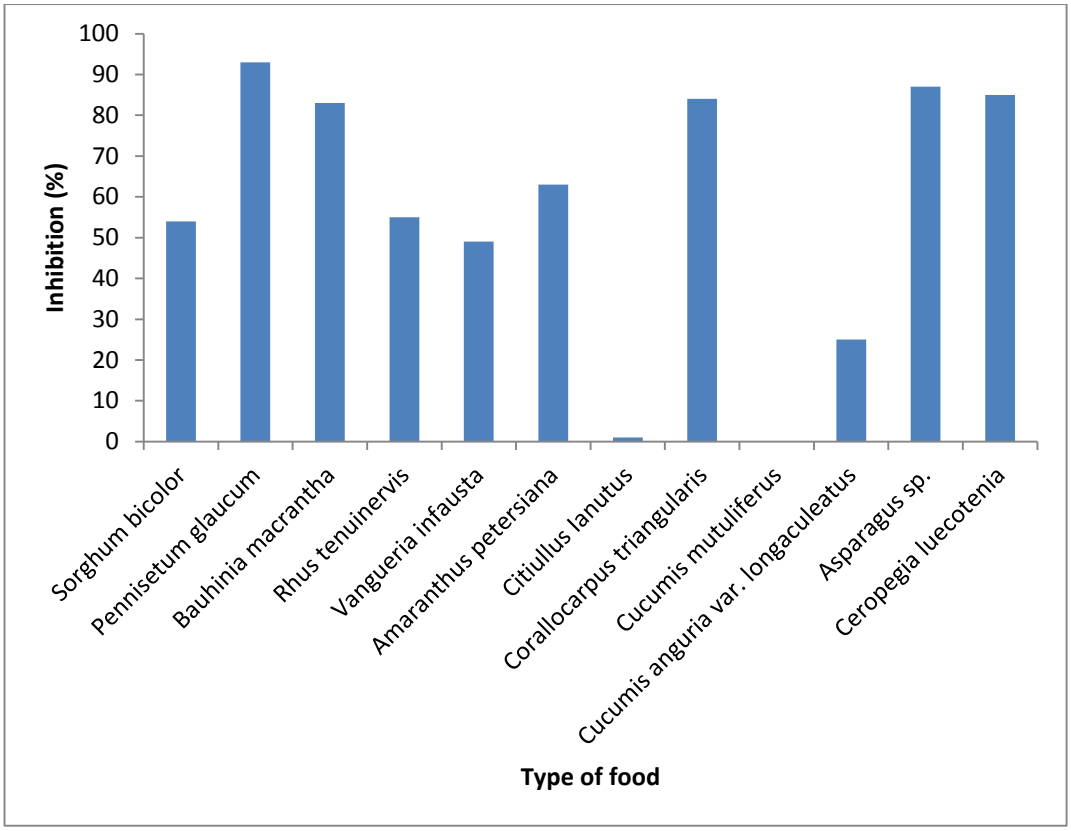


Figure 4.16 The antioxidants in the nutrients from various San foods

The figure shows the amount of antioxidant that can fight or inhibit actions of free radicals in the body. All the food samples show high percentages of antioxidants inhibition factors except *Cucumis metulifierus* and *Citiullus Ianutus* having 1 and 0 % antioxidants inhibition factors respectively. The results of this study show that the food that the San eat are rich in antioxidants.

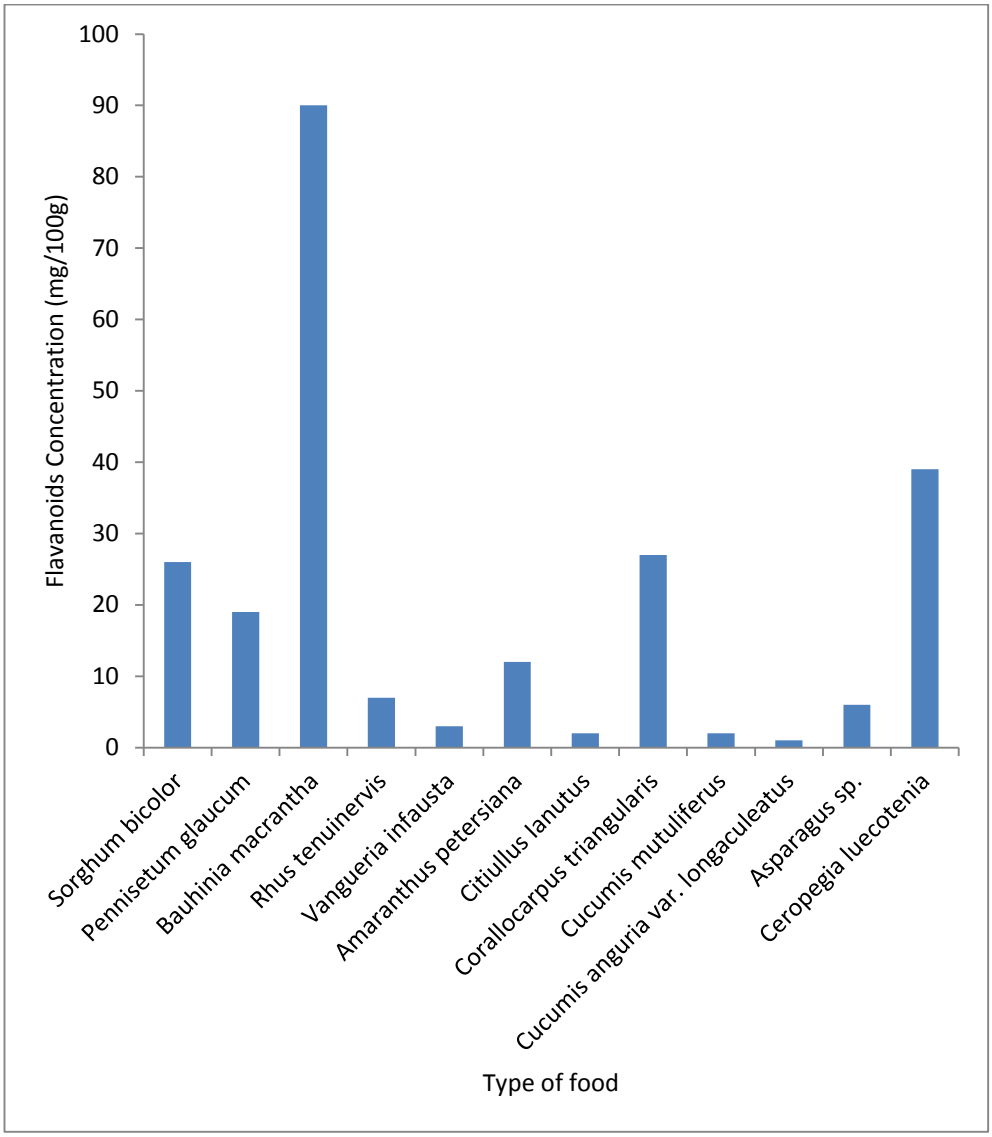


Figure 4.17 Phenolic Substances in the nutrients Flavonoids.

From figure 4.17, *Bauhinia macrantha* had the highest amount of flavonoid 90mg/100g, *Ceropegia luecoteania*, *Corallocarpus triangularis*, *Sorghum bicolor*, all had a concentration of 39mg/100g, 27mg/100g, and 26mg/100g flavonoid respectively. The remaining food samples had concentrations that were lower than 20mg/100g.

4.5 Microscopy Results

The data below was observed using the thick and thin blood smears to determine the existence of malaria parasite, the existence of spikes on the surface of the red blood cells and the presence of haemoglobin C. These are factors that contribute to a person's immunity to malaria. The Chi-square test was carried out to test the relationship between the Groups of people and shapes and structure of the blood cells and haemoglobin. This plays a significant role in comparing immunity among the two groups.

4.5.1 The existence of malaria parasites

Figure 4.18 indicates the group of the San studied (100%) tested negative for the existence of the malaria parasite in the blood streams and only 7% of the other ethnic group tested positive.

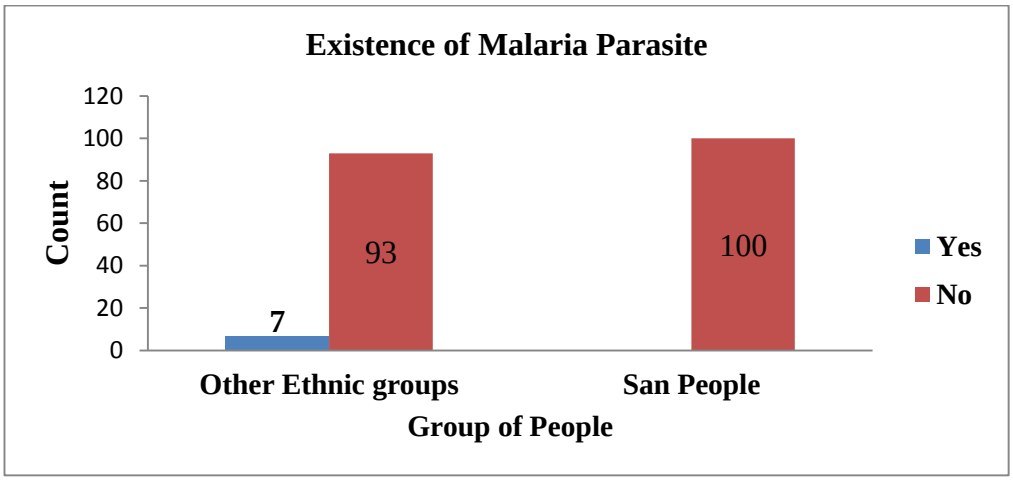
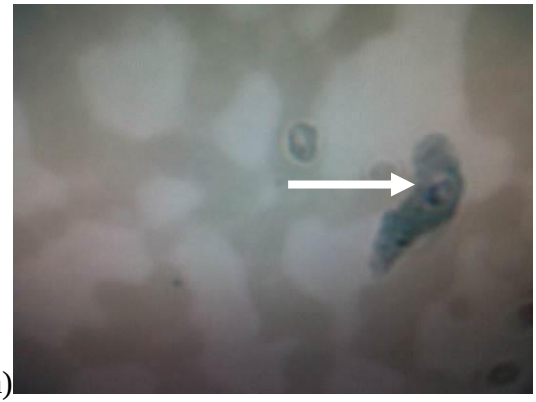


Figure 4.18 Existence of the Malaria Parasite

(a) 

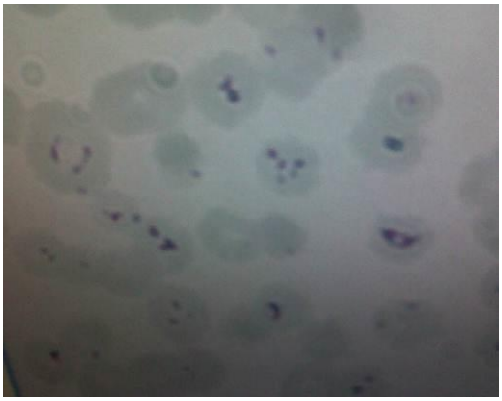
(b) 

Figure 4.19 (a) and (b) different developmental stages of malaria parasite.

(a) Malaria parasite: arrow pointing at *P. falciparum* gametocyte from the other ethnic groups.

(b) Different stages of the parasite: *Plasmodium falciparum* gametocyte from the other ethnic groups

4.5.2 Shapes of Red Blood Cells

Figure 4.20 below indicates the total count of both the normal and abnormal shapes of the red blood cell in the San and the other ethnic groups. In this study the San had

a high number of abnormal RBC (82) compared to the other ethnic groups (21). The other ethnic groups had a high number of normal RBC (79) compared to the San people (18).

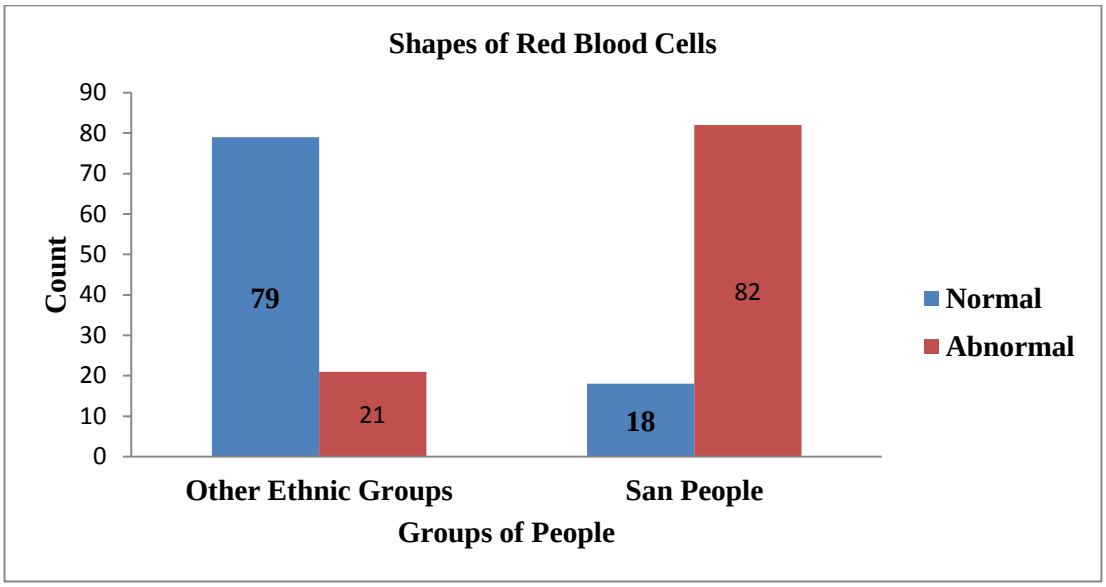


Figure 4.20 The shapes of the red blood cells of the San and the other ethnic groups

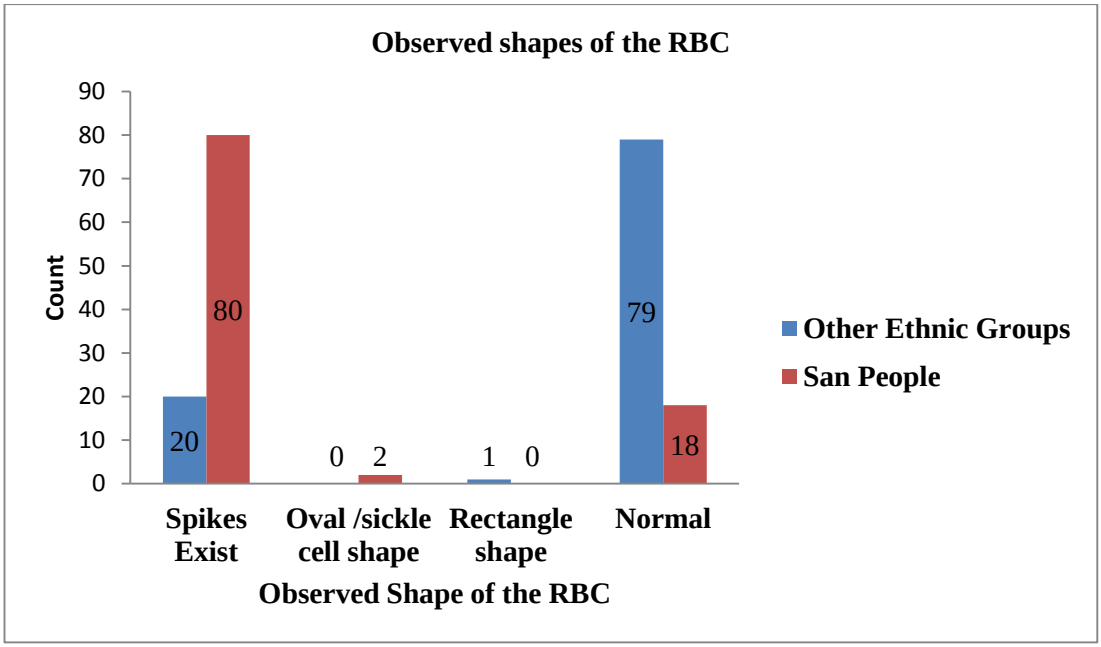


Figure 4.21 The observed shapes of the RBC

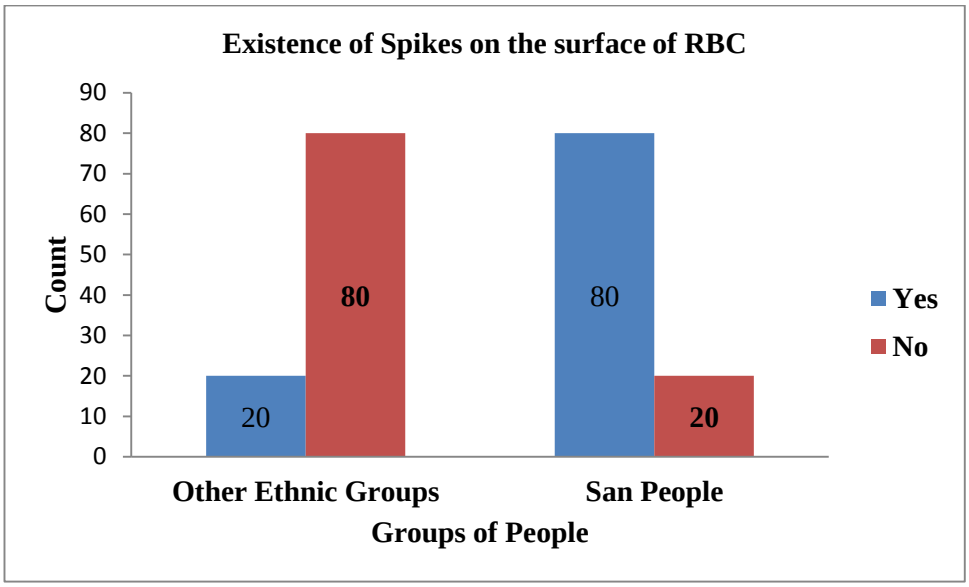
The shapes of the red blood cells in both groups were classified as normal and abnormal cells. The researcher observed that the abnormal shapes were existence of spikes on the surface, oval shaped and sickle cell shaped cells in the San blood samples. In the other ethnic groups blood samples had some spiculated cells and other irregular shaped red blood cells. In the process, the presence of HbC was also observed.

4.5.2.2 The existence of spikes on the surface of the red blood cell of the San and other ethnic groups

The existence of spikes was particularly investigated because it was the outstanding abnormalities on the surface of the red blood cells of the San people see fig. 4.21.

Out of the 100 San people blood samples, 82 were abnormal and out of the 82 abnormal cells, 80 had spikes on the surface of the red blood cells. The other two abnormal cells were oval and sickle cell shaped red blood cells amongst very few normal RBC see fig 4.21. Further, out of the 100 blood samples of the other ethnic group, there were 21 abnormal blood samples 20 of which had spikes on the surfaces of the red blood cells whilst only one had an irregular shaped RBC. There were phenotypic variations in the San red blood cells which most likely as a result of genetic influences.

Figure 4.22 The percentages of the existence of spikes on the Red blood cells



A chi square test of association was run to test if existence of spikes on the red blood cells of the San does confer their immunity against the malaria parasites.

H_o; The existence of spikes on the red blood cells of the San does not confer their immunity against the malaria parasites.

$H_{a;}$ The existence of spikes on the red blood cells of the San does confer their immunity against malaria parasites. α; 0.05

Decision Rule; reject H_o if P value < alpha value

Conclusion; we observed a p value of 0.000 which is less than 0.05 and therefore we reject our null hypothesis and conclude that the existence of spikes on the surface of

the red blood cells of the San does confer their immunity against the malaria parasites. Table 4.4 is a summary of the chi square results.

Table 4.4 Summary of chi square results for existence of spikes on the RBC of the San people.

Variable	Chi Square Value	P Value
Existence Of Spikes	72.000	0.000

4.5.2.3 The Presence Of Haemoglobin C in the RBC.

The presence of haemoglobin in the San people was a unique phenomenon that was observed in the course of the study. Therefore a new hypothesis was created and tested using the chi-square test of association.

H_o; The genetic deformation of HbC in the red blood cells of the San does not confer their immunity against the malaria parasites.

H_a; The genetic deformation of HbC in the red blood cells of the San does confer their immunity against malaria parasites. α; 0.05

Decision Rule; reject H_o if P value < alpha value

Conclusion; we observed a p value of 0.002 which is less than 0.05 and therefore we reject our null hypothesis and conclude that the presence of HbC in the red blood cells of the San does confer their immunity against malaria parasite.

Figure 4.23 indicates that 47 of the San did not show haemoglobin C in their red blood cells and 69 of the other ethnic group did not have haemoglobin C. 53 of the San did show the presence of haemoglobin C and only 31 of the other ethnic groups had haemoglobin C.

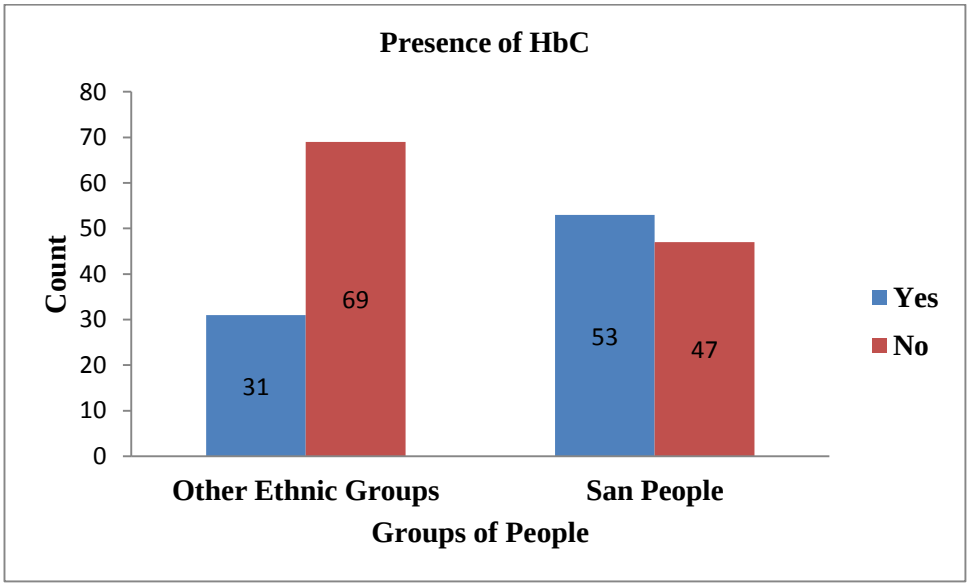


Figure 4.23 The count of the presence of HbC

Table 4.5 The summary of the chi-square results on the presence of HbC in the RBC of the San people.

Variable	Chi-Square Value	P Value
Presence Of Hbc	9.934	0.002

The figures below are some of the microscopical results of the normal and abnormal RBC, including the presence of haemoglobin C.

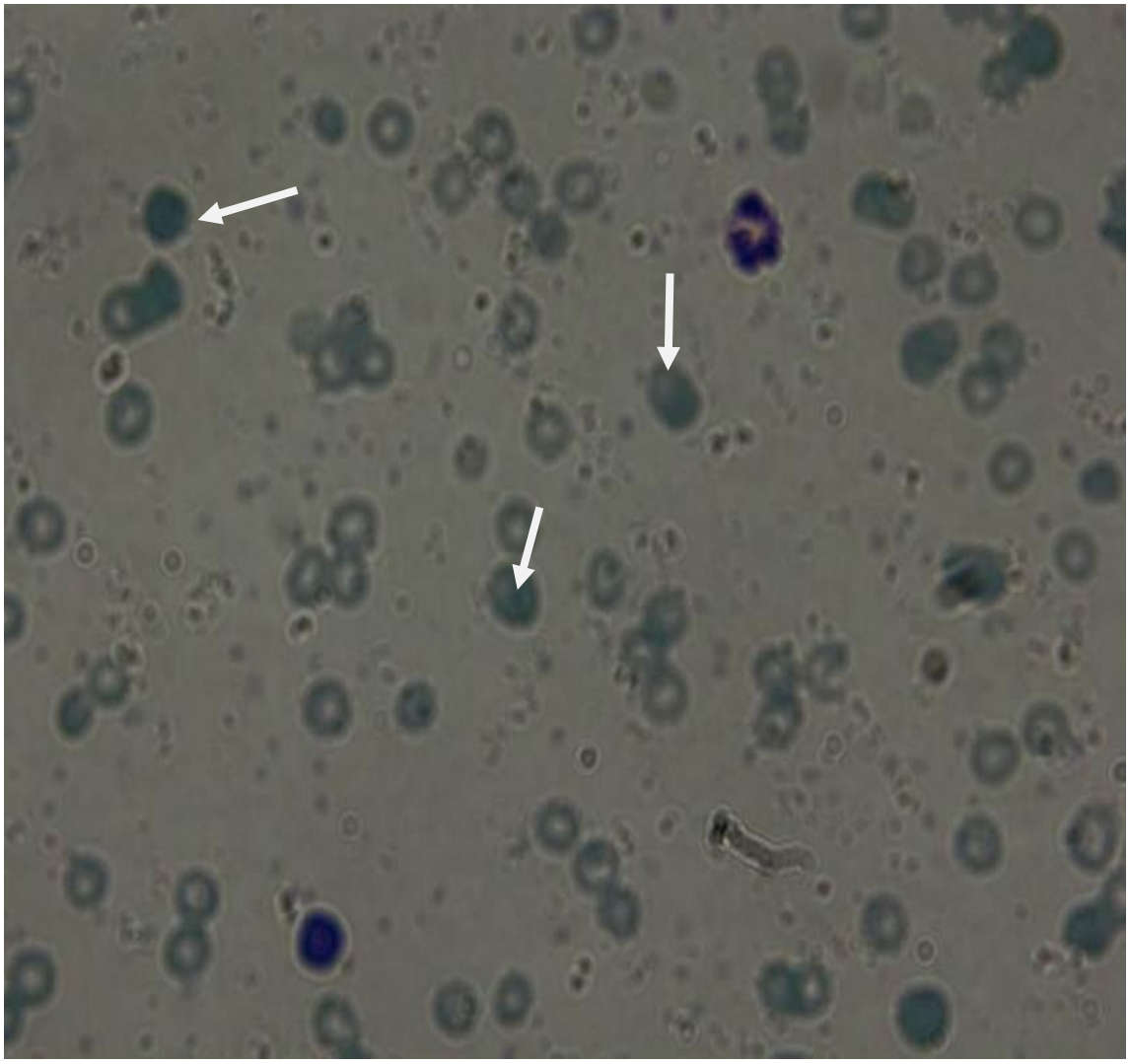


Figure 4.24 HBC and normal red blood cells of the San people.

(The arrows are pointing at normal red blood cell as well as HBC).

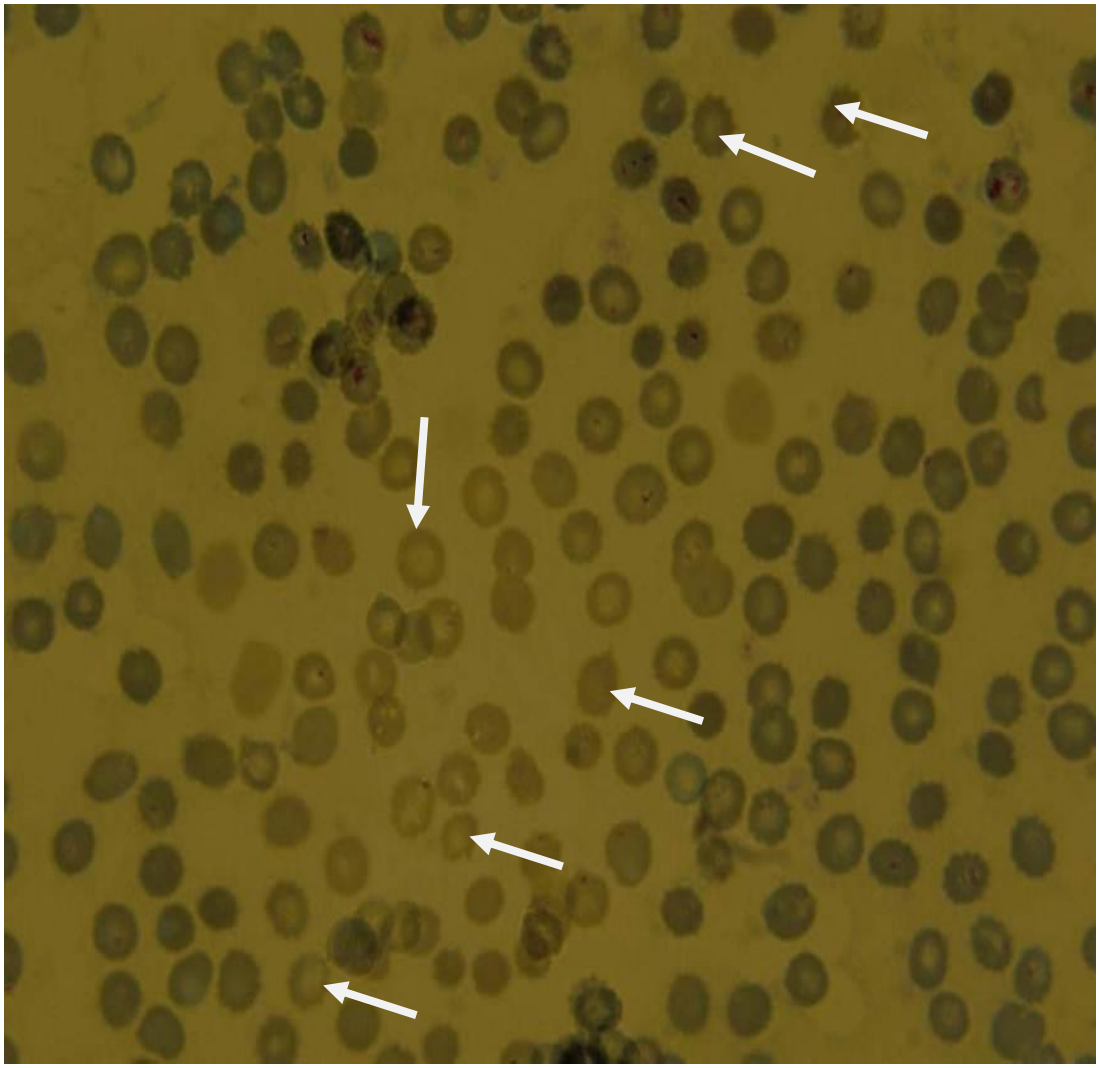


Figure 4.25 The spiculated surface of the red blood cells and Haemoglobin C in the San people.

(The arrow points at haemoglobin C and speculated cells).

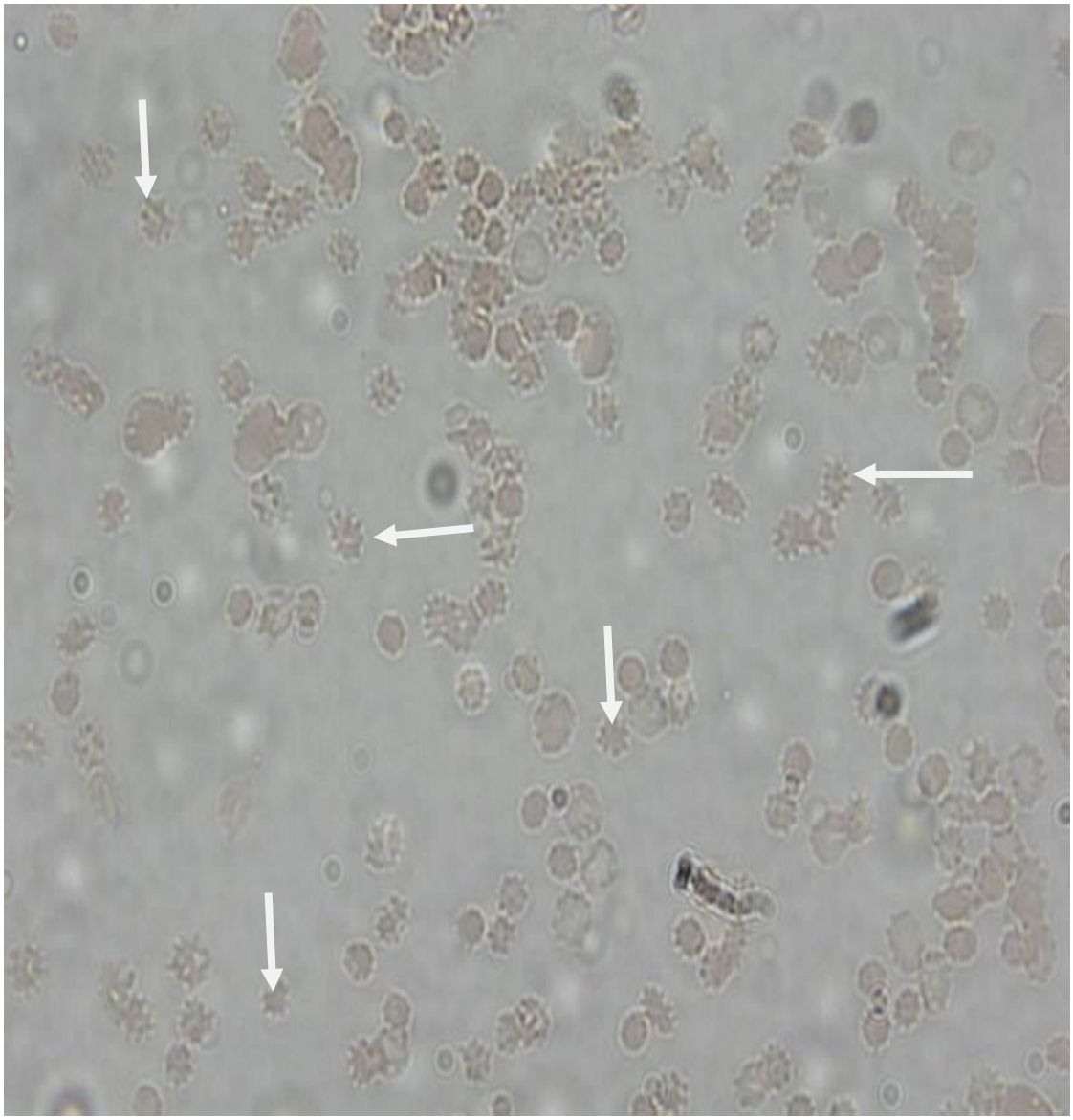


Figure 4.26 Abnormal Red blood cells with spikes from the San blood sample

(Abnormality indicated by arrows).

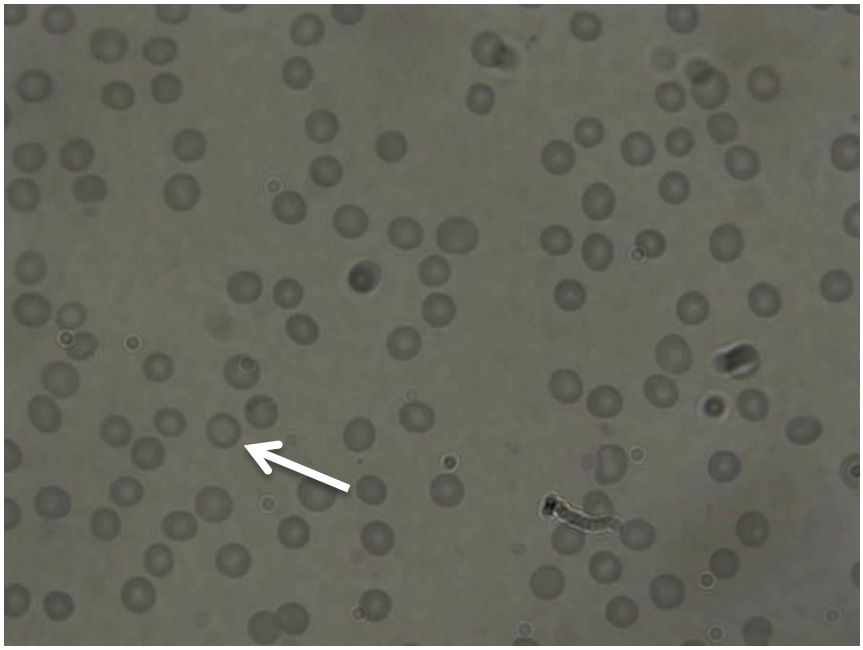


Figure 4.27 About 99 percent of red blood cells with HbC of the San people.

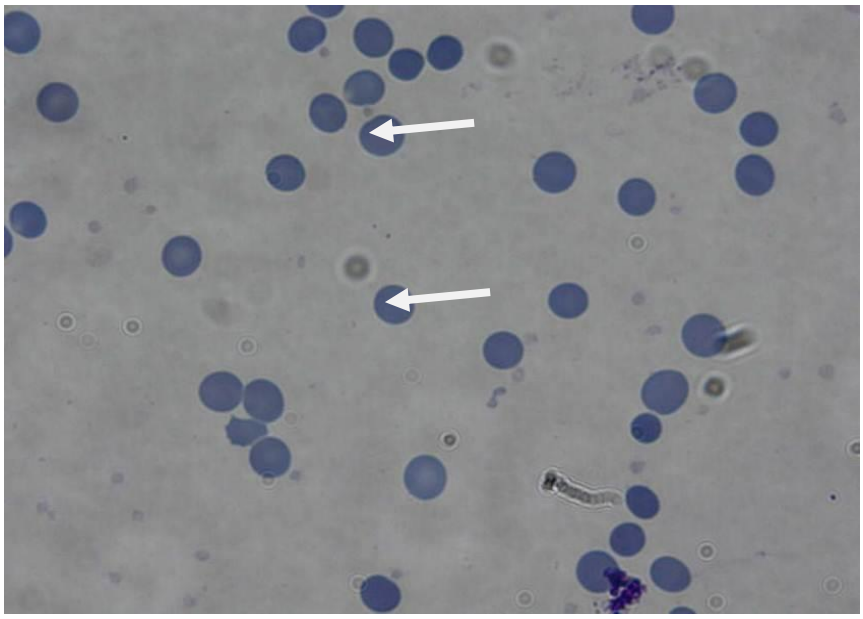


Figure 4.28 A slide with 100 percent normal red blood cells from the other ethnic groups.

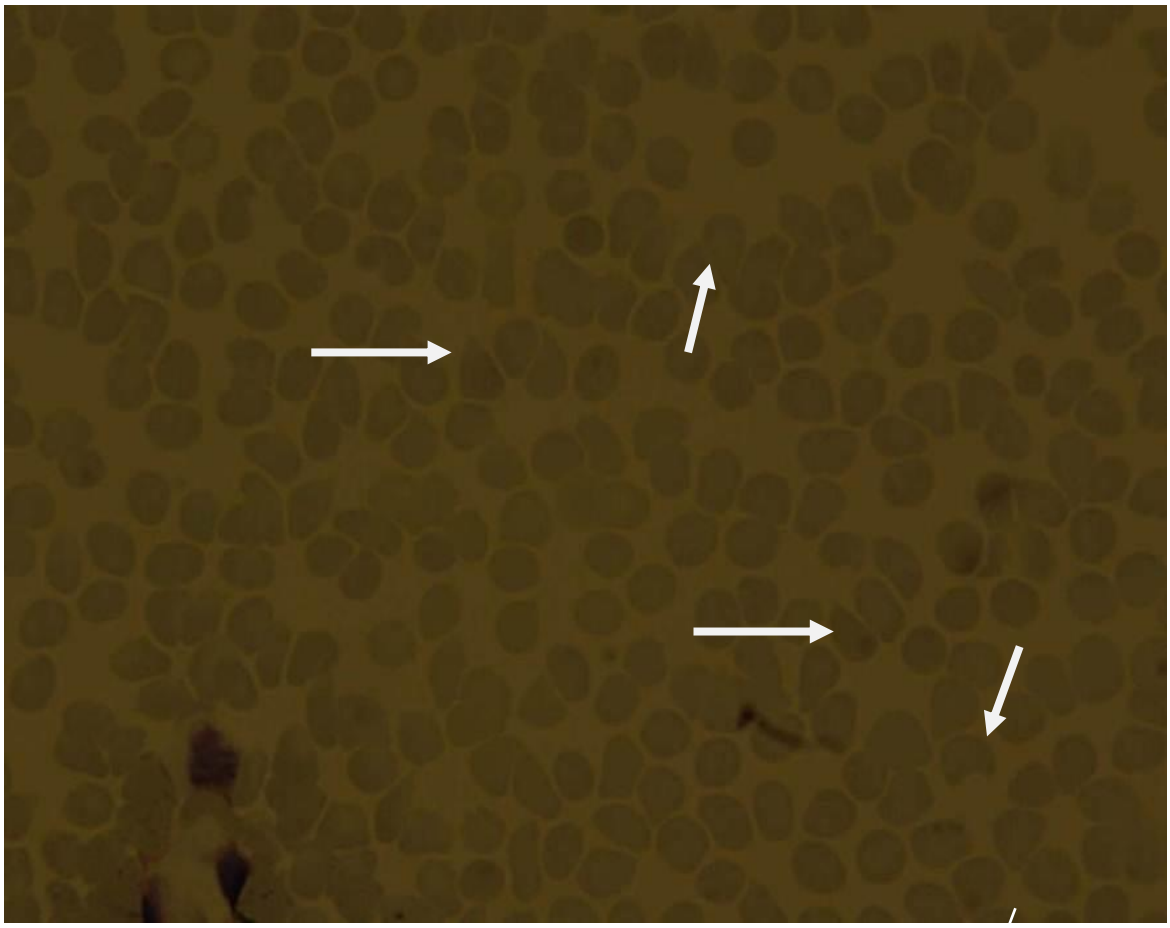


Figure 4.29 Abnormal shapes of red blood cells of the San people

(The arrows are pointing to the abnormal shapes)

4.6 Immunity verification in full blood cell counts

4.6.1 Full blood cell count of the San people and the other ethnic groups

On Table 4.6 a descriptive summary of statistics for blood component levels is presented. There are differences in the mean blood component levels between the San and the other ethnic groups. RBC, Hb, HCT, MCV, MCHC, RDW, PLT, MPV,

PCT, PDW, WBC were within normal range for both San and the other ethnic groups. It is observed that the means of RBC, HGB, HCT, RDW, PLT, PCT, NEU and LYM are higher in the other ethnic groups than in the San. The means of MCV, MCH, MCHC, MPV, PDW, WBC, MON, EOS, BAS, LIC AND IgG are higher in the San than in the other ethnic groups. The means of ALY were equal in both the San people and the other ethnic group. After the t-test was carried out, it was observed that means of RBC, HGB and HCT were significantly higher in the other ethnic groups than in the San.

Table 4.6 Full blood cell count results of the San people and the other ethnic groups

Blood Component	**Mean**	**95% Confidence Interval**	**Normal Interval**	**Units**
RBC				
San	4.47	4.37 – 4.58	3.90 – 5.72	$10^{6/}mm^3$
Other	4.96	4.81 – 5.11		
HGB				
San	13.23	12.82 – 13.63	12.0 -17.50	g/dL
Other	13.85	13.40-14.28		

HCT San Other	 38.78 40.74	 37.75–39.82 39.64– 1.85	34.9 – 50.00	%
MCV San OTHER	 86.90 82.64	 85.37-88.43 80.14-85.13	83.00-107	μm^3
MCH San OTHER	 32.37 28.07	 26.89-37.84 27.08-29.06	27.00-32.00	Pg/cells
MCHC San OTHER	 34.02 33.92	 33.81-34.22 33.65-34.19	31.50-34.50	g/dL
RDW San OTHER	 12.33 12.56	 12.08-12.58 12.03-13.08		%
PLT San OTHER	 262.85 300.28	 242.46-283-24 274.59-325.96	150-450	$10^3/mm^3$
MPV San OTHER	 9.70 9.21	 9.50-9.90 8.95-9.45		μm^3

PCT				
San	0.25	0.23-0.26		%
OTHER	0.27	0.25-0.30		
PDW				
San	17.48	16.88-18.09		%
OTHER	15.79	14.97-16.62		
WBC				
San	7.84	7.38-832	3.5-10.5	$10^3/mm^3$
OTHER	6.37	5.84-6.89		
NEU				
San	34.60	30.19-39.00	20-70	$10^3/mm^3$
OTHER	37.74	35.09-40.38		
LYM				$10^3/mm^3$
San	45.77	43.76-47.78	10-30	
OTHER	46.67	46.30-51.05		
MON				
San	9.90	9.45-10.35	2-10	$10^3/mm^3$
OTHER	8.93	8.35-9.51		
EOS				
San	10.20	8.79-11.60	2-5	$10^3/mm^3$
OTHER	3.74	2.68-4.81		
BAS				

San OTHER	1.61 0.91	1.42-1.78 0.68-1.13	0.5-1	$10^3/mm^3$
ALY San OTHER	 1.52 1.52	 1.45-1.60 1.42-1.62		$10^3/mm^3$
LIC San OTHER	 1.21 0.93	 1.10-1.32 0.72-1.13		$10^3/mm^3$
IgG SAN OTHER	 17.44 17.19	 16.54-18.35 15.86-18.52	14.00-18.00	g/dl

4.6.2 Data analysis of full blood cell counts of the San and other ethnic groups.

The T-test confirmed that there was a significant difference in the means of MCV *, PDW **, WBC ***, MON **, EOS ***, BAS ***, LIC***, MPV **, RBC***, Hb** and HCT**. The means of RBC, Hb and HCT were significantly higher in the other ethnic groups than in the San people and the means of MCV, MPV, PDW, WBC, MON, EOS, BAS AND LIC were significantly higher in the San people than in the other ethnic groups. The rest of the blood components of both the San and the other ethnic groups had no significant differences between them.

*P<0.05(significant at 5% level)

**p<0.01(significant at 1% level)

***p<0.001(significant at 0.1% level)

The table below shows the T-statistic tests for differences between the means of blood component levels for the San and other ethnic groups using Independent sample t-tests.

Table 4.7 T-Tests statistics for the San and the other ethnic groups

Blood Component	**Abbreviation**	**T-statistic**	**Significant group**
Red Blood Cells	RBC	-5.844***	Other Ethnic group
Haemoglobin	HB	-2.573**	Other Ethnic group
Hematocrit	HCT	-3.003**	Other Ethnic group
Mean Cell Volume	MCV	2.325*	San people
Mean Corpuscular Haemoglobin	MCH	1.022	
Mean Corpuscular Haemoglobin Concentration	MCHC	-0.338	
Red cell Distribution Width	RDW	-0.371	
Platelets	PLT	-1.585	
Mean Platelet Volume	MPV	3.039**	San people
Platelet Crit (% volume of blood occupied by Platelets)	PCT	-1.004	
Platelet Distribution Width	PDW	3.258**	San people
White Blood Cells	WBC	4.296***	San people
Neutrophils	NEU	-1.190	

Blood Component	Abbreviation	T-statistic	Significant group
Lymphocytes	LYM	-1.828	
Monocytes	MON	3.153**	San people
Eosinophil's	EOS	6.425***	San people
Basophils	BAS	5.240***	San people
Alymphoplastic Cells	ALY	-0.142	
Large Immature Corpuscles	LIC	2.8532**	San people
Immunoglobin G	IgG	0.253	

*** $p<0.001$ (significant at 0.1% level) **$p<0.01$ (significant at 1% level)

*$p<0.05$ (significant at 5% level)

4.7 The haemoglobin variants for the San and other ethnic groups.

When the haemoglobin was investigated, the following variants (abnormal) were separated during electrophoresis; A1a, A1b, LA1cCHb1, A1c, P3, A_o and A2. From independent samples t-test, there was no significant difference among the variants (A1a ($p=0.546$), A1b ($p=1.00$), A1c ($p=0.862$), P3 ($p=0.893$), AO ($p=0.809$), A2 ($p=0.849$) except in LA1cCHb1 with a p value of 0.003 and it was higher in the San people. The table below shows the data analysis of the abnormal haemoglobin blood components of the San and other ethnic groups.

Table 4.8 The differences between the means of abnormal haemoglobin components for the San and Other the other ethnic group using Independent sample t-tests

Haemoglobin Component	**Mean**	**T-statistic**
A1a		
San	1.28	0.610
Other	1.16	
A1b		
San	1.44	0.000
Other	1.44	
LA1cCHb1		
San	0.66	3.251*
Other	0.44	
A1c	5.50	0.175
San	5.52	
Other		
P3		
San	3.54	0.135
Other	3.52	

AO San Other	 86.79 86.88	 -2.44
A2 San Other	 2.80 2.83	 -0.192

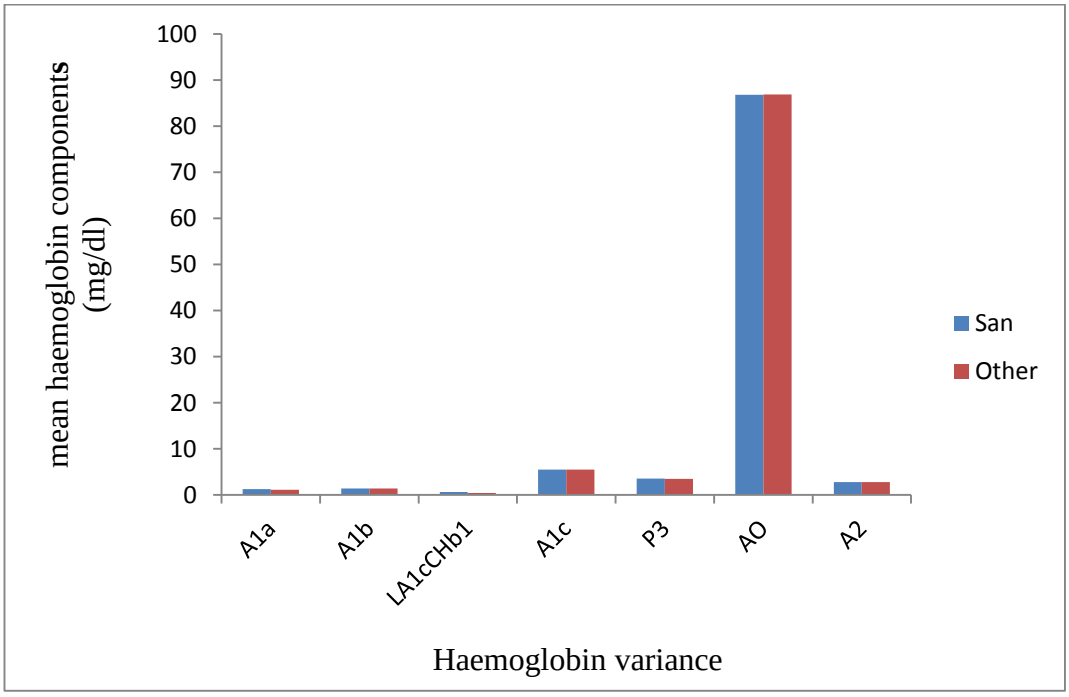


Figure 4.30 The differences between the means of abnormal haemoglobin components for the San and Other ethnic groups

NOTE: the significant difference in the presence of LA1cCHb1 found in the San led to further investigations of the presence of Haemoglobin C which was confirmed microscopically.

4.8 Summary of fungal growth on San food cultured on Potato dextrose agar

The list of the different fungi and bacteria isolated from the sample of the collected San foods. Table 4.9 indicates different species of fungi observed in San food which included tubers, leaves and roots of various plants. Some of these were; *Aspergillus, Rhizopus, Penicillium, and Saccharomyces. Rhizopus* was black with a lot of lymphae and mycelium, *Aspergillus* was also black with fine texture, *Penicillium* was greyish green and yellowish sometimes with boarders to it and a fuzzy appearance and Saccharomyces had yellowish slime streaks on the agar plate.

Table 4.9 Different species of fungi found on the different foods of the San

Sample ID/Plate No	Name of fungus/ bacteria isolated from sample
Medicinal Mixture that heals kidney ailments	Yeast cells- *Saccharomyces cerevisiae*
Tuber - *Aspergillus*	Gram positive and Gram negative Rod-shaped bacteria Aspergillus spp. , *Penicillium notatum* and *Rhizopus stolonifer*
Roots	*Penicillium notatum* and *Rhizopus stolonifer*
Vangueria infausta	Yeast *Saccharomyces cerevisiae*, *Penicillium notatum* and gram negative rod-shaped bacteria
Baikeiaea plurijuga	*Penicillium notatum* and *Rhizopus stolonife*
Sclerocarya birrea	*Aspergillus* 100%
IHU Fruits 5	*Aspergillus* 100%

Amaranthus petersiana 14	*Aspergillus, Saccharomyces cerevisiae*
IHU Roots	*Aspergillus, Saccharomyces cerevisiae* and unspecified bacterial colonies.

The figures below were observed during the analysis of fungi that on the food and herbs of the San people.

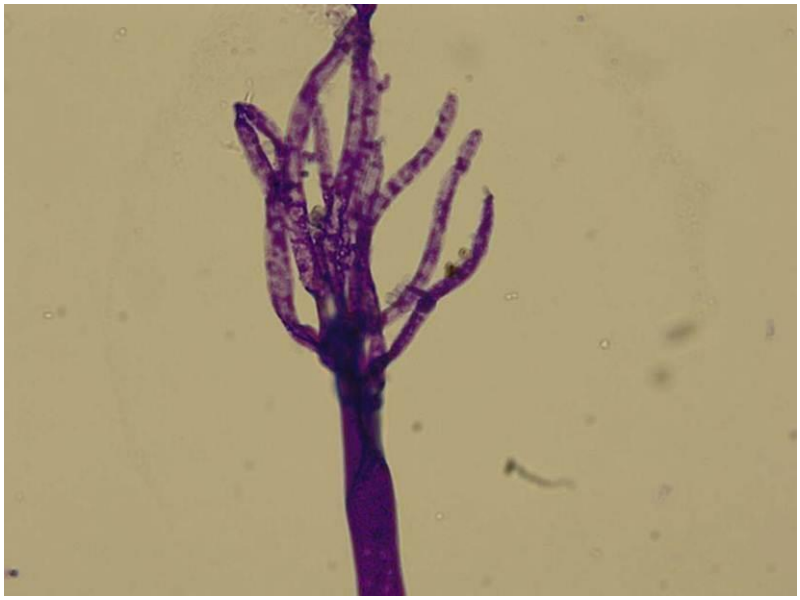


Figure 4.31 *Penicillium spp*

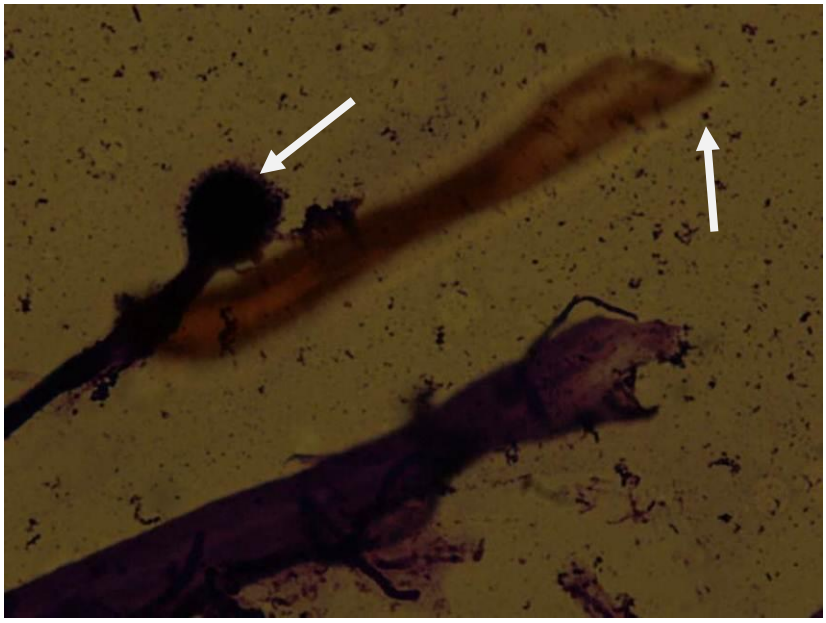


Figure 4.32 *Aspergillus spp* with a mature sporangium (arrow) and a lot of conidia spores

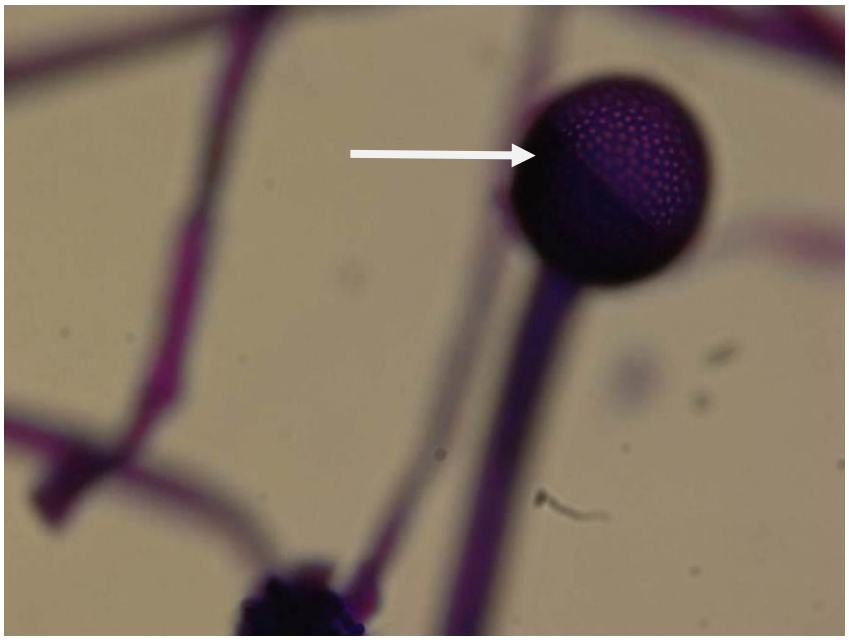


Figure 4.33 Unidentified *Rhizopus spp* of fungi with a unique sporangium

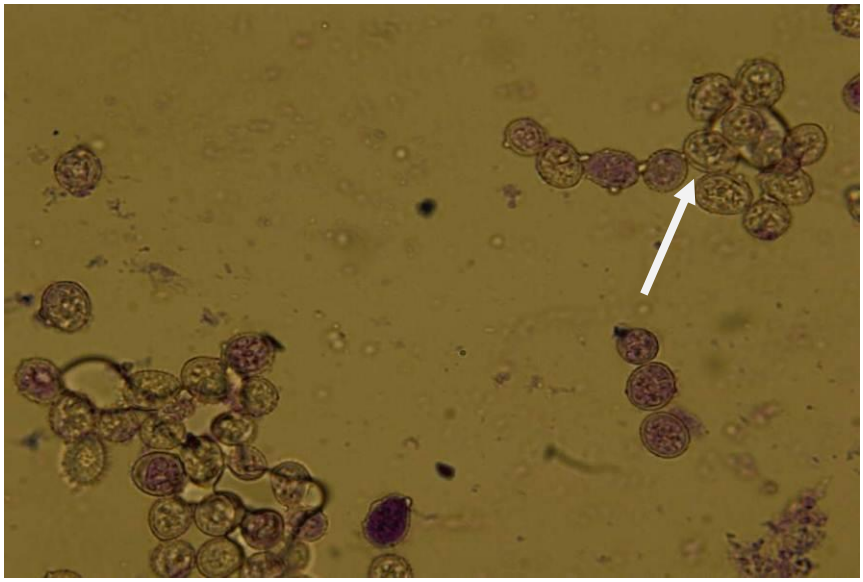


Figure 4.34 Yeast/ *Saccharomyeces cerevisiae* found in the concoction of the San used to treat kidney problem.

CHAPTER 5: DISCUSSION

5.1 Overview

This study highlighted interesting phenomena like the presence of HbC, spikes on the surfaces of the red blood cells of the San people and the higher level of both humoral and cell mediated immunity compared to that of the other ethnic groups. The nutrition of the San people was found to have more antioxidants that boost their immune system compared to the other ethnic groups. The research also observed crystallization of the red blood cells and a dark pigment in the blood samples of the San people although these were not investigated.

This study of the immune system to malaria among the San in the Oshikoto and Kavango Regions gave answers to certain interrogations leading to the following findings and results. The results obtained from the discussion indicated that the San had no knowledge of what malaria disease was about and 55.6% of the other ethnic group had knowledge of the malaria disease. This was supported by the data from the Tsumeb clinic and the locals where the malaria incidence of morbidity or mortality in the San people was almost nil. Microscopic results further confirmed that there were no malaria parasites found in the blood samples of the San people. Micronutrients especially antioxidants, flavonoids, iron and zinc played a role to boost up the immunity of the San as most of the plants they eat are consumed in their raw state. The traditional medicines of the San were not necessarily targeted towards malaria disease since they had no knowledge of the disease.

The shapes of both the red blood cell membrane and HbC in the San people had a key role to play in their natural immunity (inherited disorder of haemoglobin and red blood cells membrane). There was a significant difference in some blood components between the San people and the other ethnic groups. In the San people's blood samples, the outstanding components were MPV, PDW, WBC, MON, EOS, BAS and LIC which are required for acquired immunity. Even though the t-test did not show significant difference in IgG between the two groups, the San people's mean was slightly higher than that of the other ethnic groups.

Some medicinal fungi were found in the foods and herbs of the San people. Antibiotics from fungi like *Penicillium, Rhizopus and Aspergillus* were some of the metabolites found in the food eaten by the San people. The protection of the San against malaria parasites is discussed below.

5.2 Output from the questionnaire and focus group

The results obtained during the focus group discussion and questionnaire showed that the San people had no knowledge of malaria but 55.6% of the other ethnic groups had knowledge of malaria. Some of them knew the scientific name of the vector (female *Anopheles spp* mosquito). This could be attributed to the level of their education as well as living in urban areas, where most of the malaria information is easily accessible through MoHSS officials, radio and television broadcast. The rest of the respondents of the other ethnic groups might not have understood the question

and they might have thought the question is asking for the scientific name of the parasite or vector.

The San managed to mention some of the symptoms of malaria like headaches and fever, yet these are common symptoms of other ailments. Since they do not visit the hospital, they all rely heavily on the traditional medicines compared to 12% of the other ethnic groups that take herbs. The San took bitter herbs when they had symptoms of fever and headaches, and therefore, one can conclude that there were some malaria substances in the herbs they ate that cured the headaches and fever which are some of the malaria symptoms. The Tsintsabis clinic data confirmed that the San people did not take modern medicine, as there were no hospital records to indicate their visits. This included the major group of the San that moved to Tsintsabis centre. One would say that the San traditional medicines are effective in curing malaria symptoms and other ailments.

According to Dan, Mchombu & Mosimane (2010) the San use medicinal herbs for prophylaxis and curing malaria disease. Whether these herbs treated malaria disease or symptoms is yet to be confirmed. It is believed that bitter plants have anti-malarial properties such as alkaloids, limonoids and quassionoids that treat malaria (Titanji, Zofou & Ngemenya, 2008). In the focus group discussion, the San responded that they take bitter herbs to treat various sicknesses.

The focus group discussion and data from the clinic did not show any deaths caused by malaria in the San group. We therefore fail to reject the null hypothesis that states that there is low incidence of morbidity and mortality due to malaria patients in the San people compared to the other ethnic groups. This accord with literature from previous studies by Gunnestad et al., (2010) who stated that, the San are very resilient people such that they are able to cope and withstand common health problems like malaria.

The report by Gunnestad et al., (2010) supports our study, which focused on the high tolerance to malaria in the San people despite the harsh physical conditions they live in, without any kind of external protection like mosquito nets and spraying of homes with DDT or repellents. There is a general decline of malaria incidence and deaths amongst the other ethnic groups due to measures put in place by the government of Namibia toward elimination of malaria by 2015 (Feachem & the Malaria Elimination Group, 2009).

5.3 Micronutrients

All the micronutrients analyzed were from the berries, seeds, leaves and tubers that made up the diet of the San. The diet of the San was found to have micronutrients such as Zinc, Iron, Vitamin C, antioxidants, flavonoids and moisture that have properties that boost up the immune system. These micronutrients are known to have an influence on the progress of malaria disease (Nmorsi, Ukwandu & Egwunyenga, 2007). Vitamin A and C are strong antioxidants that play a role in protection against

malaria. Akpotuzor et al., (2007) quoted by Nmorsi et al., (2007) discovered that children that had severe malaria had a low concentration of both vitamins A and C thus confirming that micronutrients are used when the malaria parasites are on the increase.

The San people have higher micronutrients in their diet due to the fact that their food is unrefined and most of it is eaten raw. Stevens (2011) in her article "Raw vs. Cooked: Understanding the effect of cooking on micronutrients" stated that eating raw food preserves most nutrients and enzymes. Stevens (2011) further pointed out that cooking extracts toxins from some of the food items that can be fatal to the body systems. From the above information, one would advocate eating raw or dried food to boost the immune system.

The Administration of the Healing Recipes (2011) state that there are natural killer cells found in the raw food that promotes the immune system of individuals. Low natural killer cell count results in lower immunity which may cause malaria infection. *Pennisetum glaucum* which is a staple food of most of the ethnic groups and being introduced into the San diet is very low in most of the micronutrients except in Zinc where it has the highest content in the diet. The other ethnic groups practice monoculture where either mahangu, maize, sorghum or finger-millet, which constitute their staple food, are either pounded, processed or refined before being eaten resulting in the loss of valuable micronutrients unlike the San people.

Iron improves the production of haemoglobin; *Plasmodium spp* destroys the haemoglobin during its life cycle in man. Iron supplements are used as antimalarial treatment in improving the anaemic status of patients suffering from malaria (Ekvall Premji & Bjorkman, 2000). Iron in haemoglobin carries oxygen throughout the body. The mean iron found in the San food was 0.83mg/100g, with the true mean lying between 0.41-1.24(mg/100g). These readings fall within the normal range value which is 1.20mg/100g thus making the San more advantaged in obtaining iron from their foods. Iron is required for the development of haemoglobin in humans.

Zinc is the second common trace elements found in the body after iron. Most of the metabolic reaction of the body involves enzymes that are activated by the presence of zinc (Zeba et al., 2008). It also helps in the immune system of the body, like in wound healing, protein and DNA synthesis. Zinc is destroyed during milling processes and when cooking. Deficiency results in zinc will weaken the immune system which will lead to open infection which includes malaria parasites since the production of white blood cells and red blood cells is decreased in the absence of Zinc (The Administration of the Healing Recipes, 2011). The San diet has the mean of Zinc as 0.039mg/100g. This is slightly below the normal value 1.9mg/100g. Most of the San diet is composed of raw materials, fruits, tubers, roots and leaves that are not refined and do not need cooking.

Vitamin C was found to be high in most fruits like *Sclerocarya birrea* (237mg/100ml), *Maeru schinzii* (41 mg/100ml) and *Citiullus Ianutus* (10mg/100ml)

which are well above the normal requirements given by internationaldrugmart (2003). Vitamin C is a very powerful immune booster, as an antioxidant, antiviral and anti-cancer nutrient. It is incorporated in the white blood cells to fight pathogens (Field, Johnson & Schley, 2002). A healthy dose of Vitamin C protects the body from harmful pathogens including malaria parasites (Zimmermann, 2010). Vitamin C maintains healthy bones as T cells and B cells are manufactured in the bone marrow and are required in the immune system of the body. As an antioxidant Vitamin C protects the body from free radicals that cause oxidative stress (Sudhanshu, 2010).

Hitchcock, Ikeya, Biesele & Lee (2006) carried out a study on the nutrition of the San and concluded that the nutritional status of the San was good. Hitchcock et al., (2006) went on to say that the food consumed was rich in vitamin C. The San consumed about 150 different species of plants and 40 species of animals (Hitchcock et al., 2006). Phytochemicals like antioxidants and flavourant found in the San food and herbs boost the immune system against malaria symptoms and diseases. Adequate micro-nutrients are necessary to make the body's immune system strong especially in children who are very vulnerable to malaria infections. The passive immune system only lasts for six months, if there are no repeated infections by the parasite which build up the immune system, the children may end up with acute malaria disease which may lead to cerebral malaria (Reis et al., 2010). Micro-nutrients taken in right quantities are the cheapest form of acquiring immunity before the body produces its own immunity.

Flavonoids and antioxidants play a very important role in scavenging free radicals in the body that weaken the immune system of individuals. Sudhanshu et al., (2010) in their article stated that antioxidants are phytochemicals that have medicinal properties in plants which reduce free radicals in the body hence strengthening the immune system. The presence of antioxidants and flavonoids in the San nutrition have helped prevent many infectious diseases like malaria. Pourmorad et al., (2006) reported that there is a relationship between the flavonoids and their phenol compounds with antioxidants in radical scavenging. All of the flavonoids found in the San diet are natural oxidants from the plants they collect unlike some that are synthetic used as supplements in most of the food of the other ethnic groups.

In this study, the food and herbs tested showed the presence of medicinal properties. This then led into rejecting the null hypothesis that states that the diet of the San has no medicinal properties that confer immunity against malaria parasites in their food. Sudhanshu et al., (2010) reported that the environment has always provided medicinal substances for a very long time and that most of the modern medicine is being extracted from plants. Therefore, one can conclude that the San are advantaged because they eat food that is not processed unlike in the other ethnic groups.

In Oshikoto and Kavango regions there are plenty of amarula fruits besides other wild fruits available which have lots of antioxidants and as previously noted they do not only attack free radicals but boost up the immune system. This statement was supported by Sudhanshu et al., (2010) who suggested that plants have antioxidants

that fight free radicals that are released during malaria attack. Zeba et al., (2008) discovered that a combination of zinc and vitamin A enhances the immune system of man and reduces the attack from *Plasmodium falciparum* parasite. In some cases antioxidants such as N-acetylcesteine and desferoxamine have been used as malarial therapeutic drugs to treat cerebral malaria (Reis et al., 2010). Iribhogbe, Agbaje, Oreagba, Aina and Ota, (2012) in their paper found out that many antioxidants such as zinc, selenium, vitamins A and E have antimalarial properties to cure malaria disease. The presence of the above micronutrients confirms that the diet of the San is very well balanced containing the essential elements that do not only protect them from malaria parasite attacks but also prevents the development of *Plasmodium falciparum* either at the pre-erythrocyte or erythrocyte invasion.

5.4 The Immune System

The results obtained from the blood analysis were used to determine the immunity in the San people compared to the other ethnic groups. The variables to measure immunity were calculated from microscopic results, which included the presence of parasites, shape of RBC and haemoglobin C. Further analysis was done on full blood cell count and the determination of haemoglobin variances.

5.4.1 The presence of *Plasmodium falciparum* in the blood samples

The blood samples analysed and observed by microscope revealed that there were no malarial parasites on all the blood samples of the San. The collection was done in

April towards the end of the rainy season, which is the malaria endemic period. Therefore, we fail to reject the null hypothesis that states that there is no malaria parasites in the blood sample of the San people compared to that of the other ethnic groups. This unique phenomenon amongst the San people considering their lifestyle without any tools to protect themselves from the mosquito bites and contracting malaria in an endemic area could be due to one of the many factors that render immunity against malaria parasite as discussed below. Very few slides were found to have malaria parasites in the blood samples of the other ethnic groups. Out of the 100 blood samples of the other ethnic groups, only 7% were found with the *Plasmodium falciparum* parasite. The results of the other ethnic groups reflected the intense malaria elimination program being carried out in Namibia through the Ministry of Health and Social Services Malaria Indicator Survey (MoHSS, 2010). Feachan & Malaria Elimination Group (2009) further confirmed that there is a general decline of malaria incidence and deaths amongst the other ethnic groups due to measures put up by the government of Namibia towards elimination of malaria by 2015.

5.4.2 The shape of the red blood cells

The normal shape of the red blood cells in human beings that carries oxygen is biconcave shape. It has a cell membrane that is made up of two layers of biphospholipids with underlying spectrum that provides the elasticity to the cell and cytoskeleton that keeps the shape of biconcave. But the long exposure to malarial parasite infection causes genetic defects or mutations to either the surface of the red blood cells receptors or other disorders of the Haemoglobin (Fortin et al., 2002).

This was also reported by Robert (2013) who reiterated that most common causes of genetic disorders on or in the red blood cells are due to repetitive exposure to malaria *Plasmodium* parasites like in Thalassemia, G6PD deficiency, haemoglobins S, C, E and all these factors confer immunity. Robert (2013) further suggested that genetic resistance works in one, two or three ways which result in stopping the entry of the parasite into the red blood cells or preventing the development of the trophozoite inside the red blood cell or by foiling the parasite from rupturing the red blood cells with mature merozoites.

The results obtained from this study showed that both the San and the other ethnic groups had normal and abnormal red blood cells. In the San group it was found that out of the 100 blood sample 82 had abnormal cells while in the other ethnic groups 21 had abnormal cells out of the 100 blood samples and this could be due to intermarriages between tribal groups, exposure to malaria infection, geographical localities and other unknown factors. Therefore we cannot conclude that the presence of abnormal cells in the other ethnic groups confers immunity against malaria since the malaria cases have been reported to be very high in 2002 (MoHSS, 2009). There was an observation that 18 San people had normal cells but there was no empirical evidence that they were not immune to malaria.

In our study we observed three abnormal shapes of the red blood cells surfaces in the San people which were oval shaped (tear drop), sickle cell shaped and spikes on the cell surfaces and two abnormal shaped RBC’s in the other ethnic groups which were rectangular shaped and spiculated. The data from our study showed that out of the 82

San people with abnormal cells, 80 had spikes on their RBC surfaces and from the 21 who had abnormal cells in the other ethnic group, 20 had spiculated cells. A detailed article by Mentzer (2012) indicated that many deformities on the surface of the red blood cells can change the morphology of the red blood cell. Mentzer (2012) mentioned three different shapes formed during mutation where the composition of lipids or proteins in the red blood cell membrane has been changed, weakening the membrane tension which results in infolding of the membrane forming spikes. Mentzer (2012) grouped spiculated cells into echinocytes, acanthocytes and target cells which are the most common cells with this genetic disorder. Stomatocytes and Xerocytes were some of the cells mentioned in the literature that have deformities on the surfaces. Mentzer (2012) defined echinocytes, as infoldings that appear as projections or thorn like structures on the surface of red blood cells and often appear to show uneven edges on a slide, whereas with the acanthocytes their projections are smaller and evenly distributed on the red blood cell surfaces. The above definitions correlated with results of this study, although the researcher could not differentiate them into echinocytes and acanthocytes because the sizes of the spikes were different on one cell let alone on a group of cells. This made it difficult to place the individual blood samples into either echinocytes or acanthocytes. From this study both types of spikes are seen as contributing factors to the natural immunity attributed to the San. There were no signs or symptoms of any disease during the whole period of the study, confirming what Mentzer (2012) wrote saying that patients with this abnormal morphology were not anaemic and did not show any signs of haemolysis.

Some of the San's red blood cells had spikes on their surfaces. In this case, one would speculate that they were formed due to harsh conditions of nomadic life and their exposure to the elements in the environment as a result of their dress code. The San were exposed to malaria parasites for a long period of time, which could have changed the general format of their genetic makeup as well as changing the shape of the red blood cell membranes. In some blood samples one would find both spiculated and normal cells and these individuals could be heterozygous cells (Figure 4.25) that have had a mutated gene in one kind of cells and the normal gene in the other cells.

During this study the results showed RBC with spikes and few other abnormal shaped RBCs mainly in the San people. Both the spiculated cells and HbC were unique to our results. A chi-square test of association was run to test if existence of spikes on the red blood cells of the San does not confer immunity against the malaria parasite. A p-value of 0.000 was observed which is less than 0.05 and therefore we reject our null hypothesis and conclude that the existence of spikes on the red blood cells of the San does confer their immunity against malaria.

The San during hunting and gathering could go on days without water (focus group discussion, 2009). They ate plants that had a lot of water in them like *Citiullus Ianutus,* cucumbers, aloes and *Hoodia gordonii*. One would speculate that it would be during these times of stress on the red blood cell that the deformity took place. These spikes which other researchers call echinocytes or acanthocytes are very visible on the red blood cells of the San with a few in the other ethnic groups.

Gallagher (2011) mentions that spikes are formed after dehydration. One would have thought, if the cause is dehydration alone then in the presence of water the cells would recover but they do not recover after taking water. The only possible theory that stands out is that this is a genetic inherited disorder. Since the malaria parasite antigens are specific to certain sites on the red blood cells, one would suggest that the infolding and formation of spikes on the surface changes the position or elimination of the protein receptors on the red blood cells and therefore conferring immunity. The deformity could have been caused by *Plasmodium falciparum* that is a common parasite that causes malaria in the two regions studied. Zimmermann, Ferreira, Howes and Mercereau-Puijalon (2013) published that mutation on human genome of the red blood cells protects against malarial infection.

It was noted that the blood of the San was very dark, almost black compared to that of the other ethnic groups and it did not matter whether the blood came from a male or female, a child or an elderly person - the dark pigment was present in all of them. It will be interesting to do a further research to investigate this component in the blood of the San. Before the blood analysis, it was assumed that the dark pigment was due to higher concentration of iron in the haemoglobin of the San. After independent sample t-test the level of red blood cells, haemoglobin and haematocrit were significantly higher in the other ethnic groups than in the San. This could suggest that the San have an unknown blood disorder that plays an important role in the protection against *Plasmodium* parasites. Since most of the individuals with this genetic disorder are asymptomatic, one cannot tell easily the impact of any of the

clinical manifestations by the presence of this pigment. One thing that is certain, is that the San do not suffer from malaria and this was confirmed from the focus group, microscopic observations and the raw data from Tsintsabis clinic (2010).

The other red blood cell cytoskeletal genetic disorders that protects against malaria parasite infection are Southeast Asian Ovalocytosis (SAO), elliptocytosis and spherocytosis. The SAO abnormality is due to the oval shape of the red blood cell found in the populations of Papua New Guinea and Aborigines of Southeast Asia. Oval shaped cells are an inherited disorder of red blood cells. Rosanas-Urgell et al., (2012) carried out a study to see if *P. vivax* was the cause of the mutation in the human genome in Papua New Guinea causing them to take the shape of an oval. This disorder causes Southeast Asian Ovalocytosis. A few of the cells were identified in the San microscopic results. The oval shape prevents the invasion of merozoites of *Plasmodium falciparum* into the red blood cell (Robert, 2013). Just like in the spiculated red blood cells the mechanism in which these cells stop the invasion is not very well understood. The interesting feature on these genetic deformities is found in the less privileged people of the society. The San and the Aborigines live in isolated harsh conditions which could have brought about this genetic deformity as a result it has protected them against malaria parasites infections.

5.4.2.1 Haemoglobin Variants

Seven different Haemoglobin variants were identified A1a, A1b, A1c, LA1c/CHb1, P3 (HbJ), AO, A2 in the San people and the other ethnic groups. The mean

differences obtained from a t-test were not significant between the San people and the other ethnic groups, in A1b the means were the same. Further t-test indicated that a significant difference only existed in LA1c/CHb1 with p-value = 0.003 whereby the mean of the San people was significantly higher than the other ethnic groups.This is a glycerated Haemoglobin with glucose and urea attached on one of the β chains. There is very little literature on the function of the urea attachment (CHb1). HbA1c is a product of non-enzymatic reaction between free aldehyde group of glucose and a free β amino chain. This glyceration takes place at position 6 of the amino –terminal valine of the β chain of the Haemoglobin (Hinzmann et al., 2012). Generally HbA1c is used as a marker for determining the glycemic status of diabetic people and it is used to diagnose people with diabetes (Hinzmann et al., 2012). This could mean that most of the San are diabetic, which without further test we are unable to confirm and neither is there any clinical data to ascertain their diabetic status. Permission was not granted by the Ministry of Health to do any other investigation besides that of malaria. The presence of HbAlc also shows the presence of variant haemoglobins that confer immunity against malaria parasite (Fortin, Stevenson and Gros, 2002).

There are about a thousand Haemoglobin variants that have been discovered so far in humans (Rhea et al., 2012). These Haemoglobins are different from the normal, in that the genes on the β chain have either been deleted or mutated. The four most common ones are Haemoglobin S, E, C and D. If the Haemoglobin variant exists as homozygous in individuals they will show clinical signs of the genetic disorder and yet in the heterozygous, they will not be any signs of the disease. They are just carriers or traits of the abnormal gene (Rhea et al., 2012).

Studies done on haemoglobin S as a variant had clinically showed signs of sickle cell anaemia due to mutation in the β chain of the haemoglobin, where the hydrophilic glutamic acid is replaced with hydrophobic valine at position 6. This change does not only affect the shape of the red blood cells but they block the small blood capillaries causing severe pain to patients diagnosed with this ailment, but also causes sickle cell anaemia (Haque, 2010). In some of the San slides abnormal red blood cells of sickle cell were observed which contradicts a statement made by Haque (2010) who noted that the geographic distribution of the deformed HbS sickle cells and the environment are inter correlated. Nelson (n.d) concluded that people with sickle cell trait have a higher chance of survival against malaria than those with normal Haemoglobin although he ruled out sterile immunity. Individuals with either one of these two Haemoglobins are protected from malaria disease by preventing entry or development of the parasite in the red blood cell (Fortin, Stevenson and Gros, 2002).

The high significant level of LA1c/CHb1 in the San people led to further investigation of the presence of abnormal haemoglobins. The microscopic results showed the presence of both homozygous and heterozygous HbC. Due to the microscopic results that showed the presence of HbC, the hypothesis that postulates that the presence of HbC in the San people will not confer immunity against malaria parasite was rejected. Haemoglobin C is formed on position 6 of β chain where lysine is replaced by glutamine and if the offspring inherits mutated gene from both parents the homozygous genotype HbC will cause haemolytic anaemia.

Schuster et al., (2010) who carried out a study on mitochondrial and nuclear markers discovered that the genetic structure of the hunter-gathering people of Southern Africa the San, is genetically divergent from other humans. Schuster et al., (2010) did not look specifically into the genetic disorder of the red blood cell composition, which the researcher of this study did. Our results agreed with what Schuster et al., (2010) observed that the San people are genetically divergent from other human beings. Further studies could help in understanding the role played by the composition of some of these Hb variants in the immune system of the San.

There is interference from other variants like HbS, HbC and HbD when either testing or measuring the level of HbA1c. The results are not always accurate so other tests are recommended to be used for the determination of glycemic molecules in the blood samples of individuals. This means that there is a correlation between Haemoglobin variants and LA1c/CHb1 (Fortin, Stevenson and Gros, 2002). The one observation to note in the results of this study was the average level of LA1c/CHb1 in the San which was significantly higher than that of the other ethnic groups. There is a high speculation that there was interference from one of the variant Haemoglobins. The results on the shapes of haemoglobin variants of the San and that of literature are the same. Rhea et al., (2012) stated that the Hispanic African Americans have both diabetes and either HbC or HbS or trait. Rhea et al., (2012) further stated that in Thailand where Tharu people are immune to malaria because of mutation on β chain of thalassemia haemoglobin are affected by the presence of variant HbE and diabetes. In this study, Haemoglobin LA1c/CHb-1 of the San

showed that its presence was significantly higher than the other variant haemoglobins identified. In the blood samples observed, the San abnormal Haemoglobin can only be that of HbC as a result of the presence of glycerated Haemoglobin as well as the microscopic results.

The presence of HbC confirms the presence of natural immunity in the San to protect them from *Plasmodium falciparum*. It features in West Africa, Central Africa as well as where there is hyperendemic of malaria. Rhea et al., (2012) noted that Haemoglobin C disease in homozygotes is rare and relatively mild. In rare cases it affects a small amount of haemolytic anaemia and minor enlargement of the spleen. The people live a normal healthy life. Rhea et al., (2012) citing Kohne (2011) confirmed that individuals with variant HbC or trait live a normal healthy life and those with the disease have minor haemolytic anaemia.

The t- test carried out on the means of blood components showed that the RBC, Hb, and HCT showed a higher significant difference in other ethnic groups p value <0.001; P value <0.01 and P value <0.1 respectively than that of the San. This is another indicator to show that the San have some kind of anaemic ailment that is featuring in the lower RBC, HB and HCT count.

A comparative study was carried out in Burkina Faso by Fortin et al., (2002) on three ethnic groups, Fulani, Mossi and Rimaibe who were all living in the same climate conditions, vegetation and the hyperendemic malaria transmission. The Fulani are

the nomadic pastoral ethnic group in West and East Africa. They leave their homes for some time with their animals in search of greener pastures but return to their homes after that. Fortin et al., (2002) did all the tests that are related to malarial infections that is parasitology, clinical and immunological tests. The results of Fortin et al., (2002) results reflected that the Fulani had higher immunity both acquired and natural that is genetic inheritance of abnormal Haemoglobin that protects them from malaria parasites.

Arama et al., (2011) confirmed the above study by carrying a similar study on the sympatric ethnic groups, the Fulani and the Dogon children in Mali. The study was carried out after rain season, which means it was during malarial season. The two communities lived in the same conditions of cultural, climatic and malaria endemic areas. Results of Arama et al., (2011) showed that the Fulani were better protected against *Plasmodium falciparum* compared to the Dogon. This was attributed to innate immunity in the Fulani though acquired immunity played an important role as there were higher levels of T- and B- cells as well as monocytes. In this study, the white blood cells comprising EOS, BAS, NUE and MON were higher in the San than the other ethnic groups confirmed by Arama et al., (2011).

In 2005, Bolad published a paper on their study between the Fulani and Mossi in Burkina Faso and Fulani and Dogon in Mali. The results between the neighbouring tribes exhibited that the Fulani tribe had higher levels of antibodies IgG and IgM due to the presence of *Plasmodium falciparum* and that in the Fulani tribe fewer parasites

were detected. Bolad et al., (2010) also carried out a similar study comparing two groups in West Africa. These were the Fulani and Mossi and Fulani and Dokonjini both in Burkina Faso where the Fulani had higher levels of antibodies IgG and IgM due to the presence of *Plasmodium falciparum*. In this study, there was no significant difference in levels of IgG between the San and the other ethnic groups.

The other Haemoglobin variant that was noted in this study was P3 which is at times called HbJ and very little is known about this haemoglobin variant. Sachdev et al., (2010) reported that it is asymptomatic and those with the disease have mild anaemia. Its mutation could be on either one of the alpha or beta chains. The role this variant and the rest play in malaria infection is not very clear. Their presence in the San people is not known. One may assume that the presence of P3 is to protect individuals against malaria (Sachdev et al., 2010).

Schuster et al., (2010) in their study of the San genomes identified about 1.3 million genetic variants in the Southern African San. The San whom they closely studied and sequenced the genomes discovered that the tribe had unique physiology with genetic variants. Schuster et al., (2010) emphasized that the genetic differences between the other ethnic groups and the San and their health conditions were different. This study yielded the same results as that of Schuster et al., (2010) on the hereditary immune system of the San being better than the other ethnic groups. Rosanas-Urgell et al., (2012) displayed that human gene mutation was a result of some protection against some repetitive diseases that put pressure on the cells of the body and force them to

mutate as a form of survival through natural selection. The San could have gone through the same experience in as far as malaria infection is concerned making them more immune to the disease caused by the *Plasmodium falciparum* than the other ethnic groups.

The studies by Bolad (2005) and Arama et al., (2011) who studied the Fulani and the other ethnic groups in West Africa showed that different ethnic groups living in similar geographical conditions have different immune response to malaria infection. In Namibia one could say the San are the indigenous group of Namibia and they have been exposed to many malarial infections that led to genetic alterations or mutation in the Haemoglobin β and α chain as well as in the membrane of the red blood cells for survival. The exposure to harsh conditions can lead to gene mutation like in the Fulani of West Africa and Aborigines of Southeast Asia and Tharu tribe in the mountainous area of Thailand. Therefore the presence of Haemoglobin C in the San confers their immunity against malaria while the presence of HbC in the other ethnic group could be as a result of inter marriages between the two groups since the study was carried out in the same geographical area. The exposure to parasites in the other ethnic group's areas has also brought about mutation of their normal haemoglobin to HbC but may not necessarily cause immunity in this group since there is no empirical evidence to support this observation.

5.4.3 The blood cell components to determine Acquired Immunity

Various parameters of blood samples were measured and statistically analyses between the San and other ethnic groups. The following variables: White blood cell, Red blood cells, platelets and Immunoglobulin were tested at 95% confidence and broken down into: Hb, HCT, MCV, MCH, MCHC, RDW, MPV, PCT, PDW, NEU, MON, EOS, BAS, ALY, LIC, IgG and LYM. Further, a T-test was done to check if there is a significant difference in means between the two groups. From the t test, it is observed that WBC have a p value of 0.000 and therefore rejecting the null hypothesis and concluding that the San have the higher mean value of white blood cells, PLT showed a p value = 0.052 which is greater than 0.05 as such failing to reject the null hypothesis and concluding that there is no difference in means between the two groups. IgG had a p value of 0.904 and therefore, here too failing to reject the null hypothesis and concluding that there is no difference in mean values of the two groups.

5.4.3.1 Cell mediated immunity

From the results of this study the presence of white blood cells, eosinophil, basophils and monocytes were significantly higher in the San than that of the other ethnic groups. This was due to the cell-mediated immune response (T cell mediated immune response). It involves many white blood cells that play various roles in inducing, combating and destroying the invading malaria parasites. The presence of the malaria parasite will invoke the reaction of T-cells to invade both the pre-erythrocyte and intra-erythrocytic parasitic stages (Kakkilaya, 2011; Shomon,

unpublished). The T cells have receptors on their surfaces called T receptors and their role is to help the T cells to recognize the parasitic merozoites and destroy the infected red blood cells (Shomon, unpublished). NIAID (2008) reported that T cells could not identify any free-floating antigens except by the help of T-receptors on their surfaces. They either respond to immunity by attacking the merozoites directly or give instructions to other cells to attack the parasite like in B cells. The T helper cells instruct them to produce specific antibodies, or communicate with phagocytes in this case the neutrophils, monocytes and macrophages to engulf and digest the infected red blood cells. The T cells can also instruct the granulated white blood cells that are eosinophil, basophils as well as the neutrophils to release the granules that will destroy the parasite. Natural Killers (NK) are white blood cells that are antigen specific cells and are armed with granules that carry chemical substances that kill the parasite on contact. This reaction is initiated by $CD8^+$ T cells. The $CD8^+$ T cells play a significant role in destroying cerebral malaria parasites as well as preventing the liver infection by the sporozoites in vitro. This is done together with $CD4^+$ T cells (Stevenson and Riley 2004; NAID 2008).

Shimon (2013) further illustrated the functions of T cells in the production of cytokines and chemokines, which are secreted by stimulation of major histocompatibility complex (MHC) instigated by the presence of T cell receptors. The information above is trying to show the sophistication of the San immune system. The complexity of the San immune system serves as further evidence that the San are immune to malaria.

5.4.3.2 Humoral immunity

Humoral immunity is when the body releases antibodies against the malaria parasite. In this case, IgG1 and IgG3 play a very important role in the fight against malaria parasites by forming a complex of IgG with the antigens of malaria parasites. These complexes are engulfed by macrophages or destroyed in the spleen (Groux and Gysin, 1990).

The t-test results showed that there is no significant difference between the groups for both IgG and Lymphocytes. It confirms the fact that there was no need for the production of both Lymphocytes and IgG to higher levels in the absence of the malaria parasites mostly in the San people. The other reason why there was some IgG in both groups was a low presence of parasitemia that induced the production of IgG or it was due to the presence of B memory cells. Many researchers agree that repetitive infection induce the production of antibodies that protect individuals from malaria attacks but the longevity of the antibodies in the blood does not determine for how long one is protected. The B cells produced by the bone marrow play a very important role in acquired immunity as they produce specific antibodies to other antigens as well as those of malaria (Cavalho and Granholm, 1990). B cells secrete antibodies that are specific to particular antigens. Antibodies play a very important role in humoral immunity against the blood stage of merozoites by either inhibiting the parasite entry into the red blood cells or can both be engulfed by phagocytes or be taken to the spleen or liver where they are destroyed (NIAID, 2012).

A debate is still going on whether the B memory cells are long-lived after initial infection or after several infections. Wipaso et al., (2010) studied the longevity of antibodies and B cell memory response and found out that infrequent malaria infections are capable of inducing long-lived antibody and memory B cell responses. In this study B memory cells were not investigated though the presence of IgG was observed in both the San and the other ethnic groups. Dorfman et al., (2005) findings state that people who are frequently exposed to malarial parasites will require antibodies to be protected from malaria disease as the level decreases when one moves from the endemic area or when seasons change. This loss of antibodies may delay acquisition of immunity should one be re-infected.

Woodberry et al., (2008) carried out a longitudinal study on antibody response in Papuans who suffered from severe *Plasmodium falciparum* malaria, *Plasmodium vivax* malaria was mixed with *P.vivax* malaria and in those with past exposure. The results indicated that there was no significant difference in the level of antibody responses between those that had acute malaria and those that had past exposure and the results obtained in our study are similar as far as IgG is concerned.

Furthermore, Dorfman et al., (2005) cited by Woodberry et al., (2008) suggested that the fall in IgG could be linked with the short half-life of the antibody or in the malfunctioning in the production of B memory cells. Wiposa et al., (2010) do not support the statement, and they said antibodies that are naturally acquired after repetitive exposure to malaria tend to last longer in the body and Shigidi et al.,

(2008) agree with Wiposa et al., (2010) after studying the pattern of IgG in patients with cerebral malaria (CM) and those with uncomplicated malaria (UM). The results of Shigidi et al., (2008) were similar to those obtained by Wiposa et al., (2010). IgG level in CM was significantly higher than in UM patients. One could also argue that the presence of IgG reflected in the results could have been induced by other antigens from other pathogens and not necessarily from malaria parasites especially in the case of the San. Whatever the explanation, one may need to carry out more tests on the antibody pattern, production pattern and the longevity of the San antibodies.

Dorfman et al., (2005) stated that even after many exposures, humans are not resistant to infection. From the results obtained, the presence of IgG from past exposure plays a significant role in the immunity of the San people. The presence of IgG could also mean that at the time when the blood was collected there were some memory cells present in the blood samples but whether these were due to recent infection or past exposure further research could enlighten this query. If there is no sterility in malaria disease are we saying that there is always a small quantity of parasitemia that will invoke the production of antibodies and therefore protect an individual against malaria disease?

Langerhorne, Ndungu, Sponaas and Marsh (2008) in their report suggested that for one to build up immunity there should be continued exposure to malarial antigens to produce more memory cells and for them to last. Langerhorne et al., (2008) further

stated that in acute malaria, immunity is present to fight the parasite in children who live in higher endemic areas than in those who live in lower endemic areas of the parasite. Tavern, Tavernier, Fiers, and Playfair (1993) reported that there was another outbreak after thirty years had lapsed and the people who were present during the first outbreak were more resistant to the second outbreak of malarial disease than the younger ones. One can say that the presence of IgG persisted after the malaria transmission season in low levels as a protective measure of the body against the disease. This supports my proposal that the San acquired their immunity long time ago and it only gets better as they continue to be exposed and hence one can safely say their immunity is sterile. Those who resettled in Tsintsabis and Bravo had no records of any San who tested positive to the malaria parasite. From the results of this study, the immunity of the San people is not affected by where they settle. This study demonstrated that the acquired immune response to *P. falciparum* infection was stronger in the San despite the fact that the two groups lived in the same endemic areas. Regardless of the fact that the results did not support the argument in as far as the production of antibodies is concerned, Oshikoto and Kavango regions used to have high incidence of malaria though now it is on the decline (MoHIS, 2011).

The low level of IgG in the case of the other ethnic groups is due to low infection from malaria parasites as their homes are sprayed with insecticides, people sleeping under treated mosquito nets and use of mosquito repellents by those who will be outdoors at night when mosquitoes are active. This keeps away the vector from

biting them and they will not be any antigens from the parasite to evoke the production of antibodies. The presence of IgG also plays an important role in production of phagocytes, which contribute to protective immunity against malaria (Groux and Gysin, 1990).

Though the level of the San IgG was lower than expected, their $CD4^+$ was significantly higher than that of the other ethnic groups see Table 4.7. This defense mechanism against malaria parasites is humoral invoked, whereas cell mediated lymphocytes play a very important role by producing either T cells which coordinate with whole immune system and destroy infected cells by phagocytosis although it is not very well understood. NK and $CD4^+$ are some of the cells produced by T cells or instruct the B cells to produce the necessary antibodies. The pre-erythrocytic parasites lasts only for a few days therefore the production of $CD4^+$ and $CD8^+$ T cells should be readily available to fight the new parasites until the next mosquito bite (Langerhorne et al., 2008). The production of these cells should be maintained to fight any new parasites or be changed into memory cells that last for some time. The $CD8^+$ normally last up to 6 months (Langerhorne et al., 2008). This is long enough to last the malarial season in Namibia.

5.4.4. Platelets

The results of the platelets mean that both the San and other ethnic groups were within the normal range (San $263x10^3mm^3$, others $300x10^3mm^3$). The t-test results of platelets indicate that there was no difference in the means between the San and the

other ethnic groups. The means between MPV and PDW both had a P value < 0.01 making the volume and distribution of platelets in the blood significantly higher in the San than in the other ethnic groups. It has been recently discovered that platelets play a role in the immune system against *Plasmodium falciparum.* This then confirms further that the San are more immune towards malaria parasites.

Platelets are small anucleate cells that are part of the blood components whose role is known to be blood clotting but recent studies have discovered that it has many other roles in the immunity of individuals by killing malarial parasites through either adaptive or innate immunity (Flaumenhaft, 2013; Aprile, 2013; Conglei et al., 2012). Aprile (2013) reported that platelets prevent the growth of intra erythrocyte parasites, and eventually kill the parasites. Foote (2009) cited by Flaumenhaft (2013) talks about platelet factor 4 (PF4, CXCL4) that attaches itself to Duffy antigen on the red blood cells there by killing the parasite. Flaumenhaft (2013) later on discovered that activated platelets adhere to the red blood cell by using CD36 and release PF4 which is packed with destructive granules. These granules are the ones that kill the merozoites. Platelets are able to kill the parasites in the blood serum by using its open canalicular system that helps it to engulf or filter out antigens or pathogens (Zaki, 2011). The platelets are capable of working with inflammatory cells such as neutrophils, eosinophils, basophils and monocytes in terminating the parasites. The combination of the inflammatory cells with platelets builds up a stronger immunity against malaria parasites in the San. Platelets are being used as indicators for the presence of malaria parasites as higher and normal levels indicate the absence of the

parasite and the lower measurements indicate the presence of the parasite Zaki cited McConkey (2010), (Chipfupa, 2012,{Personal communication} Zaki, 2011).

In some individuals the level of platelets is lower due to a disorder in which platelets destroy themselves making individuals at risk of getting malaria disease, but this is not the case in this study. Conglei et al., (2012) noted that the low levels of platelets were caused by some factors like autoimmune thrombocytopenia. Thrombocytopenia is a disorder by either the platelets themselves where they produce auto-antibodies that destroy themselves or when the macrophages in the spleen destroy the platelets causing thrombocytopenia. The association of thrombocytopenia and the presence of malaria parasites are an indicator for the presence of *P. falciparum* and *P.vivax* (Conglei et al., 2012).

5.5 Fungi

Different species of fungi were found on both fresh and dried food including herbs that are used as traditional medicines of the San. Many of the species identified had some metabolites that play an important role in the fight of some pathogens like bacteria and some other parasites like *Plasmodium falciparum* as it has been recently reported. It has always been known that *Penicillium* and *Aspergillus spp* have some metabolites that fight bacteria in the form of antibiotics.

The food of the San tribe did not only contain micro nutrients and antioxidants but fungi also grew on them. The San people do not store their food in modern facilities

like refrigerators or spray with fungicides. The foods are stored in the open and eaten when it's either fresh or dried. The food of the other ethnic groups has minimal fungal growth in the sense that most of it is kept in refrigerators but one would not rule out that the metabolites found in the fungi of both groups played a role in general immunity of individuals. The Kavango and Oshikoto regions have higher summer rainfalls and the heat and moisture encourages the growth of fungi. Ecologically fungi are important to the environment as they recycle nutrients into the soil through decomposition of organic matter. Fungi also affect human health in various ways (Rivera & Seifert, 2011).

Recent studies have shown that fungi can now be used as a biopesticide against *Anopheles* mosquitoes, that is a vector for *Plasmodium falciparum.* Two species that were discovered, *Beauveria bassiana* and *Metarhizium anisopliae,* have ingredients that are capable of killing the mosquito as the spores grow the mycelium through the endoskeleton into the body, killing both the mosquito and the parasite in the salivary glands.

In as much as fungi can be a nuisance it is playing a very important role in a new tool that is able to fight the parasite that has devastating results on man (Behne, 2012). Currently in Namibia DDT is being used to fight against malaria, in that it is used as indoor spray (MoHIS, 2009). The results together with treated mosquito nets are very encouraging and this strategy seems to be very effective in reducing the incidence of malaria. The use of DDT observed from other studies seems to have adverse effects

on the environment as well as in human being. It destroys the ecosystem by contaminating the environment in air, water and land. The chemicals can enter the food or water in the homes or the ponds and wells which supply water to both humans and wildlife which will have adverse effects in the body of these organisms (Van Dyk, Bouwman, Barnhoorn, Bornman, 2010). Though the effects of fungi on the San immune system have not been studied from the research above, it is believed that the fungi have a great role as endophytes as well as destroying the parasite either at erythrocyte or liver stages (Rivera & Seifert, 2011). What is required is further research on the effects of fungi and malaria parasites.

St. Leger, Joshi and Roberts, (1998) reported that *M. anisopliae* can cure malaria parasite by exposing the spore of the fungi to the mosquitoes carrying the sporozoites. The fungi do not only kill the mosquito but the sporozoites in the mosquito as well. There have not been any side effects reported by using the wild fungi but there are some doubts on the use of transgenic fungi that seem to be more effective than the wild fungi. Fungi are already in use as a bio pesticide against locusts and other agricultural pests in some African countries (Chinnock, 2011). Judging from this research there is no evidence of side effects observed in the San as they eat the fungi as part of their diet. McNeil (2005) stated that the two species of fungi that kill malaria parasites in the mosquito are harmless to humans. Fang et al., (2011) also discovered that the transgenic fungi do not only kill *Anopheles* mosquitoes but release a protein that prevents the multiplication of the parasite in the blood cells.

Therefore from the study the use of fungi as a bio pesticide or antimalarial medication on mosquito nets is highly recommended by the researcher since there seems to be no evidence of side effects from the research carried out. Fungi grow and multiply rapidly in this kind of climate that encourage the breeding of mosquitoes especially in the endemic areas of malaria disease the Sub Saharan regions. It does not cost much to grow and can be reproduced in large numbers in such a short time.

CHAPTER 6: CONCLUSIONS

Despite the fact that the San people and the other ethnic groups live in an endemic area of malaria, results from the research indicated that the San had no knowledge of malaria and had never suffered from the disease. Focus group discussion, questionnaire and experiments of the San people indicated an absence of malaria parasites in their blood samples and had no knowledge of malaria disease. The clinic data also showed no sickness or deaths caused by malaria ever recorded at the clinic of the San people. The other ethnic groups' parasites were present in some of the blood samples and had knowledge of malaria. Results from the questionnaire indicated that some respondents not only suffered from malaria but had relatives who died from the disease.

This led to further investigation on the immunity of the San people, by analyzing micronutrients and antimalarial substances found in their food and herbs. Their food was found to have antioxidants, flavonoids, Vitamin C, Zinc and Iron which boost up immunity of an individual. The San eat their food raw, without being cooked or peeled and therefore there were large quantities of micronutrients observed. The presence of these substances is assumed to have an influence on the immune system of the San. On the other hand, the other ethnic groups have to refine and cook their food before it is eaten and in this way micronutrients are lost.

The capacity of human body to adjust to adverse conditions was confirmed by the mutation of the genetic makeup of the red blood cell membranes and haemoglobin.

Blood analysis showed that the red blood cells of the San and a few of the other ethnic groups had an abnormal shape that had spikes on the surface of the red blood cells. These structures were not found in the normal red blood cells of both the San and other ethnic groups. Just like in the sickle cell the spiked shape of the red blood cell of San people confers immunity against malaria parasite. Haemoglobin C which was found in the San people plays a role in the immune system of the San. There is partial anaemia in the San people as indicated from the results where the presence of haemoglobin C was higher in the San as compared to the other ethnic groups. This disorder of the anaemia caused by the haemoglobin C in the San does not show any clinical manifestation making the San healthier than those who have sickle cell anaemia. The RBC count of the other ethnic groups was significantly higher than those of the San showing that the group studied were not anaemic.

Acquired immunity proved to be significantly important in the San people. The size of the white blood cells ($CD4^{+}$), platelets distribution width and mean platelet volume, are a requirement for both cell mediated and humoral immunity, were very high in the San people compared to the other ethnic group. One could argue that the longer exposure to the parasites has made the San to acquire both the natural and acquired immunity due to their life style. The level of IgG in both the San people and the other ethnic groups did not show a significant difference.

Fungi have been discovered to have some medicinal properties like *Pennicilium* and *Rhizopus spp* found in the San people's food. Metabolites and endophytes that boost immunity of individuals could be found in the fungi that grow on the San's food and

herbs that they eat. Storage of the food of the San people is always in the open or stored in sacks. These environmental conditions encourage fungal growth.

In conclusion, this study has shown that the immune system comes from various aspects of both the natural and acquired immune system. This is the first study that has shown empirical evidence of the San people's immunity to malaria disease in Namibia in the two regions studied, Oshikoto and Kavango. The various variables were measured both microscopically and statistically. Both t-test and chi-square verified the significance of the results confirming the knowledge that was being speculated on the immunity of the San. The unique observations noted on the presence of spikes on the RBC and the shapes of the haemoglobin C will draw attention for further research.

CHAPTER 7: RECOMMENDATIONS

Now that the San people are being relocated and some are living with the other ethnic groups, their lifestyle has changed therefore there is need to do a follow up study on the San people's immunity. A longevity study would be recommended to cover a wider area of San immunity.

Patients' records at the health centers should indicate their ethnicity so that the researchers will be able to access information on which ethnic groups are more vulnerable to diseases like malaria. These will confirm with the data collected on the field for better comparisons and conclusions. There are other minority groups in Namibia like the Himbas of Opuwo, Ovahimba, Ovazemba and Ovatue people who have a similar lifestyle to that of the San (Anaya, 2013). The empirical evidence of the above ethnic groups on malaria is not known therefore studies on their immune system should be carried out.

Nutrition of the San people has valuable nutrients which can be very beneficial to the nation and could be grown on large scale for various purposes like exporting, research and consumption. Like in the case of *Hoodia gordonii* which is now grown for commercial purposes, traditional medicines of the San can be scientifically analyzed, tested to check if there are any antimalarial properties that can be used as drugs to treat malaria and the data should be recorded. Documentation of these plants will avoid research being done repeatedly on one plant or confirm on its efficacy.

The information will also help in determining the reliability of the traditional antimalarial compounds and to compare by carrying out similar research programs.

It would be of interest if the role of haemoglobin P variant, the dark pigment observed in the San people's blood samples and the crystallization of the red blood cells of the San could further be studied.

Further studies could help if IgG could be analysed in its various components to assess which immunoglobulin is more effective against malaria parasites. Recommendation on the study of the mechanism of the immune system of the San may give a long lasting vaccine against malaria especially the transfer of the serum with IgG antibodies into a mouse or in vitro and observe the responses. Since nothing has been tried using the San blood samples in developing vaccine, it will be worth trying. The dark pigment in the blood of the San people if investigated could bring a solution to this puzzle. There could be some memory cells in the blood of the San that can be used to develop the malaria vaccine and this will trigger immunity to the recipients. This will be an addition to other research work that has been going on to find a suitable vaccine against malaria.

Development of drugs vaccines should be tried with the rudimentary apparatus that is available and with the data collected from this study there should be some answers to this problem especially against the deadliest malaria parasite *Plasmodium falciparum*. There is so much potential in this study for us to explore and utilize the knowledge that has been made available than for it to be shelved it.

It is recommended that is important make ultra-structural investigations on the spiculated red blood cells of the San group of people using scanning electron microscopy. This will help in detecting other structural details of the membranes of the red blood cells that may have an immunological effect.

The University of Namibia Malaria laboratory is too small in terms of space and manpower to carry out such an activity. The nation needs a much bigger and modern institute that is able to carry out research and train its own personnel as well as net working with other institutions regionally and internationally. The technology acquired should be decentralized to places where there is a higher incidence of malaria and in that way the nation would confidently deal with any situation as far as malaria transmission is concerned be it current or resurging of the parasite.

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APPENDICES

Appendix 1: The Questionnaire of the other ethnic groups

Malaria Questionnaire

NAME:_____

SEX_____

AGE_____

DISTRICT-_____

TRIBE:_____

1. What is Malaria?_____

_____

_____

_____

2. What are the symptoms? _____

_____

3. Have you ever suffered from malaria (if yes how many times)? _____

4. Were you taken to the hospital, which hospital?_____

5. How many members of your family suffered from malaria?_____

6. Did they all go to the hospital or clinic for treatment?_____

7.Have you or any member of your family taken traditional medicine to cure the disease?

_____

_____

8. If yes can you write down the name or names of the medicine? _____

_____

_____

9. Were they effective?

_____

10. Is malaria a common disease in your community?_____

11. Are your homes sprayed with insecticides by Government officials?_____

12. How often do they visit your homes in a year? _____

13. Are you provided with mosquito nets by the Government, do you use them?_____

14. As a community what efforts are being done to clear away the breeding places of mosquito?_____

_____

15. Do you have any relatives that died from malaria?_____

16. Which year?_____

17. What is the staple food in your community? _____

_____

Appendix 2: Percentages of respondents showing different symptoms of the other ethnic group

Fever

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	41	35.0	35.0	35.0
	Present	76	65.0	65.0	100.0
	Total	117	100.0	100.0	

Headache

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	64	54.7	54.7	54.7
	Present	53	45.3	45.3	100.0
	Total	117	100.0	100.0	

Vomiting

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	23	19.7	19.7	19.7
	Present	94	80.3	80.3	100.0
	Total	117	100.0	100.0	

Nausea

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	113	96.6	96.6	96.6
	Present	4	3.4	3.4	100.0
	Total	117	100.0	100.0	

Diarrhoea

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	89	76.1	76.1	76.1
	Present	28	23.9	23.9	100.0
	Total	117	100.0	100.0	

Flu

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	114	97.4	97.4	97.4
	Present	3	2.6	2.6	100.0
	Total	117	100.0	100.0	

Joint Pains

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	110	94.0	94.0	94.0
	Present	7	6.0	6.0	100.0
	Total	117	100.0	100.0	

Loss of Appetite

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	102	87.2	87.2	87.2
	Present	15	12.8	12.8	100.0
	Total	117	100.0	100.0	

Weak

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	111	94.9	94.9	94.9
	Present	6	5.1	5.1	100.0
	Total	117	100.0	100.0	

Anaemia

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	115	98.3	98.3	98.3
	Present	2	1.7	1.7	100.0
	Total	117	100.0	100.0	

Dizziness

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	111	94.9	94.9	94.9
	Present	6	5.1	5.1	100.0
	Total	117	100.0	100.0	

Joint Inflammation

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	115	98.3	98.3	98.3
	Present	2	1.7	1.7	100.0
	Total	117	100.0	100.0	

Sweating

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	109	93.2	93.2	93.2
	Present	8	6.8	6.8	100.0
	Total	117	100.0	100.0	

Hallucination

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	115	98.3	98.3	98.3
	Present	2	1.7	1.7	100.0
	Total	117	100.0	100.0	

Cough

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	113	96.6	96.6	96.6
	Present	4	3.4	3.4	100.0
	Total	117	100.0	100.0	

Temperature

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Normal	109	93.2	93.2	93.2
	Hot	8	6.8	6.8	100.0
	Total	117	100.0	100.0	

Blood Pressure

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Normal	116	99.1	99.1	99.1
	High	1	.9	.9	100.0
	Total	117	100.0	100.0	

State of Feeling

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Normal	93	79.5	79.5	79.5
	Cold	24	20.5	20.5	100.0
	Total	117	100.0	100.0	

Drowsiness

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	116	99.1	99.1	99.1
	Present	1	.9	.9	100.0
	Total	117	100.0	100.0	

Tiredness

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	116	99.1	99.1	99.1
	Present	1	.9	.9	100.0
	Total	117	100.0	100.0	

Shivering

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Absent	107	91.5	91.5	91.5
	Present	10	8.5	8.5	100.0
	Total	117	100.0	100.0	

Appendix 3: The results from the Questionnaire (Q3-Q16)

Q3

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	YES	64	54.7	56.1	56.1
	NO	50	42.7	43.9	100.0
	Total	114	97.4	100.0	
Missing	System	3	2.6		
Total		117	100.0		

Q4

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	YES	67	57.3	69.1	69.1
	NO	30	25.6	30.9	100.0

	Total	97	82.9	100.0	
Missing	System	20	17.1		
Total		117	100.0		

Q5

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1.00	12	10.3	13.0	13.0
	2.00	38	32.5	41.3	54.3
	3.00	13	11.1	14.1	68.5
	4.00	6	5.1	6.5	75.0
	5.00	9	7.7	9.8	84.8
	6.00	2	1.7	2.2	87.0
	7.00	5	4.3	5.4	92.4
	10.00	4	3.4	4.3	96.7
	23.00	2	1.7	2.2	98.9
	25.00	1	.9	1.1	100.0
	Total	92	78.6	100.0	
Missing	System	25	21.4		
Total		117	100.0		

Q6

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	YES	88	75.2	85.4	85.4
	NO	15	12.8	14.6	100.0
	Total	103	88.0	100.0	
Missing	System	14	12.0		
Total		117	100.0		

Q7

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	YES	15	12.8	13.6	13.6
	NO	95	81.2	86.4	100.0
	Total	110	94.0	100.0	
Missing	System	7	6.0		
Total		117	100.0		

Q10

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	YES	95	81.2	81.9	81.9
	NO	21	17.9	18.1	100.0
	Total	116	99.1	100.0	
Missing	System	1	.9		
Total		117	100.0		

Q11

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	YES	90	76.9	78.9	78.9
	NO	24	20.5	21.1	100.0
	Total	114	97.4	100.0	
Missing	System	3	2.6		
Total		117	100.0		

Q12

Number of times sprayed		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1.00	75	64.1	75.0	75.0
	2.00	18	15.4	18.0	93.0
	3.00	4	3.4	4.0	97.0
	4.00	1	.9	1.0	98.0
	7.00	1	.9	1.0	99.0
	12.00	1	.9	1.0	100.0
	Total	100	85.5	100.0	
Missing	System	17	14.5		
Total		117	100.0		

Q13

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	YES	74	63.2	65.5	65.5
	NO	39	33.3	34.5	100.0
	Total	113	96.6	100.0	
Missing	System	4	3.4		
Total		117	100.0		

Q15

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	YES	39	33.3	34.5	34.5
	NO	74	63.2	65.5	100.0
	Total	113	96.6	100.0	
Missing	System	4	3.4		
Total		117	100.0		

Q16

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	1993.00	1	.9	2.6	2.6
	1996.00	1	.9	2.6	5.1
	1997.00	1	.9	2.6	7.7
	1998.00	2	1.7	5.1	12.8
	1999.00	3	2.6	7.7	20.5
	2000.00	6	5.1	15.4	35.9
	2001.00	1	.9	2.6	38.5
	2002.00	2	1.7	5.1	43.6
	2003.00	3	2.6	7.7	51.3

	2004.00	2	1.7	5.1	56.4
	2005.00	3	2.6	7.7	64.1
	2006.00	4	3.4	10.3	74.4
	2007.00	2	1.7	5.1	79.5
	2008.00	5	4.3	12.8	92.3
	2009.00	1	.9	2.6	94.9
	2010.00	2	1.7	5.1	100.0
	Total	39	33.3	100.0	
Missing	System	78	66.7		
Total		117	100.0		

Appendix 4: Summary of focus group discussion on the knowledge of Malaria with the San people in both Mangetti post and Bravo (2009).

Q: Do they know what malaria is?

A: Malaria causes headaches.

- ✓ After explaining the symptoms to them

A: They have seen people having fever but for a long time they did not know it

- ✓ It's not close

Q: Do they ever fall sick?

A: Isolated cases- TB /AIDS now

Q: Do you take any medication?

A: Traditional herbs and if it persist they now go to the clinic women are the ones that gather the herbs

Q: Are they still hunting?

A: Yes, but restricted and arrested by commercial farmers

- ✓ The Oshiwambos restrict them from the occupied places
- ✓ They have been restricted to a small space

Q: Why are some of their homesteads abandoned?

A: Yes but they were told by the Government to abandon them because of winter and rain. Some of our relatives remained in the bush

Q: Are they happy about the relocation?

A: There are not happy about it, but no choice but to please the Government

Dress code

Q Your old dressing code what were your clothes made of?

A From animal hides that men made.

Q Why have you stopped dressing like you used to?

A Our parents – we started looking for jobs and needed clothes and blankets. The employers gave them them to us.

Kind of Jobs

Q What are you doing for life?

A Hunting or gathering and taking care of goats and cattle of farmers who occupied our land. Some of the San people scare off birds and baboons from big fields as an occupation but they do not till the land.

Q: What is the impact of this change?

A: This is not our lifestyle but we are forced. The Government has been providing food and clothes; we just wait for the handouts and are very sad with this change.

The handouts only come after probably a year, meanwhile – they go back to hunting and gathering.

Food

Q: What is your staple food?

A: Wild fruits, roots, tubers, some seeds of leguminous plants meat, spinach

Q: Have you adjusted to Mahangu, maize and sorghum?

A: They are gradually adjusting though it has not been easy, its now eating for survival.

Q: How do you store your food?

A: Dry continuously in the open and keep in empty bags that contained food hand outs.

Malaria

Q: Are there any mosquitoes in the area?

A: Plenty, they bite us a lot especially at night.

Q: How do you protect yourselves from the mosquito bites?

A: We used to use some tree barks and shrubs that we burned to repel the mosquitoes away. These days, there have been restrictions from using these repellants by the new owners of the land.

Q: Did the mosquito repellants work?

A: Sometimes.

Q: Do they know what malaria disease is?

A: Most of them were not aware of the disease.

Q: Did they know that mosquitoes can cause malaria?

A: It causes headaches only,

Q: Do you go to the hospital or clinic when you are ill

A: "They also do not go to the hospital or clinics due to lack of transport and traditionally, they depend on herbs for treatment of various ailments.

Q: Has any member of the community died of malaria?

A: No, but of late some of our people are dying from TB.

Q: Do they get mosquito nets?

A: No they have no communication with Government officials except for those who are living in Tsintsabis Center.

Q: If they were given, would they use them?

A: Yes

Q: Where are the women or men (depending on the day and/or where the discussion was conducted)?

A: Women had gone to look for food (like in Bravo)

A: Men had gone hunting or drinking (like in Mangetti)

Q: What has made you survive up until now with this kind of life style?

A: God, The Creator of mankind.

School

Q Do your children go to school?

A: Mangetti Post had their own traditional bush school to which they sent their children. In Bravo and Tsinstabis, there are Government schools to which some of the children attend.

Water

Q: Where do you get your water from?

A: At water points that are approximately 2.3 km away

A short interview carried out on Thimbukushu man from Kavango who relocated to Mangetti Post living amongst the San.

Q: Do you suffer from malaria?

A: Yes frequently in fact my wife is in bed suffering from malaria?

Q: Do they as a family take medication?

A: Yes both traditional as well as tablets from Tsintsabis Clinic.

Appendix 5: The assessment of different micro elements in the San foods at the analytical laboratory in Windhoek.

Test / Sample I.D.	Plant part analysed	Moisture % m/m	Iron as Fe mg/100 g	Zinc as Zn mg/100g	Vitamin C mg/100g
1. *Pentarrtinum insipidum*	seeds	73.5	5.5	2.0	-
2. *Ceropegia tentaculata*	tuber	76.0	3.6	0.4	-
3. *Hypaene petersiana*	Leaves	61.5	3.3	0.7	-
4. *Amaranthus petersiana*	Leaves	84.8	1.5	0.6	-
5. *Vangueria infausta*	Fruit	59.6	66	1.7	-
6. *Fockea angustifolia*	Tuber	53.4	0.5	0.7	41
7. *Maeru schinzii*	Tuber	80.5	1.6	0.3	9

8. *Lapeirousia coeculea*	Tuber	80.0	1.1	0.5	12
9. *Sclerocarya birrea*	Flesh	88.3	0.4	0.3	237
10. *Grewia bicolor var. bicolor*	Fruit	79.9	0.4	0.1	6
11. *Bauhinia macrantha*	Fruit	61.6	0.7	0.5	12
12. *Corallocarpus triangularis*	Tuber	73.5	1.1	0.3	1
13. *Citrillus sp.*	Fruit	92.4	0.3	0.2	8
14. *Citiullus Ianutus*	Fruit	96.4	0.3	0.1	10
15. *Pennisetum glaucum*	Seeds	12.0	1.0	3.2	1

Appendix 6: The assessment of different micro elements in the San foods at the Biochemistry laboratory at the University of Namibia

	Abs				
Test tube	Trial 1	Trial 2	Trial 3	Average	% inh.
Control	0.933	0.932	0.932	0.932	0
S_1	0.424	0.425	0.425	0.425	54
S_2	0.068	0.068	0.068	0.068	93
S_3	0.158	0.160	0.161	0.160	83
S_4	0.424	0.423	0.424	0.424	55
S_5	0.470	0.472	0.472	0.471	49
S_6	0.347	0.347	0.347	0.347	63

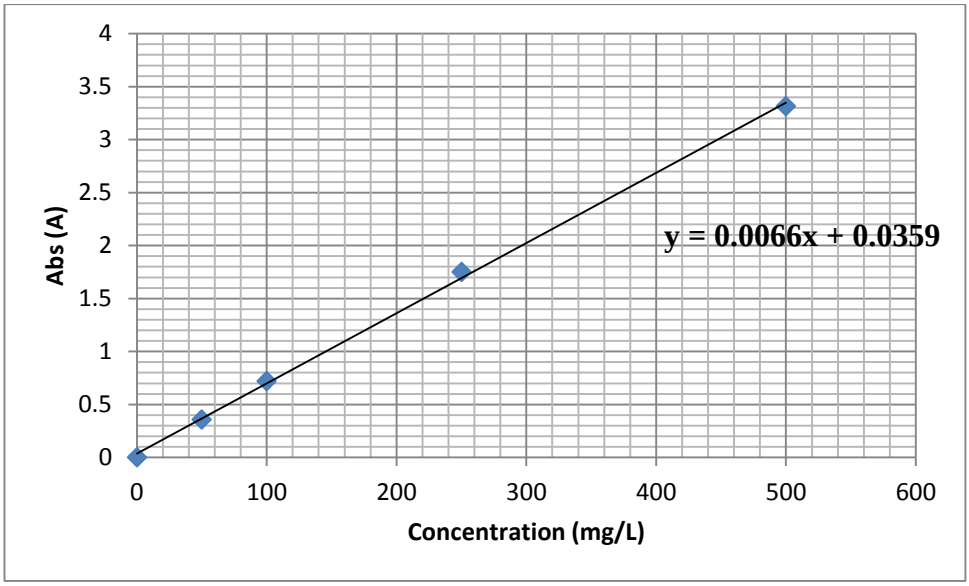
S_7	0.941	0.942	0.942	0.942	1
S_8	0.146	0.146	0.142	0.145	84
S_9	0.931	0.932	0.932	0.932	0
S_{10}	0.700	0.700	0.700	0.700	25
S_{11}	0.119	0.120	0.119	0.119	87
S_{12}	0.144	0.144	0.145	0.144	85

Appendix 7: (A) Antioxidant results from the University of Namibia Biochemistry laboratory

Test tube	Trial 1	Trial 2	Trial 3	Average
St1 (50 mg/L)	0.356	0.356	0.356	0.356
St 2 (100 mg/L)	0.719	0.718	0.719	0.719
St 3 (250 mg/L)	1.751	1.750	1.751	1.751
St 4 (500 mg/L)	3.316	3.316	3.315	3.316
S_1	1.735	1.735	1.735	1.735
S_2	1.231	1.231	1.230	1.231
S_3	5.999	6.000	5.999	5.999
S_4	0.432	0.433	0.433	0.433
S_5	0.227	0.228	0.228	0.228
S_6	0.765	0.766	0.766	0.766

S_7	0.138	0.139	0.139	0.139
S_8	1.765	1.766	1.766	1.766
S_9	0.109	0.110	0.110	0.110
S_{10}	0.070	0.071	0.071	0.071
S_{11}	0.424	0.425	0.425	0.425
S_{12}	2.555	2.556	2.557	2.556

Appendix 7: (B) Phenolic results from Unam Biochemistry laboratory



Calibration curve from which the results of the sample were calculated.

Appendix 7: (C) Phenolic results calculated from calibration curve at Unam Biochemistry Laboratory

Sample Nº	Concentration from curve	Real concentration (mg/L)	Real concentration (mg/100g)
S_1	257.439	261.610	26
S_2	181.076	185.615	19
S_3	903.500	904.554	90
S_4	60.167	65.290	7
S_5	29.106	34.379	3
S_6	110.621	115.501	12
S_7	15.621	20.959	2
S_8	262.136	266.285	27
S_9	11.227	16.586	2
S_{10}	5.318	10.706	1
S_{11}	58.955	64.083	6
S_{12}	381.833	385.404	39

Appendix 8: Machine used for full blood count in Tsumeb NIP

PENTRA XL 80

Features

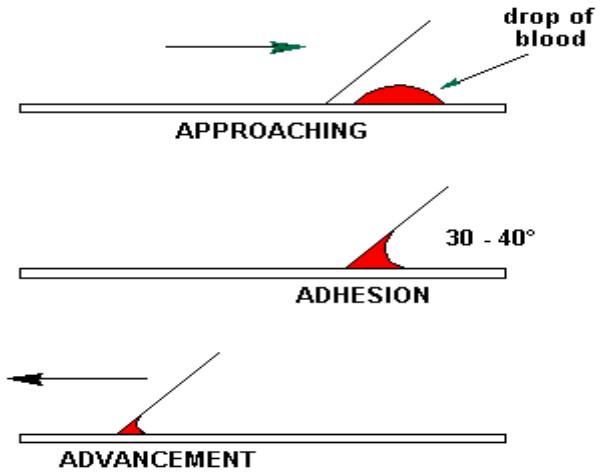
Pentra XL 80

- Throughput: Up to 80 samples/hour
- Large capacity auto-loader (100 tubes)
- Stat sampling on open or closed tubes
- Automatic Sample Re-run
- Reagents: Only 4 onboard reagents and 1 diluent
- Perfect differentiation of the 5 WBC sub-populations with DHSS* Technology
- 3 histograms for RBC, BAS/WBC and PLT together with the 5 DIFF Matrix.
- Basophils counted through specific channel
- High resolution matrix includes the determination of 2 additional subpopulations (% and #): Atypical Lymphocytes (ALY***) and Large Immature Cells (LIC***)
- Customized Dilution Ratio (CDR)
- Integrated Validation Station

Appendix 9: Taking the Blood

Cleanse a finger. With a sterile lancet, make a puncture on a fingertip. If you have difficulties in doing this, you can wait until you have a casual wound. In the meantime, keep all the materials needed ready and protected from dust, particularly the clean microscope slides.

Making the Smear



Picture acessed on 15/04/04 from

www.funsci.com/fun3_en/blood/blood_07.gif

How to prepare a blood smear

Place a small drop of blood near an end of a slide. According to the figure above, bring the edge of another slide in contact with the drop and allow the drop to bank evenly behind the spreader. The angle between the two slides has to be 30-40 degrees. Now, push to the left in a smooth, quick motion. The smear should cover about half the slide. It is important that the quantity of blood is not excessive; otherwise the red cells could hide the leukocytes. So, if you succeed in making a gradual transition from thick to thin in your smear, you should get a zone with a satisfactory distribution of cells. With a single drop of blood, you can make several smears. In fact, to make a smear, it is enough to leave a spot of blood of 3 mm about in diameter on the slide. It is useful to perform many smears. In fact, not always they

are successful, and with some attempts, it is easier to get one well prepared. To avoid producing clots, you must make each smear with fresh blood and straight after having deposited it. To this purpose, it is useful to be helped by another person where one deposits the blood, and the other makes the smears. With the microscope, you should observe the smears to check that some of them are properly made. The red cells must not overlap each other, nor be so scarce as to be too spread out (Tagliasacchi & Carboni, 1997).

Appendix 10 Full blood cell count of the samples by Gender

Blood Component	Mean	95% Confidence Interval	Normal Level	Units
RBC				
Male	4.71	4.52 - 4.89	4.32 – 5.72	$10^{6/}mm^3$
Female	4.59	4.50-4.71	3.90 - 5.03	
HB				g/dL
Male	13.46	12.94-13.97	13.5 - 17.5	g/dL
Female	13.42	13.04-13.81	12.0 -15.5	
HCT				
Male	39.45	36.10-40.80	38.8 - 50.0	%
Female	39.47	38.49-40.45	34.9-44.5	
MCV				

Male	84.58	82.03-87.14	83.00-100.00	μm^3
Female	85.89	84.31-87.47	83-107	
MCH				
Male	33.97	23.69-44.25	27.00-32.00	pg/cell
Female	29.20	28.54-29.86	27.00-32.00	
MCHC				
Male	34.06	33.79-34.33	315-345	g/dL
Female	33.94	33.73-34.10	315-345	
RDW				
Male	12.58	12.14-13.02		%
Female	12.32	12.03-12.61		
PLT				
Male	277.73	248.83-306.63	150-450	$10^3/mm^3$
Female	274.72	255.01-294.44	150-350	
MPV				
Male	9.53	9.26-9.80		μm^3
Female	9.53	9.32-9.73		
PCT				
Male	0.26	0.24-0.28		%
Female	0.26	0.24-0.27		
PDW				

Male	16.97	16.15-17.78		%
Female	16.87	16.22-17.51		
WBC				
Male	6.96	6.29-7.63	3.5-10.0	$10^3/mm^3$
Female	7.54	7.09-7.99	3.5-10.5	
NEU				
Male	31.91	28.69-35.13	2.0-7.0	$10^3/mm^3$
Female	37.74	33.45-42.03	2.0-7.0	
LYM				$10^3/mm^3$
Male	48.95	46.20-51.69	1.0-3.0	
Female	45.59	43.73-47.45	1.0-3.0	
MON				
Male	10.12	9.49-10.75	0.2-1.0	$10^3/mm^3$
Female	9.26	8.83-9.69	0.2-1.0	
EOS				
Male	7.72	5.85-9.59	0.02-0.5	$10^3/mm^3$
Female	8.10	6.70-9.51	0.02-0.5	
BAS				
Male	1.30	1.11-1.49	0.05-0.1	$10^3/mm^3$
Female	1.40	1.20-1.61	0.05-0.1	

ALY				
Male	1.53	1.43-1.63		$10^3/mm^3$
Female	1.52	1.44-158		
LIC				$10^3/mm^3$
Male	1.15	0.96-1.35		
Female	1.09	0.97-1.21		
IgG				
Male	17.15	15.71-18.58	688-1251	g/l
Female	17.47	16.62-18.33	720-1038	

Appendix 11: Sample Results from NIP Laboratory

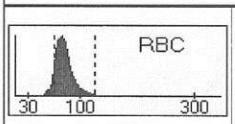
158

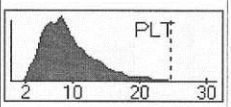
NIP TSUMEB S/N 101 PXL 4575

Report Printout

Validated

Final report date 04/04/2012 10:07:55	Sample ID 500089146	Collection Date
Type Standard	Department	Physician
Comments		
Patient ID	Patient Name	First Name
Date of Birth	Age	Gender Unknown
Comments		
Operator NIP TSUMEB		

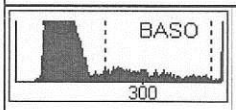




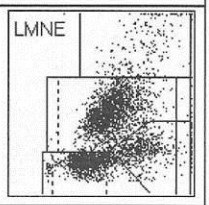
RBC	5.29	$10^6/mm^3$
HGB	13.8	g/dL
HCT	40.1	%
MCV	76 l	μm^3
MCH	26.2 l	pg
MCHC	34.5	g/dL
RDW	11.9	%
PLT	273	$10^3/mm^3$
MPV	9.5	μm^3
PCT	0.258	%
PDW	17.8	%

Flags and Alarms

Morphology Flags
MIC
Analyzer Alarms
LMNE+
Suspected Pathology
Neutropenia
NRBCs
Basophilia
Microcytes
Platelet Aggregates
Remarks
RBC of the Run 04/04/2012 10:07:54
WBC of the Run 04/04/2012 10:07:54
PLT of the Run 04/04/2012 10:07:54
DIFF of the Run 04/04/2012 10:07:54
Reagent Expired



WBC	4.5*	$10^3/mm^3$	
	%	#	
NEU	37.0!	1.66*L	
LYM	33.2!	1.49*	
MON	9.5!	0.43*	
EOS	4.4!	0.20*	
BAS	15.9!	0.71*H	
ALY	2.4!	0.11*	
LIC	1.1!	0.05*	



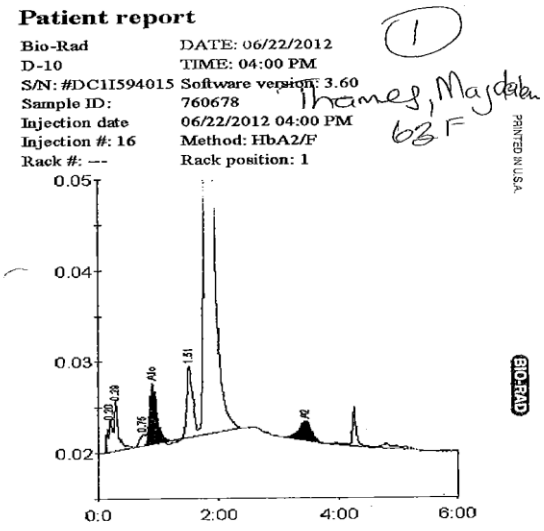
Microscopic Examination

	+	++	+++		%	#		
Anisocytosis	☐	☐	☐	Neutrophils			Metamyelocytes	
Hypochromia	☐	☐	☐	Bands			Myelocytes	
Polychromasia	☐	☐	☐	Lymphocytes			Promyelocytes	
Poikilocytosis	☐	☐	☐	Monocytes			Blast	
Microcytosis	☐	☐	☐	Eosinophils			ATY LYM	
Macrocytosis	☐	☐	☐	Basophils			Other	
PLT Clumps	☐	☐	☐				Total(100%)	
							NRBC's	

Out of Panic Range XX.X* Out of Normal Range XX.X

Printed on 04/04/2012 10:07:59 Operator : NIP TSUMEB Page 1

Appendix 12: The results of Hbvariances of one of the partcipants blood sample analysed.

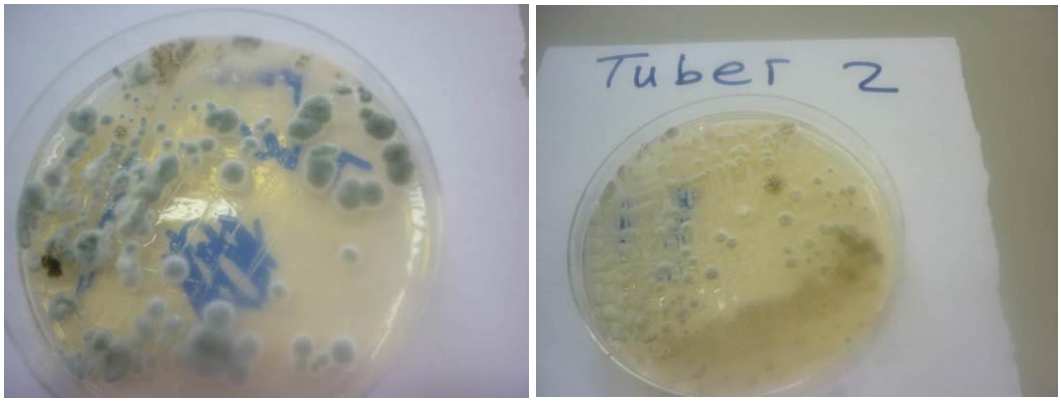


Peak table - ID: 760678

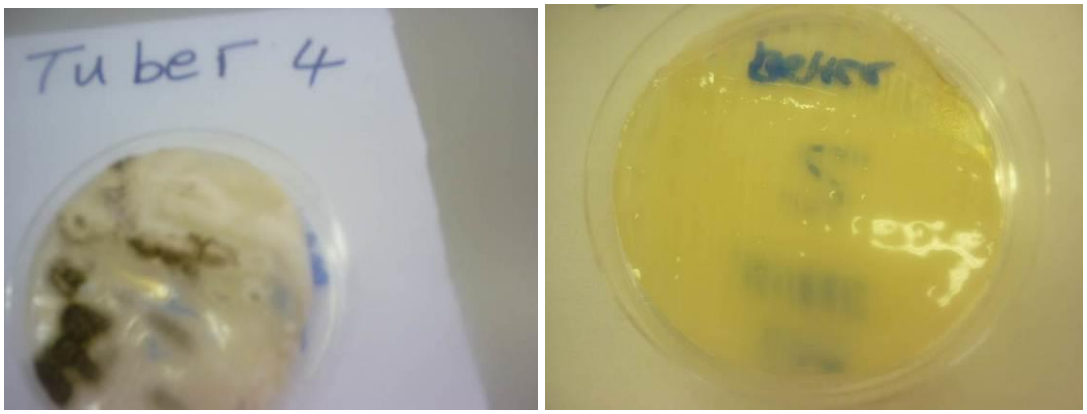
Peak	R.time	Height	Area	Area %
A1a	0.20	3642	20469	1.5
A1b	0.29	5716	20899	1.5
LA1c/CHb-1	0.75	1283	7933	0.6
A1c	0.90	6674	57631	6.0
P3	1.51	7834	53632	3.8
A0	1.80	194283	1208685	86.3
A2	3.44	2114	31149	2.4
Total Area:			1400397	

Concentration:	%
A1c	6.0
A2	2.4

Appendix 13: The diagrams below shows fungi from the San food nutrient



(a)



(b)

Appendix 14a, b and c; Reference letters from the Head of Biological Sciences at the University of Namibia addressed to various authorities.

a.

UNIVERSITY OF NAMIBIA

Private Bag 13301, 340 Mandume Ndemufayo Avenue, Pionierspark, Windhoek, Namibia

Department of Biological Sciences

20 April 2010

TO WHOM IT MAY CONCERN

Ms. Catherine Amoo is a lecturer in the Department of Biological Sciences at the University of Namibia She is also a PhD candidate and is currently doing her research on Malaria, and is particularly interested in the Oshikoto Region.

I would therefore like to request you and your good office to afford Ms Amoo all the help and assistance she requires, in order to support her in this very important endeavour.

I would like to use this opportunity to thank you sincerely for your help and support.

Should you have any question or would like further information, please, do not hesitate to contact the undersigned.

Sincerely

Dr. R. Bock
Head of Department
Department of Biological Sciences
University of Namibia

Tel. 061-2063423
Fax: 061-2063791
Cell: 0814328757
Email: rbock@unam.na

DEPARTMENT OF BIOLOGICAL SCIENCES
UNIVERSITY OF NAMIBIA
Private Bag 13301, Windhoek

b.

UNIVERSITY OF NAMIBIA

Private Bag 13301, 340 Mandume Ndemufayo Avenue, Pionierspark, Windhoek, Namibia

Department of Biological Sciences

10 October 2012

TO WHOM IT MAY CONCERN

Ms. Catherine Amoo is a registered PhD candidate in the Department of Biological Sciences at the University of Namibia. Ms Amoo's research is comparing the occurrence of malaria among the San people in comparison to other Namibian groups, to establish a link between nutrition and apparent resistance to malaria.

I would therefore like to request you and your good office to afford Ms Amoo all the help and assistance she requires, in particular access to health statistics, in order to support her in this very important endeavour.

I would like to use this opportunity to thank you sincerely for your help and support.

Should you have any question or would like further information, please, do not hesitate to contact the undersigned.

Sincerely

DEPARTMENT OF BIOLOGICAL SCIENCES
UNIVERSITY OF NAMIBIA
Private Bag 13301, Windhoek

Dr. R. Bock
Department of Biological Sciences
University of Namibia

Tel. 061-2063423
Fax: 061-2063791
Cell: 0814328757
Email: rbock@unam.na

c.

Appendix 15: A permit to carry out the study from the ministry of Health Social Services

9 - 0/0001

REPUBLIC OF NAMIBIA

Ministry of Health and Social Services

Private Bag 13198	**Ministerial Building**	**Tel: (061) 2032510**
Windhoek	**Harvey Street**	**Fax: (061) 227786**
Namibia	**Windhoek**	**E-mail: eshaama@mhss.gov.na**
Enquiries: Ms. E.N. Shaama	**Ref.:** 17/3/3	**Date:** 04 August 2011

OFFICE OF THE PERMANENT SECRETARY

Ms. Chipo Catherine Amoo
University of Namibia
P/Bag 13301
Windhoek

Dear Ms. Amoo

Re: A study of the apparent immunity to Malaria among the San people in Tsumeb area of Oshikoto and Kavango regions of Namibia

1. Reference is made to your application to conduct the above-mentioned study.

2. The proposal has been evaluated and found to have merit.

3. **Kindly be informed that permission to conduct the study has been granted under the following conditions:**

3.1 The data to be collected must only be used for completion of your PhD Degree;
3.2 No other data should be collected other than the data stated in the proposal;
3.3 A quarterly report to be submitted to the Ministry's Research Unit;
3.4 Preliminary findings to be submitted upon completion of study;
3.5 Final report to be submitted upon completion of the study;
3.6 Separate permission should be sought from the Ministry for the publication of the findings.

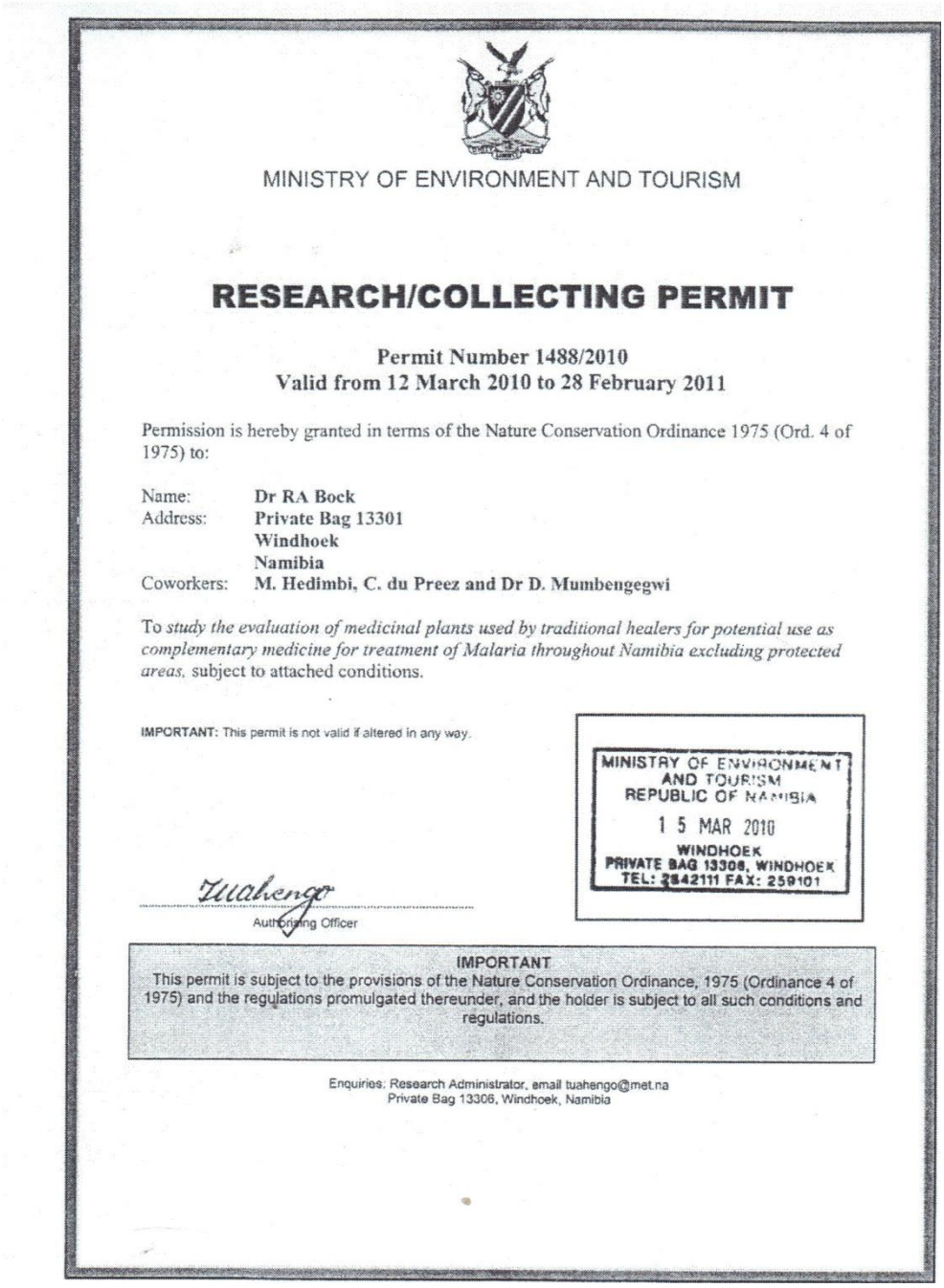
Yours sincerely,

MR. K. KAHUURE
PERMANENT SECRETARY

"Health for All"

Appendix 16: Plant collection permit

Appendix five: Plants collection permit and pictures of plants



MINISTRY OF ENVIRONMENT AND TOURISM

RESEARCH/COLLECTING PERMIT

Permit Number 1488/2010
Valid from 12 March 2010 to 28 February 2011

Permission is hereby granted in terms of the Nature Conservation Ordinance 1975 (Ord. 4 of 1975) to:

Name: **Dr RA Bock**
Address: **Private Bag 13301**
Windhoek
Namibia
Coworkers: **M. Hedimbi, C. du Preez and Dr D. Mumbengegwi**

To *study the evaluation of medicinal plants used by traditional healers for potential use as complementary medicine for treatment of Malaria throughout Namibia excluding protected areas,* subject to attached conditions.

IMPORTANT: This permit is not valid if altered in any way.

MINISTRY OF ENVIRONMENT AND TOURISM
REPUBLIC OF NAMIBIA
15 MAR 2010
WINDHOEK
PRIVATE BAG 13306, WINDHOEK
TEL: 2842111 FAX: 259101

Tuahengo
Authorising Officer

IMPORTANT
This permit is subject to the provisions of the Nature Conservation Ordinance, 1975 (Ordinance 4 of 1975) and the regulations promulgated thereunder, and the holder is subject to all such conditions and regulations.

Enquiries: Research Administrator, email tuahengo@met.na
Private Bag 13306, Windhoek, Namibia

Appendix 17: Permit from Namibia Institute of Pathology

2.2 **MS CHIPO CATHERINE AMOO**

Shall pay NIP for services provided upon presentation of an invoice;

DATED at Windhoek this 30th day of January 2012

AS WITNESS

1. _____
2. _____

_____
Harold T. Kaura

General Manager: TO for and on behalf of NIP

AS WITNESS

1. _____
2. _____

_____
Ms Chipo Catherine Amoo

NAMIBIA INSTITUTE OF PATHOLOGY (NIP) LTD
HEAD OFFICE
2012 -02- 14
PO BOX 277 WINDHOEK
TEL: +264 - 61 - 2954200
FAX: +264 - 61 - 255566
REPUBLIC OF NAMIBIA

1. DEFINITIONS

In this Memorandum of Agreement, unless the context otherwise indicates, the following terms shall have the meaning set out hereunder unless otherwise expressly stated.

1.1 "MoHSS' – Shall mean the Ministry of Health and Social Services

1.2 "NIP" – Shall mean the Namibia Institute of Pathology

1.3 "MOA" – Shall mean the Memorandum of Agreement

1.4 "NAMAF" – Shall mean the Namibia Association of Medical Aid Funders

2. OBLIGATIONS OF THE PARTIES:

2.1 NIP

Shall provide diagnostic testing on specimens provided by Ms Chipo Catherine Amoo at the following laboratories: Rundu and Tsumeb;

Shall provide the following testing(Full Blood Count (FBC); Malaria parasite identification; IgG) on a total specimen of approximatel 150-200;

Shall invoice Ms Chipo Catherine Amoo for services provided at NAMAF tariff rate;

MEMORANDUM OF AGREEMENT

ENTERED INTO

BY AND BETWEEN

THE NAMIBIA INSTITUTE OF PATHOLOGY (NIP) LTD

(Company Registration No: 2000/431)

AND

MS CHIPO CATHERINE AMOO

(Id No:BN706363)

(Jointly hereinafter collectively referred to as "Parties")

PREAMBLE:

WHEREAS Ms Chipo Catherine Amoo has submitted a research proposal to the MoHSS Research Committee for approval;

WHEREAS this research proposal was approved by the MoHSS Research Committee;

WHEREAS Ms Chipo Catherine Amoo requested the use of NIP laboratories in Tsumeb and Rundu;

WHEREAS The Namibia Institute of Pathology has agreed to carry out laboratory testing on samples collected by Ms Chipo Catherine Amoo for her PhD thesis;

WHEREAS the parties wish to record the terms and conditions which will govern and regulate the relationship;

NOW THEREFORE the parties agree as follows:

C.C.

Appendix 18: Lancet laboratories, Johannesburg, South Africa

LANCET LABORATORIES

www.lancet.co.za

	JOHANNESBURG	PRETORIA	DUR
Switchboard	(011) 358 0600	(012) 483 0100	(031
Client Services	(011) 358 0888	(012) 483 0110	(031

Po Box 8477, Johannesburg, 2000

Quotation Number : 2516119 **20-Jun-1**

TRADING TERMS AND CONDITIONS OF LANCET LABORATORIES PTY LTD

CONTRACT: The contract for the supply of the goods incorporates all of these conditions and the customer's written or verbal order is deemed to be an acceptance hereof.

ITC/CIPRO: All applications will undergo ITC/Cipro approvals.

STOCK: In the event of Lancet Laboratories providing stock, the samples must be returned to Lancet Laboratories

PRICES: Prices are based on the Company's acquisition price and is affected by the Rand/US Dollar exchange rate. Therefore prices are subject to change without notice.

METHOD OF PAYMENT: Eft payments and cheques are accepted.

CREDIT TERMS: The Company's credit terms are strictly 30days from date of invoice.
Interest will be raised on all accounts over 60 days, calculated at the ruling prime overdraft rates two point five percentage points (2.5%).

I/We warrant that the Directors/Proprietors/Partners have never been insolvent or associated with any business failure.

I/We acknowledge that should credit facilities be granted as a result of this application that they may be withdrawn by the Company at any time without prior notice and that the decisions as to whether or not to grant credit facilities to the purchaser as at the sole discretion of the Company.

I/We the undersigned do hereby warrant that all the information recorded in this application is true and correct, that I/WE sign of my/our free will and with full knowledge and understanding of the contents thereof, and that I/We are daily authorised in doing so.

I/WE DO HEREBY ACCEPT THE TRADING TERMS AND CONDITIONS OF SUPPLY AS SET OUT ABOVE AND ACKNOWLEDGE THAT I HAVE READ AND UNDERSTOOD THE CONTENTS THEREOF.

C. AMOO

Authorised Signature **Name in Block Letters and Capacity**

Appendix 19: Letter of consent sent to parents of Otjikoto Senior School learners

To donate blood nurses from Tsumeb hospital and Tsintsabis volunteered to collect the blood samples from their communities. Equipment came from NIP as part of the payment. At Otjikoto Secondary, the chiefs and elders in the communities were made aware of the intention to collect blood from individuals for the research well in advance and verbal consent sought from them. This gave them time to think about it before get permission from MoHSS. Two application letters were written one to the Ministry of Health and Social Services to request for a permit to collect blood from the Oshikoto and Okavango regions and the other was sent to Namibia Pathological Institute (NIP) for blood analysis since UNAM laboratories were not built to handle such large volumes of blood samples. A contract had to be signed between NIP and myself for the analysis of blood. Both permits were granted. 183 blood samples were collected blood in two vacutainers glass tubes, one for whole blood and the other for plasma.80 samples from the San in Tsintsabis and 50 samples from Bravo communities. The other 50 came from the other group as a control. School children from Otjikoto Secondary School in Tsumeb volunteered to donate blood and a written consent form from the Headmistress was given to them to give to their parents for their approval by signing to allow the students to donate blood. A total of 50 students came forward. School a donation in the form of money was given to both the school and the students who donated blood. The same was done to the other communities in Tsintsabis and Bravo were given groceries to the households that came.

Appendix 20: Authorization of parents to draw blood from the learners.

OTJIKOTO SENIOR SECONDARY SCHOOL

PRIVATE BAG 2003, TSUMEB TEl: (067) 220391 FAX: (067) 221559 E-Mail: otjikoto@iway.na

REF. NO: 703

ENQUIRIES: MS. E.M. KAONDE DATE: 30 MARCH 2012

Dear parent

RE: Authorization to draw blood from your child for research purposes

This letter seeks permission from you as a parent for your child to participate in this exercise on Tuesday, 03 April 2012 at school. The purpose of this exercise is to help a PHD student, Ms Chipo Catherine Amoo , from the university of Namibia to carry out her research . She is investigating the apparent immunity to malaria among the san people in Oshikoto [Tsumeb area] and Okavango regions of Namibia, respectively.

Studies such as this one are very vital for the development of the country. Your assistance in this regard will therefore be highly appreciated.

Yours faithfully

H.H Shingo
Acting Principal

MINISTRY OF EDUCATION
OTJIKOTO SENIOR SEC. SCHOOL
30 MAR 2012
Private Bag 2003, TSUMEB
Tel: 067 - 220 391 / Fax: 067 - 221 559
OSHIKOTO REGION

Name of Learner

Signature of Learner

Name of Parent

Signature of Parent

Appendix 21: Summary of Independent samples T-Test results

Name of Blood Component	**Groups of People**	**Means**	**Mean Difference**	**P Value**	**Confidence Interval**
Red Blood Cells	San	4.4576	-0.50122	0.000	-0.64, -0.36
	Other ethnic groups	4.9588			
Haemoglobin	San	13.0558	-0.84421	0.001	-1.35, -0.34
	Other ethnic groups	13.9000			
Hematocrit	San	38.0859	-2.81611	0.000	-4.23, -1.40
	Other ethnic groups	40.9020			
Mean Cell Volume	San	86.2526	3.27263	0.005	0.98, 5.57
	Other ethnic groups	82.9800			

Mean Corpuscular Haemoglobin	San	33.8811	3.66789	0.151	-1.35, 8.69
	Other ethnic groups	33.9460			
Red cell Distribution Width	San	12.3979	-0.6495	0.667	-0.36, 0.23
	Other ethnic groups	12.4968			
Platelets	San	267.2947	-0.9892	0.664	-0.55, 0.35
	Other ethnic groups	293.9400			
Mean Platelet Volume	San	9.6947	-26.64526	0.052	-53.5, 0.22
	Other ethnic groups	9.2060			
Platelet Crit (% volume of blood occupied	San	0.2545	0.48874	0.000	0.24, 0.74
	Other ethnic	0.2680			

by Platelets)	groups				
Platelet Distribuiton Width	San	17.3011	-0.01359	0.250	-0.04, 0.01
	Other ethnic groups	15.8240			
White Blood Cells	San	7.9474	1.47705	0.001	0.61, 2.35
	Other ethnic groups	6.3620			
Neutrophils	San	34.0189	1.58537	0.000	1.01, 2.12
	Other ethnic groups	37.1014			
Lymphocytes	San	45.6179	-3.08245	0.183	-7.63, 1.42
	Other ethnic groups	48.5160			
Monocytes	San	9.8937	-2.89811	0.023	-5.38, -0.41

	Other ethnic groups	8.7160			
Eosinophils	San	10.4011	1.17768	0.000	0.57, 1.78
	Other ethnic groups	3.7634			
Basophils	San	1.6126	6.63765	0.000	5.12, 8.16
	Other ethnic groups	0.8928			
AlymphoPlastic Cells	San	1.5074	0.71983	0.000	0.50, 0.94
	Other ethnic groups	1.5160			
Large Immature Corpuscles	San	1.2260	-0.00863	0.861	-0.11, 0.09
	Other ethnic groups	0.9340			

Immunoglobin G	San	17.2714	0.29200	0.001	0.12, 0.46
	Other ethnic groups	17.1944			

Appendix 22: The existence of the Malaria Parasite in the blood samples of the San and other ethnic groups

Groups of People		**Existence of Malaria Parasite**		**Total**
		Yes	**No**	
San people	**Count**	0	100	100
Other ethnic Groups	**Count**	7	93	100

Appendix 23: The shapes of the red blood cell of the San and other ethnic groups

Ethnic group	**Shapes of red blood cell**		**Total**
	Normal	**Abnormal**	
San	18	82	100

Other ethnic groups	79	21	100

Appendix 24: The count of the existence of spikes on the Red blood cells

Groups of People	**Existence of Spikes on the surface of the red blood cells**		**Total**
	Yes	**No**	
San people	80	20	100
Other ethnic Groups	20	80	100

Appendix 25: The percentages of the presence of HbC in the RBC.

		Presence of Hbc		**Total count**
		% No	**% Yes**	
Respondents Ethnicity	**San**	47	53	100
	Other Ethnic Groups	69	31	100

Appendix 26: Limitations of the study

1. The financial resources.
2. Language barrier.
3. Accessibility to the groups as they are nomadic.
4. Patients who visit hospitals are not admitted according to tribes
5. Identification papers for most of the San people were non-existent.
6. Other ethnic groups were not really forth coming with information and donating blood.

Annexures

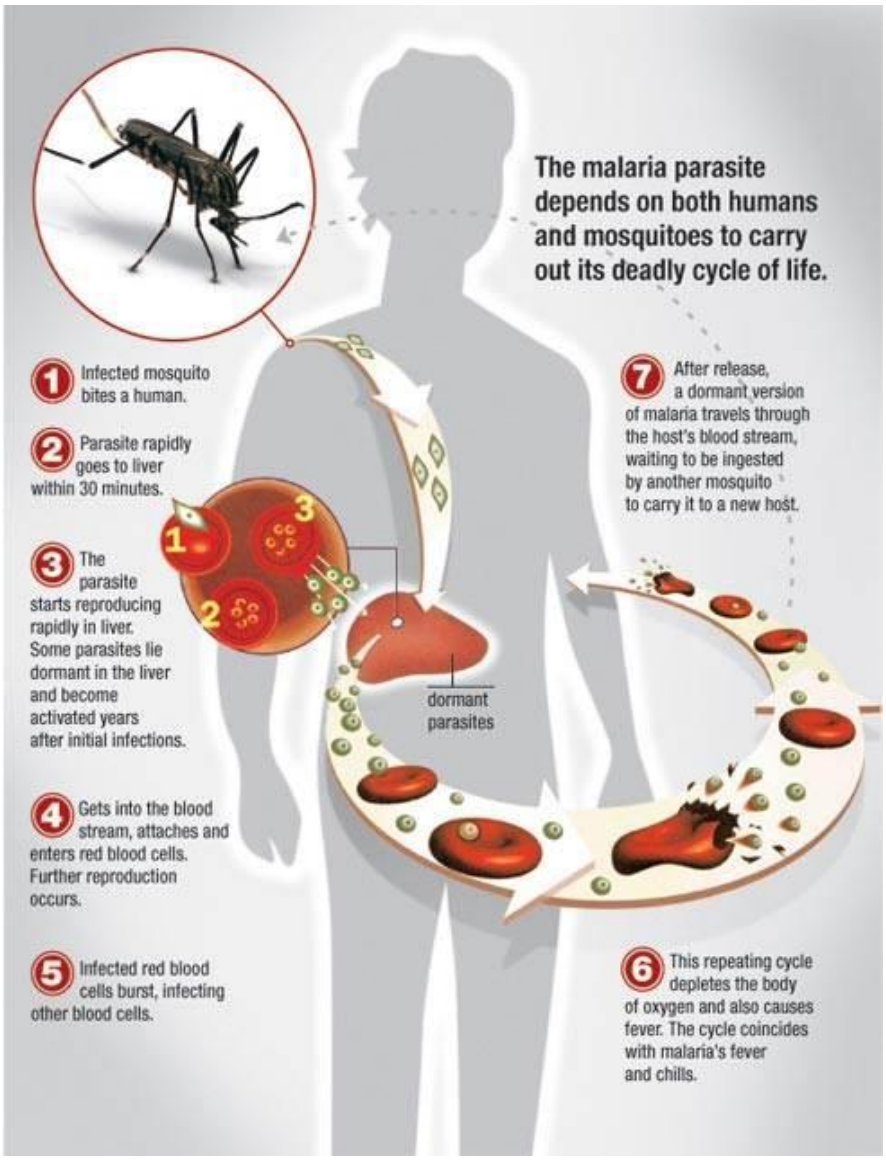
Annex 1: In patients Malaria Deaths in Kavango and Oshikoto Regions.

Region	District	Facility	2008	2009	2010	2011	Grand Total
Kavango Region	Andara	Andara Hospital	2	3	9	0	14
	Nankudu	Nankudu Hospital	4	7	5	0	16
	Nankudu	Nkurenkuru Health Centre	0	0	1	0	1
	Nyangana	Nyangana Hospital	4	1	2	0	7
	Rundu	Bunya Health Centre	1	0	0	1	2
	Rundu	Mupini Health Centre	1	0	0	1	2
	Rundu	Rundu Hospital	24	22	25	19	90
Oshikoto Region	Onandjokwe	Onandjokwe Hospital	2	0	0	2	4
	Tsumeb	Tsumeb Hospital	1	0	0	1	2

Annex 2: Inpatient malaria cases in Kavango and Oshikoto Regions.

Region	District	Facility	2008	2009	2010	2011	Grand Total
Kavango Region	Andara	Andara Hospital	174	157	194	22	547
	Nankudu	Mpungu Health Centre	151	120	104	10	385
	Nankudu	Nankudu Hospital	75	108	49	46	278
	Nankudu	Nkurenkuru Health Centre	222	308	221	43	794
	Nankudu	Rupara Health Centre	80	62	52	22	216
	Nankudu	Tondoro Health Centre	129	120	88	36	373
	Nyangana	Nyangana Hospital	133	32	61	9	235
	Rundu	Bunya Health Centre	149	67	33	51	300
	Rundu	Mupini Health Centre	316	98	122	37	573
	Rundu	Rundu Hospital	520	319	331	219	1389
	Rundu	Shambyu Health Centre	311	144	33	36	524
Oshikoto Region	Onandjokwe	Okankolo Health Centre	7	0	0	1	8
	Onandjokwe	Onandjokwe Hospital	70	49	17	15	151
	Onandjokwe	Onayena Health Centre	39	13	9	0	61
	Onandjokwe	Onyaanya Health Centre		2	2	0	4
	Tsumeb	Tsumeb Hospital	24	19	4	14	61

Annex 3: Life cycle of *Plasmodium falciparum.*



Science Primer-exploring malaria, exploreable.wordpress.

Annex 4: Haematology Standard Operating Procedures for blood full cell count.

It helps to divide the field into quadrants by imaginary lines and estimate cells per quadrant.

Calculate the percentage of infected erythrocytes as follows:

$$\frac{\text{No. of infected erythrocytes in 10 fields}}{\text{Total number of erythrocytes in 10 fields}} \times 100\ (\%)$$

EXAMPLE:

20 infected erythrocytes counted in ten fields ...X 100

1000 erythrocyte counted in ten fields =2%

One parasite seen in more than 1 000 RBC is reported as *0.1 %*

Method 2:

Determination of parasites/µl of blood is accomplished by counting the number of parasites on a thick film in relation to the patient's actual number of white blood cells per µl of blood. If the patient's WBC count is not available then an assumed average number of 8000 is used, however this is less accurate. Two tally counters are required, one for counting WBC and one for counting asexual forms.

Before starting the count, complete a thorough examination of the thick and thin films to identify any parasite present.

On the thick film find a field with 12 WBC's or more and start the count here.

Count the number of parasites until reaching the field with the 200th WBC (many experts believe that a minimum of 500 WBCs should rather be counted to be more accurate).

In this field, count all of the WBCs present. Thus the total WBCs counted may be slightly more than 200.

If 10 or more parasites were found then calculate the results as follows:

$$\frac{\text{no of parasites counted}}{\text{no of WBC counted}} \times 8000 \text{ (or patient's own WBC)} = \text{no of parasites/µl of blood.}$$

If after counting the 200th WBC the number of parasites is 9 or fewer, then continue counting until you reach at least 500 WBC and use the above formula to calculate the result.

In very high infections count all the parasites in one HPF where there are more than 12 WBCs. The HPF can be divided into ¼ and the number of parasites multiplied by 4.

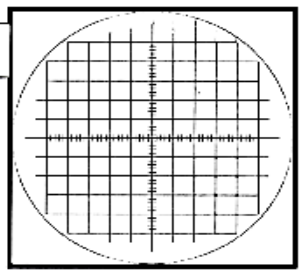
LIMITATIONS:

Usually only *Plasmodium falciparum* parasites are quantified, not any of the other malaria species. This is because the non-falciparum species seldom reach densities of grater then 1%, and the information is not clinically relevant.

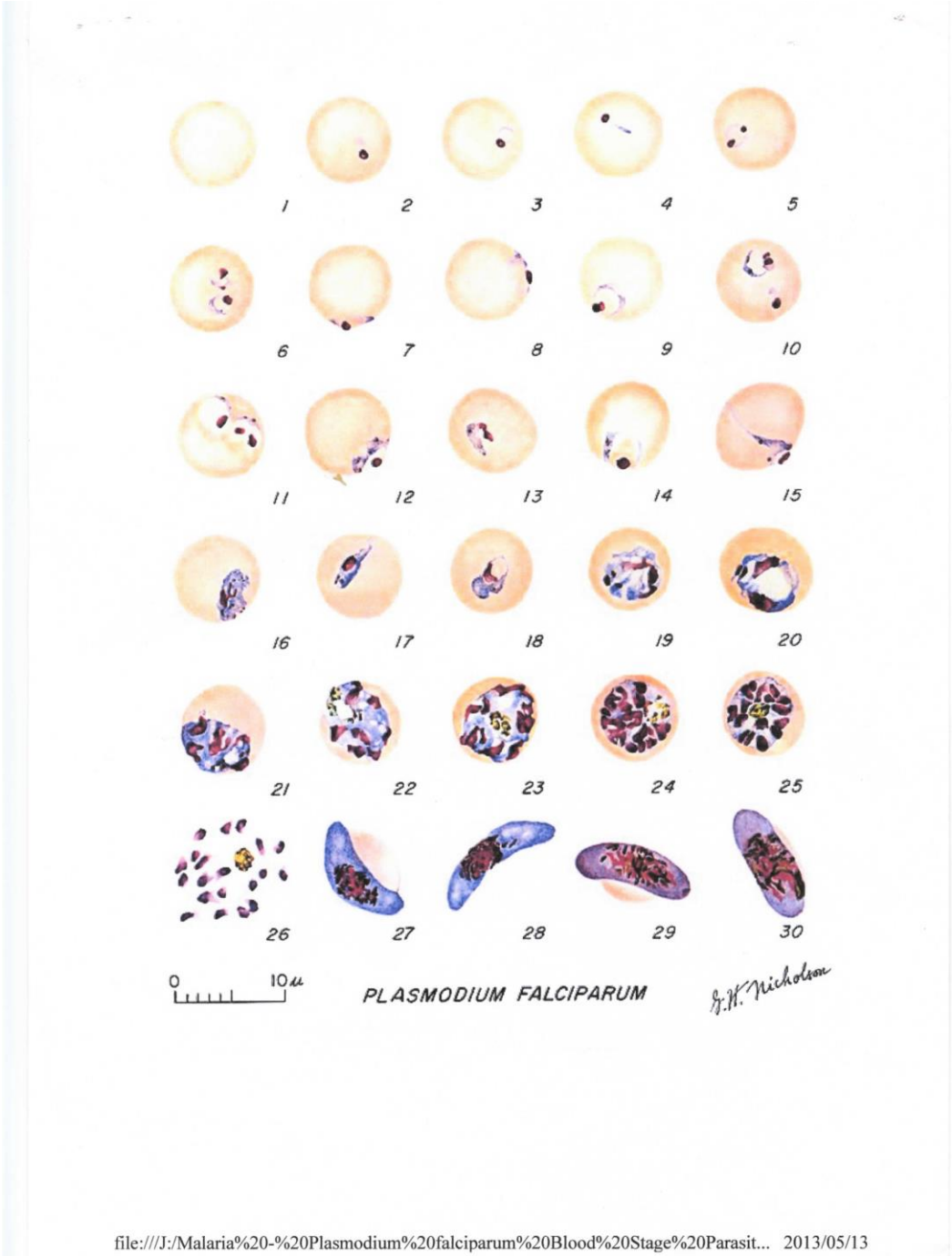
REFERENCES:

1. WHO/NICD EQA Program. Feb. 2007. Prof John Frean.

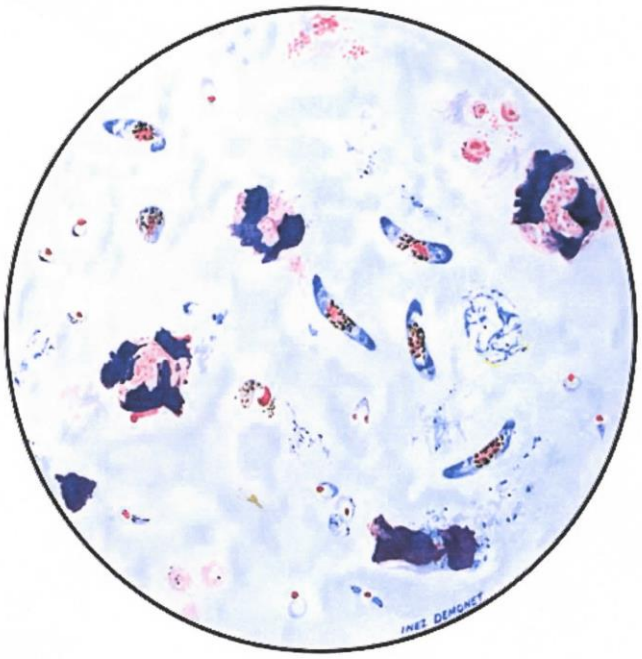
Graticule



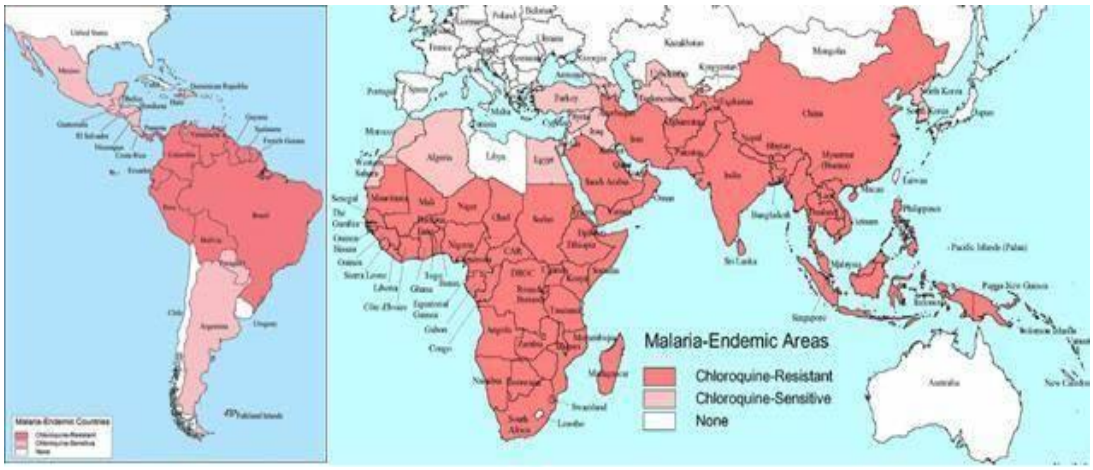
Annex 5 (A): The different stages of Malaria infection in Man



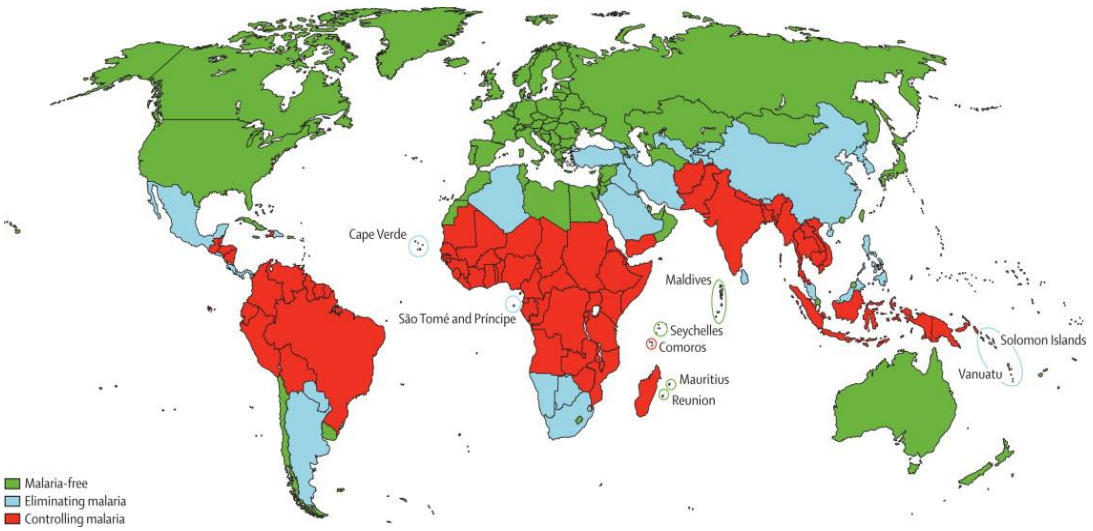
Annex 5: (B) Gametocytes of *Plasmodium falciparum*



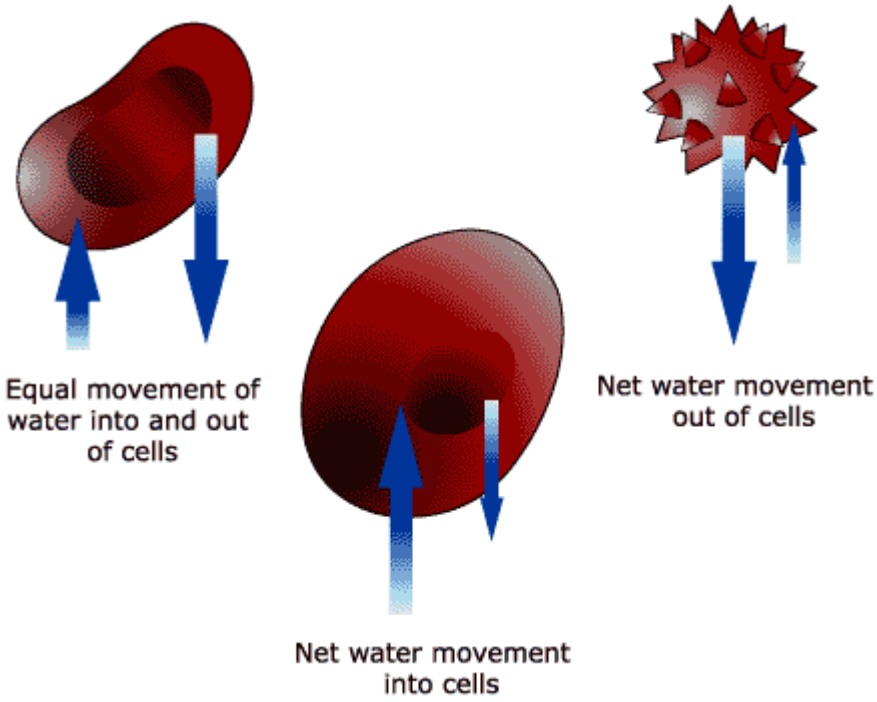
Annex 6 (A) The map showing the malaria endemic areas of the world (Feachan and malaria elimination group, 2009)



Annex 6 (B): Map showing the shrinking map of malaria of the world (Feachan and malaria elimination group, 2009)



Annex 7: The formation of spikes on the surface of the red blood cells due to dehydration



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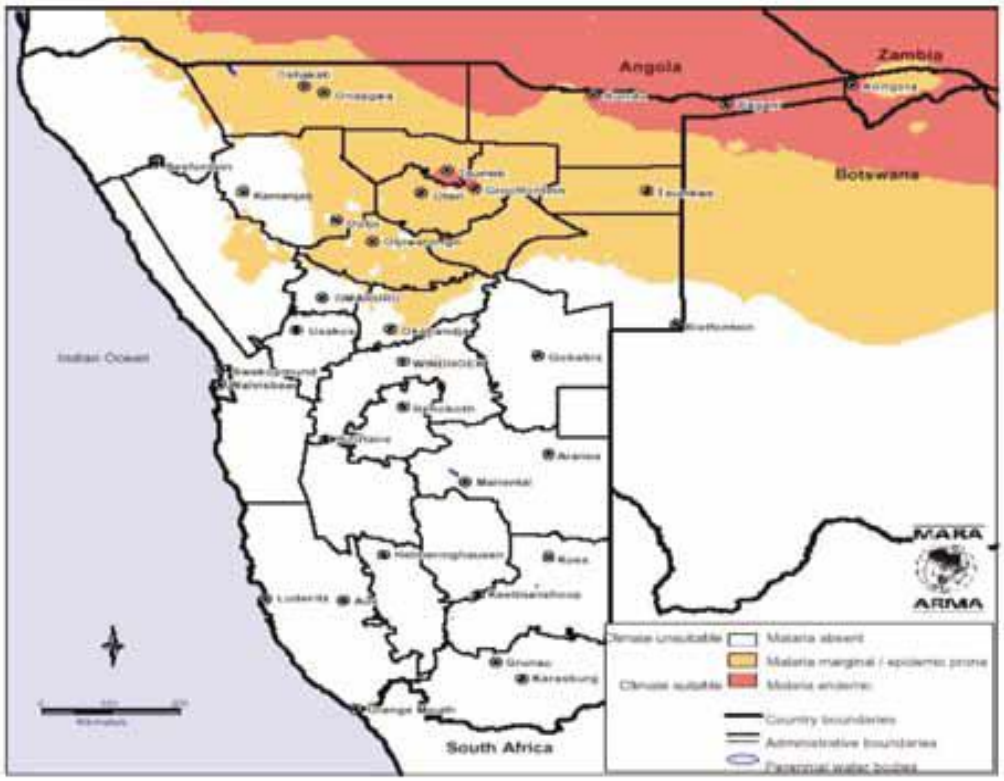
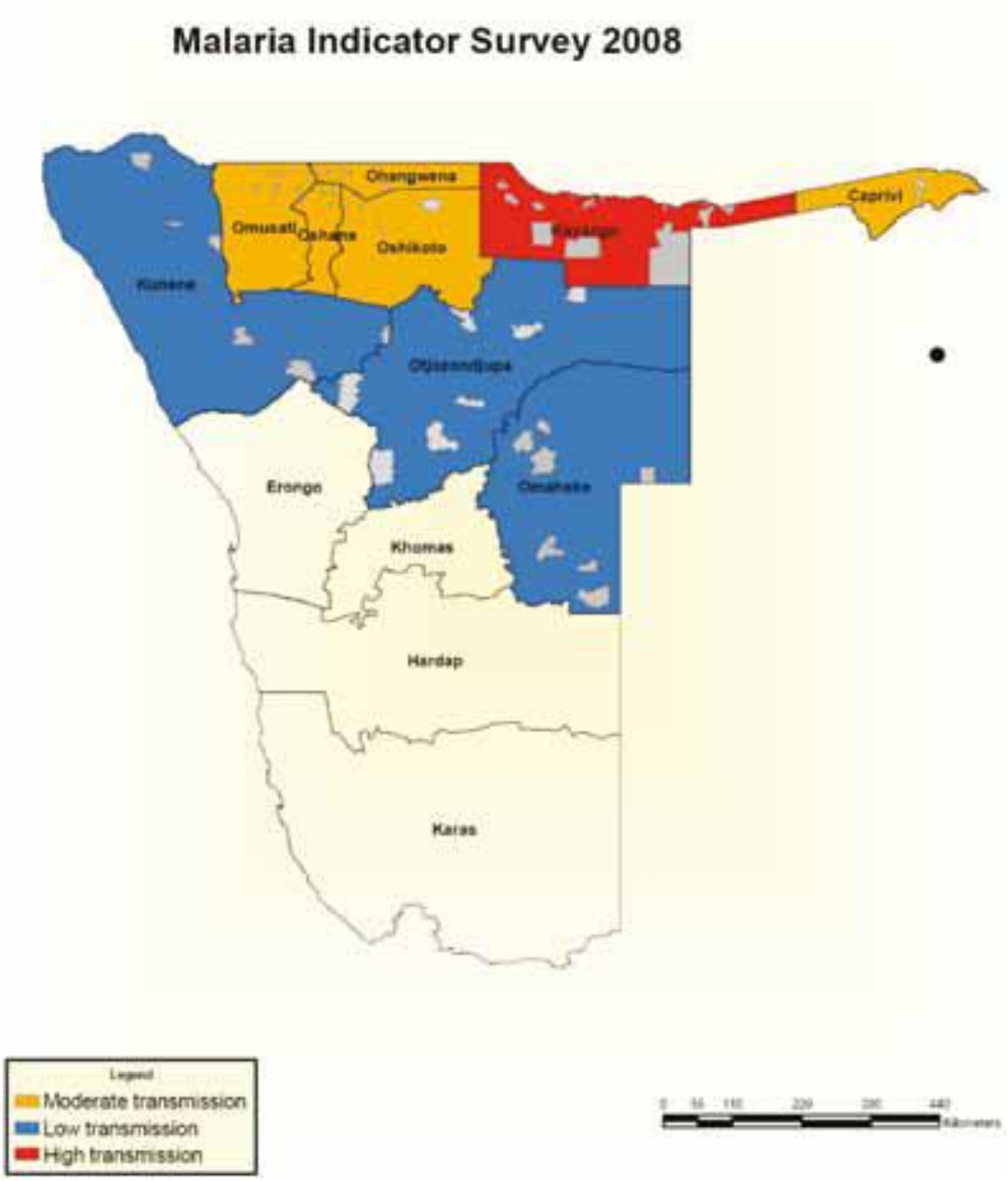
http://toolboxes.flexiblelearning.net.au/demosites/series3/308/laboratory/personalstudy/ps-DiluentsAndIsotonity.htm Retrieved on 12th December 2013

Annex 8: Malaria risk stratification in Namibia (MARA ATLAS project, 2002)



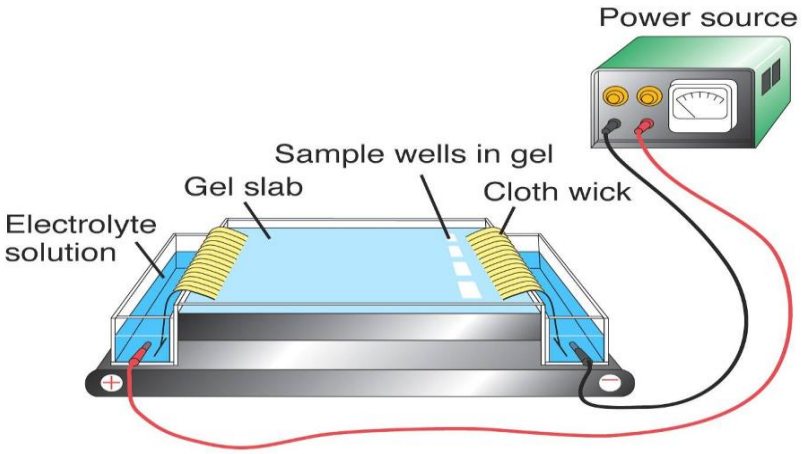
Annex 9: The transmision rate of malaria in Namibia



Annex 10 (A) The Biorad D-10 machine for the analysis of Haemoglobin variance

Source: http://bio-rad.com/en-bh/category/d-hemoglobin-testing-system. Retrieved 8 October 2013.

Annex 10 (B) The Haemoglobin electrophoresis machine used to separate abnormal Haemoglobins



Annex 11: Traditional Herbs used by the San for treatment of various ailments

(a) *Hoodia officinalis*

(b) *Hoodia Currorii*

Source:http://www.google.com.na/search? Retrieved on 8 October 2013

(a) The devil claws

(b) The devil claw species

Source:http://www.webmd.com/vitamins-supplements/ingredientmono-984-claw

Retrieved on 8th October 2013

Annex 12: The San drinking *Hoodia spp* liquid

Source:https://www.google.com.na/search?hl Retrieved on 8th October 2013

Annex 13: The recommended daily intake

Annex 13a: Recommended dietary allowances- Men

Age	11 – 14	15 – 18	19 – 24	25 – 50	+51
Calories (kCal)	2500	3000	2900	2900	3000
Protein (g)	45	59	58	63	63
Vitamin A (ug)	1000	1000	1000	1000	1000
Vitamin D (ug)	10	10	10	5	5
Vitamin E(mg)	10	10	10	10	10
Vitamin K (ug)	45	65	70	80	80
Vitamin C (mg)	50	60	60	60	60
Thiamin (mg)	1.3	1.5	1.5	1.5	1.2
Riboflavin (mg)	1.5	1.8	1.7	1.7	1.4
Niacin (mg)	17	20	19	19	15
Vitamin B6 (ug)	1.7	2	2	2	2
Folate (ug)	150	200	200	200	200
Vitamin B12 (mg)	2.0	2.0	2.0	2.0	2.0
Calcium (mg)	1200	1200	1200	800	800
Phosphorous (mg)	1200	1200	1200	800	800
Magnesium (mg)	270	400	350	350	350
Iron (mg)	12	12	10	10	10
Zinc (ug)	15	15 – 18	15	15	15
Iodine (ug)	150	150	150	150	150
Selenium (ug)	40	50	70	70	70

Annex 13b: Recommended dietary allowances- Female

Age	11 – 14	15 – 18	19 – 24	25 – 50	+ 51	Pregnant	Lactating (First 6 months)	Lactating (Second 6 months)
Calories (kCal)	2200	2200	2200	2200	1900	+ 300	+ 500	+ 500
Protein (g)	46	44	46	50	50	60	65	62
Vitamin A (ug)	800	800	800	800	800	800	1300	1200
Vitamin D (ug)	10	10	10	5	5	10	10	10
Vitamin E(mg)	8	8	8	8	8	10	12	11
Vitamin K (ug)	45	55	60	60	60	65	65	65
Vitamin C (mg)	50	60	60	60	60	70	95	90
Thiamin (mg)	1.1	1.1	1.1	1.1	1	1.5	1.6	1.6
Riboflavin (mg)	1.3	1.3	1.3	1.3	1.2	1.6	1.8	1.7
Niacin (mg)	15	15 – 18	15	15	13	17	20	20
Vitamin B6 (ug)	1.4	1.5	1.6	1.6	1.6	2.2	2.1	2.1
Folate (ug)	150	180	180	180	180	400	280	260
Vitamin B12 (mg)	2.0	2.0	2.0	2.0	2.0	2.2	2.6	2.6
Calcium (mg)	1200	1200	1200	800	800	1200	1200	1200
Phosphorous (mg)	1200	1200	1200	800	800	1200	1200	1200
Magnesium (mg)	280	300	280	280	280	320	355	340
Iron (mg)	15	15	15	15	10	30	15	15
Zinc (ug)	12	12	12	12	12	15	19	16
Iodine (ug)	150	150	150	150	150	175	200	200
Selenium (ug)	45	50	55	55	55	65	75	75