POPULATION GENETICS AND TRANSMISSION DYNAMICS OF *PLASMODIUM FALCIPARUM* IN THE KAVANGO EAST AND ZAMBEZI REGIONS OF NAMIBIA

A RESEARCH DISERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN SCIENCE

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ABSTRACT

In 2010, Namibia, declared a goal to eliminate malaria within its borders, this was revised in 2017 to 2022 due to new challenges in achieving elimination. Some of the key challenges associated with malaria control and elimination are; 1) a lack of comprehensive data including malaria case distribution and resources especially in Africa where the burden is highest 2) there is no accurate classification of imported and local malaria cases or quantification of the level of importation 3) a lack of validated tools to supplement transmission estimates. Malaria positive samples tested with rapid diagnostic tests (RDTs) and dried blood spots (DBS) were collected with corresponding epidemiological data from health districts in the Kavango East and Zambezi regions of Namibia. DNA was extracted using the chelex DNA extraction method, the parasite DNA was genotyped with capillary electrophoresis using a 26 microsatellite marker set to determine *P. falciparum* genetic structure in the Kavango East and Zambezi regions of Namibia

The study through population genetics analysis showed that the genetic structure of P. falciparum in Namibia follows the pattern of a high transmission setting, there are high levels of genetic diversity, low genetic relatedness, random mating and population admixture. Secondly, there are high levels of importation contributing to local transmission. Lastly, population genetic matrices are good surrogate markers for measuring malaria transmission intensity and importation.

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TABLE OF CONTENTS

ABSTRACT i
LIST OF PUBLICATIONS ii
LIST OF CONFERENCE AND POSTER PRESENTATIONS iii
LIST OF TABLES viii
LIST OF FIGURES ix
LIST OF ABBREVIATIONS AND/OR ACRONYMS xi
ACKNOWLEDGEMENTS xiii
DEDICATION xiv
DECLARATION xv
CHAPTER 1: INTRODUCTION 1
1.1 Background of the malaria the disease 1
1.2 Problem statement 5
1.3 Objectives 7
1.4 Significance of the study 8
1.5 Limitations of the study 10
1.6 Delimitations of the study 10
1.7 Thesis overview and scope 11
1.8 References 13
CHAPTER 2: LITERATURE REVIEW 17
2.1 Malaria burden 17
2.2 Malaria transmission and clinical presentation 19
2.3 Malaria parasite life cycle 19
2.4 Global malaria trends 22
2.5 Regional initiatives for malaria elimination 24
2.6 Malaria elimination 26
2.7 Malaria elimination challenges and available tools 28
2.7.1 Diagnosis of malaria 28
2.7.2 Molecular diagnosis of malaria 31
2.7.3 Alternative diagnostic methods 33

2.7.4 Vector control 33
2.8 Malaria treatment 35
2.9 Heterogeneous and cross boarder transmission 37
2.10 Population genetics of *Plasmodium* (Hardy-Weinberg theorem) 39
2.10.1 *Plasmodium* genome 41
210.2. Size polymorphisms 43
2.10.3 Partial sequencing 43
2.10.4 Multilocus genotyping 44
2.10.5 Whole genome sequencing 44
2.11 Plasmodium population diversity variation dogma 45
2.12 Manuscripts addressing specific objectives 48
2.13 References 50
CHAPTER 3: GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* IN THE KAVANGO EAST AND ZAMBEZI REGIONS OF NAMIBIA 75
3.1 Abstract 75
3.2 Background 76
3.3 Methods 78
3.3.1 Study sites and *P. falciparum* isolates 78
3.3.2 DNA extraction 81
3.3.3 Genotyping 82
3.4 Population genetics analysis 88
3.4.1 Within-host diversity 88
3.4.2 Population genetic diversity 88
3.4.3 Population structure and Genetic differentiation 89
3.4.4 Ethical approval 89
3.5 Results and discussion 90
3.5 Conclusion 97
3.6 References 99
CHAPTER 4: A COMPARISON OF MALARIA CASE DISTRIBUTION AND TRANSMISSION DYNAMICS IN ANGOLAN AND NAMIBIAN RESIDENTS IN THE KAVANGO EAST REGION OF NAMIBIA 124
4.1 Abstract 124
4.2 Background 125

4.3 Methods 128
4.3.1 Study sites and sample collection. 128
4.3.2 Malaria case distribution 130
4.3.3 DNA extraction and genotyping 131
4.3.4 Multiplicity of infection 132
4.4 Ethical approval 133
4.5 Results and Discussion 133
4.5.1 Malaria case distribution by age 133
4.5.2 Within host diversity in Namibian and Angolan residents 135
4.5.3 Relative risk to travel to a malarious region by age and primary district residence .. 138
4.6 Conclusion 141
4.7 References 143
CHAPTER 5: GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* IN THE ZAMBEZI REGION OF NAMIBIA AS A MEASURE OF TRANSMISSION INTENSITY AND IMPORTATION 168
5.1 Abstract 168
5.2 Background 169
5.3 Methods 171
5.3.1 Study sites and sample collection. 171
5.3.2 Microsatellite genotyping of P. falciparum 172
5.3.3 Within-host diversity 173
5.3.4 Population genetic diversity 173
5.3.5 Genetic relatedness of *P. falciparum* infections 174
5.4 Results and Discussion 174
5.5 Conclusion and recommendations 179
5.6 References 181
CHAPTER 6: OVERALL CONCLUSIONS 206
CHAPTER 7: CONTRIBUTION TO NEW KNOWLEDGE 210
CHAPTER 8: RECOMMENDATIONS 211
CHAPTER 9: APPENDICES 212
9.1 Appendix A: Population genetics analysis code chapter 3 212
9.2 Appendix B: Data analysis Chapter 4 222

9.3 Appendix C: Data analysis Chapter 5 243
9.4 Appendix D: Figure 8, page 75, map code 256
9.5 Appendix E: Raw data 259

LIST OF TABLES

Chapter 2

Table 1: Molecular criteria used to characterize epidemiology in low and high transmission malaria settings and expected outcomes (Anderson et al., 1999; Auburn & Barry, 2017; Escalante et al., 2015; Mobegi et al., 2012; Vardo-Zalik et al., 2013) 47

Chapter 3

Table 2: Showing the physical distance in kilometers between health facilities, sufficient distance to detect genetic differences in *P. falciparum* population genetics 81

Table 3: Primer, probe sequences and cycling conditions for varATS qPCR to determine parasite density 83

Table 4: Master mix for the 1st round of PCR and 10X Primer Mixture for Groups 1-4 84

Table 5: Master mix for the 2nd round of PCR 86

Table 6: G_{ST} metric analysis of genetic differentiation between populations 96

Chapter 4

Table 7: A comparison of within host diversity by age group between Namibian and Angolan residents 137

Table 8: Relative risk to travel by age and residents between Namibian and Angolan residents 140

LIST OF FIGURES

Chapter 2

Figure 1: World malaria map showing the distribution of malaria cases 2017 (IAMAT, 2017; https://www.iamat.org/risks/malaria)....18

Figure 2: Transmission intensity defined from high to zero (WHO, 2017)....18

Figure 3: *Plasmodium* life cycle in the mosquito vector and vertebrate host. Source: http://ocw.jhsph.edu/index.cfm/go/imageLib:il.viewImageDetails/resourceID/438DCC50-FFE9-0B64-8515BF619797AA48/browseTopic/Malaria/topicID/16/ 21

Figure 4: Varying levels of malaria incidence in the highly connected E8 region. Source: https://malariaelimination8.org/state-malaria-e8/....26

Figure 5: Malaria elimination continuum showing the 4 phases and their respective thresholds (Moonasar et al., 2013)....28

Figure 6: Non-uniform decrease of malaria across boarders; a challenge for elimination. Source: https://malariaelimination8.org/namibia/....39

Figure 7: Haploid *P. falciparum* genome with 14 chromosomes. Source: https://www.researchgate.net/figure/Haploid-Plasmodium-falciparum-genome-containing-14-chromosomes-The-whole-genome-of-23-Mb_fig1_271225415 [accessed 21 Apr, 2019]....42

Chapter 3

Figure 8: A map showing the study sites, namely, Rundu, Nyangana, Andara and Sibbinda....80

Figure 9: Distribution and location of the 26 microsatellite markers on the parasite genome (Liu et al., unpublished data)....83

Figure 10: Used positive RDTs and epidemiological data on the RDT that were collected and stored at -20℃ until further genetic analysis....87

Figure 11: Increase in genetic differences with an increase in MOI (F_{WS}: p-value < 2.2e-16 at 95%CI)....92

Figure 12: Distribution of population level genetic diversity across the 4 populations...94

Figure 13: Analysis of the association of alleles across the 4 populations....95

Figure 14: Highly admixed populations showing no structure with the maverick software. 97

Chapter 4

Figure 15: Study site showing the 24 health facilities in the Kavango East region where the samples were collected and the specific clinics 130

Figure 16: Proportion of malaria case distribution by age between Namibian and Angolan residents 135

Figure 17: An overall comparison of within host diversity between Namibian and Angolan residents 138

Chapter 5

Figure 18: Map of the Zambezi region showing the locations of the 6 health facilities in this study 172

Figure 19: Panel A showing positive correlation between multiplicity of infections and reported number of cases. Panel B shows an inverse correlation between Fws and the reported number of cases 176

Figure 20: Panels A and B show a positive correlation between population level genetic diversity with reported number of malaria cases 177

Figure 21: Panels A and B show the correlation of proportion of travel and genetic relatedness with the reported number of cases respectively 178

LIST OF ABBREVIATIONS AND/OR ACRONYMS

ACT Artemisinin Combination Therapy

ALMA African Leaders Malaria Alliance

APLMA Asia Pacific Leaders Malaria Alliance secretariat

APMEN Asia Pacific Malaria Elimination Network

Bp Base Pairs

Bst *Bacilus strearothermophilus*

COI Complexity of Infections

CQ Chloroquine

DBS Dried Blood Spots

DNA DeoxyriboNucleic Acids

E8 Elimination 8 (A regional initiative of 8 countries in the Sub-Saharan region)

EIR Entomological inoculation rate

ELISA Enzyme-Linked Immunosorbent Assay

GEE Generalized Estimating Equations

GMEP Global Malaria Elimination Program

GPIRM Global Plan for Insecticide Resistance Management

HIV Human Immunodeficiency Virus

HIVDR Human Immunodeficiency Virus Drug Resistance

IFA ImmunoFluorescent Antibody assay

IRS Indoor Residual Spraying

ISA Index of ASsociation

ITNs Insecticide Treated Nets

IVM Integrated Vector Management

LAMP Loop mediated isothermal AMPlification

LD Linkage Disequilibrium

LLINs Long Lasting Insecticide Nets

Mb Mega Bases

MoHSS Ministry of Health and Social Services

MOI	Multiplicity Of Infections
MQ	MefloQuine
NMCP	National Malaria Control Program
nPCR	nested Polymerase Chain Reaction
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PLP	Perforin-Like Protein
QBC	Quantitative Buffy Coat
qPCR	quantitative Polymerase Chain Reaction
RACD	Reactive Case Detection
RDTs	Rapid Diagnostic Tests
RIs	Regional Initiatives
RNA	Ribonucleic Acid
SNPs	Single Nuclear Polymorphisms
SP	Sulfadoxin – Pyrimethane
SPECT	Sporozoite microneme Protein Essential for Cell Traversal
TB	Tuberculosis
uRDT	ultra-sensitive Rapid Diagnostic Tests
WHO	World Health Organisation

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My Family that is always loving, supportive and encouraging in all areas of life. The joy of our Lord is our strength. A special mention to my wife, Natasja, for the sacrifices and moral support through it all

DEDICATION

I dedicate this work to the malaria elimination goal and teams globally. To my family, our vision and prayers for a fruitful life through our Lord.

Kukomborerwa kunotobva kuna Ishe!

DECLARATION

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

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CHAPTER 1: INTRODUCTION

1.1 Background of the malaria the disease

Malaria is a major public health concern to date although it is both treatable and preventable. It is transmitted through bites by female *Anopheles* mosquitoes that are infected by protozoan parasites of the genus *Plasmodium.* A total of 3.3 billion people are estimated to be at risk of exposure to malaria annually (Cowman *et al.*, 2016). To date, the highest burden of the disease, in terms of both morbidity and mortality is in Africa (~88% of cases), largely due to economic and climatic factors that give an optimal environment for transmission (Lee *et al.*, 2017). In Sub-Saharan Africa, temperatures that go above 30°C and good rainfall provide an optimum environment for *Anopheles* mosquito breeding and spreading. The poor economic performance hinders implementation of malaria interventions, access to health facilities and building of appropriate infrastructure such as health facilities (Gueye *et al*., 2014).

However, malaria has garnered a global interest for elimination and has seen recent significant declines in incidence and prevalence with a number of countries moving from malaria control to the pre-elimination and elimination phases (Cohen *et al.*, 2010). The significant decline in malaria is chiefly credited to the introduction of rapid diagnostic tests (RDTs) and artemisinin combination therapies (ACTs) (Awoleye and Thron, 2016; Froeschl *et al.*, 2018). Following this decline, Southern African countries, namely, Namibia, Angola, South Africa, Zambia, Eswatini, Botswana, Mozambique and Zimbabwe have formed a block of countries with the objective of eliminating malaria by

2030, called the Elimination 8 (E8) (Newby *et al.,* 2016). The formation of the E8 is based on the realization that malaria is not bound by geographical delineations. In 2010, Namibia, declared a goal to eliminate malaria within its borders by 2020 (Namibia Malaria Indicator Survey, 2009). This goal to eliminate malaria by 2020 was revised to 2022 after a series of outbreaks in 2016 (Chanda *et al.*, 2018).

As malaria declines and countries move from control of malaria to pre-elimination and elimination, it becomes increasingly difficult to maintain the low prevalence and to ultimately eliminate the residual malaria infections. The key challenges that hinder progress toward malaria elimination are: 1) Failure of the WHO recommended diagnostic tools RDTs and expert performed microscopy to detect low parasite density (asymptomatic) infections which are reported to make up the majority of cases (up to 80%) in low transmission settings *(Sturrock et al.*, 2015; Tambo *et al.*, 2018; Tambo, Mwinga & Mumbengegwi, 2018). Most countries routinely use the two diagnostic tools. 2) Emergence of parasite resistance to the recommended first line drugs including Artemisinin combination therapies (ACTs) (Amambua-Ngwa et al., 2018; Cerqueira et al., 2017) 3) A lack of comprehensive data and resources especially in Africa where the burden is highest (Chiyaka *et al.*, 2013; Lover *et al.*, 2017) and, 4) Inaccurate and incomplete information used as measurement of progress and basis for resource allocation (Galappaththy, Fernando and Abeyasinghe, 2013; Escalante *et al.*, 2015; Reiner *et al.*, 2015).

Accurate information on malaria transmission is required for developing effective malaria elimination strategies, monitoring progress towards zero local malaria transmission, and

verifying and maintaining elimination status. Passive surveillance where malaria cases are counted and recorded where they are detected does not provide sufficient information on transmission, especially in elimination settings, since infections may be acquired far from where they ultimately present due to health facility preference or a lack of health facilities in one geographical area (Boncy et al., 2015). Furthermore, passive surveillance does not detect asymptomatic malaria infections as these individuals do not present at health facilities (Baliraine *et al.*, 2009;Hofmann *et al.*, 2015; Smith et al., 2017). Additional surveillance tools, including data on travel history and human population movement have been proposed to fill this gap, but the accuracy of the primary data and of the underlying assumptions made in their analysis are unclear (Greenhouse & Smith, 2015). The underlying assumptions are that if an individual travelled to a malarious region, the infection is imported although the individual resides in a malaria endemic region. Additionally, there is an assumption that self-reported travel history is accurate and that reported cases are a true reflection of malaria transmission intensity. Lastly, an assumption is made that malaria cases originated from where they are reported and are fragmented geographically in a low transmission setting such as Namibia.

Therefore, this study employed population genetics to determine multiplicity of infections (MOI), genetic diversity, linkage disequilibrium and parasite flow. The study also sought to assign *P. falciparum* parasites which make up the majority of infections in Namibia to a geographical location and population structure 1) within and between the Kavango and Zambezi regions of Namibia, 2) between individuals that reported travel and those that did not report travel and 3) within malaria hotspots and outside the hotspots. Results

from the study will inform the elimination strategy and resource allocation in the fight against malaria.

1.2 Problem statement

Firstly, there are no data to-date on the population genetic structure of the *P. falciparum* parasite in Namibia which could aid in creation of tailored interventions. The population genetic matrices used to determine population structure include the Multiplicity of infection (MOI), genetic diversity of *P. falciparum,* linkage disequilibrium and admixture of parasite populations. These matrices can be used to determine parasite transmission intensity, population connectivity and they all have implications on the clinical presentation of malaria cases, development of genetic traits such as drug resistance and the spread of these traits.

Secondly, there is no measure of the importation from Angola and a measure of how the imported cases contribute to local transmission in Namibia. Without accurate data on levels of importation, resources are focused on targeted interventions in Namibia only and the continuous importation reduces the efficacy of the targeted interventions. A comparison of Angolan malaria infections and Namibian malaria infections could give insight into the level of importation and the contribution of imported cases to further malaria transmission.

Lastly, currently used prevalence and transmission estimates such as travel history and environmental variables are based on assumptions with varying degrees of subjective evidence available to support them. The assumptions that include self-reported travel history are inaccurate and incomplete as some individuals cannot recollect their previous travel (Escalante *et al.*, 2015; Reiner *et al.*, 2015), additionally there is an assumption that

cases originate where they are reported and reported cases are a representation of the prevalence of malaria. In Namibia, malaria cases are detected using RDTs and microscopy using reactive case detection (RACD). These diagnostic tools have been reported as failing to detect a significant number of malaria cases at low parasite density (up to 80%) in low transmission settings and RDTs miss malaria infections due to deletions in the *HRP2* gene of *P. falciparum* (Tambo et al., 2018). As a result, the number of malaria cases is underestimated and there is no further useful information such as parasite flow and connectivity of malaria infections. Malaria transmission intensity measures need to be complemented by genetic data in order to accurately determine the transmission intensity, levels of importation and to account for malaria cases that do not present at health facilities from a representative sample size. These challenges lead to inaccurate transmission estimates and ineffective allocation of limited resources moving toward malaria elimination.

1.3 Objectives

This study employed the use of microsatellite genotyping with the following objectives:

a) To determine *P. falciparum* genetic diversity across district health facilities in the Kavango East and Zambezi regions of Namibia through
    - determining multiplicity of infections as a measure of within host *P. falciparum* genetic diversity
    - calculating the population level genetic diversity,
    - determining *P. falciparum* random or nonrandom mating patterns through calculation of linkage disequilibrium
    - describing the *P. falciparum* clustering patterns geographically

b) To compare the malaria case distribution in Angolan and Namibian residents in the Kavango East region in Namibia by age and district residence using primary residency as a proxy for residency

c) To investigate the usefulness of *P. falciparum* genetic diversity as a measure of malaria transmission intensity

1.4 Significance of the study

This study is important because the current data such as malaria transmission estimates and classification of cases as local or imported is insufficient. The current data will be supplemented with information derived from genotyping which will increase confidence in the data being used to drive the allocation of substantial resources, and identify important limitations of existing estimates. Firstly, chapter 3 investigates the *P. falciparum* population genetic structure variation across the Kavango East and Zambezi regions of Namibia. The *P. falciparum* genetic diversity is an indicator of adaptability of the parasite to the host and counteraction with interventions (Nabet *et al*., 2016). Highly polymorphic populations of *P. falciparum* have better potential to adapt to the host and counteract interventions such as developing resistance against anti-malarial drugs (Auburn & Barry, 2017). In addition, *P. falciparum* population genetic structure will indicate how admixed the parasite populations are within the region and across regions (Schultz et al., 2010); highly admixed parasite populations show connectivity and greater potential to spread resistance traits rendering local targeted control measures ineffective.

Secondly, chapter 4 compares the malaria case distribution patterns by age and residence between the Kavango East region population in Namibia and Southern Angolan population. The malaria case distribution patterns show the peak ages of infection in both populations and informs control programs on the individuals at highest risk of acquiring an infection. Additionally, a comparison of the case distribution patterns shows how connected two adjacent geographical locations are and the risk of importation. Furthermore, the study determines the age groups and residence at highest risk of

travelling to a malarious region and the associated risk of acquiring a malaria infection. Data from this study will directly feed back into surveillance activities and resource allocation in Namibia, and more broadly, the E8 region. The E8 region is highly connected as a result of porous borders amongst the countries. The porous boarders allow free human movement and there is no restriction to mosquito carrying parasites across boarders.

Thirdly, chapter 5 investigates the use of *P. falciparum* population genetic diversity matrices as surrogate markers for transmission intensity and levels of importation in the Zambezi region of Namibia. Parasite genetic measures have the potential to accurately determine transmission dynamics (Auburn & Barry, 2017) and predict transmission intensity and importation risk in a geographical area based on a small representative sample size. With important strategic decisions being made based upon these estimates, the ability to validate or refine them against an alternative benchmark such as molecular epidemiology would be of great value. There is currently limited *P. falciparum* population genetics analysis across E8 the region with one study carried out in Eswatini (Roh *et al*., 2019).

Additionally, this study through genetic and genomic surveillance will aid in the detection of new forms of adaptation (adaptive molecular changes) such as drug resistance potential and genetic fitness as they arise in the parasite population.

1.5 Limitations of the study

The limitations of this study were firstly, Rapid Diagnostic Tests (RDTs) were used as the DNA source for genotyping. The RDTs miss a significant number of cases (up to 80%) that are low parasite density infections as they have a low threshold for malaria infection detection. Additionally, RDTs could miss malaria infections were the *HRP2* gene in the *P. falciparum* has been deleted. Only symptomatic cases or those presenting at a clinic were included, this leaves out all the asymptomatic cases which make up a significant proportion of malaria cases. In this study, all age groups included, therefore the genetic data from asymptomatic cases was obtained from children under the age of 5 years who have not developed immunity. In addition, genetic data does not require deep sampling to estimate prevalence as this can be done with a representative sample size. Secondly, the data from health facilities could not be complete as there are manual records that are prone to error and there could be cases that were not recorded or are missing. Lastly, the data were collected from a health facility based survey in Namibia therefore the malaria burden in Angolan residents could be underestimated.

1.6 Delimitations of the study

The collection of samples was limited to the Kavango East and Zambezi regions of Namibia as that is where malaria is most prevalent, accounting for up to 90% of all malaria cases in Namibia (Nghipumbwa et al., 2018)

1.7 Thesis overview and scope

The scope of this thesis was to determine currently unknown malaria transmission dynamics in Namibia through determining the genetic structure of *P. falciparum*. Chapter 1 provides a background of malaria the disease globally, regionally and nationally. Chapter 2 reviews the current literature focusing on malaria elimination challenges, specifically, diagnosis, treatment, vector control and unknown transmission dynamics. Additionally, this chapter explores the use of population genetics analysis to address some of the malaria elimination challenges.

Namibia has declared a goal to eliminate malaria within its borders by the year 2022 but to achieve this, several challenges have to be overcome including determining malaria transmission patterns and networks. The generation of genetic data on malaria transmission networks will aid in identifying and implementing appropriate interventions to achieve the goal of malaria elimination.

A study was conducted in Kavango East and Zambezi regions, Namibia to i) describe the population genetic structure of *P. falciparum* parasite in Kavango East region (Chapter 3), ii) to determine malaria importation networks across health district borders and across national borders with Angola (Chapter 4), iii) validate the use of high resolution genotyping of *P. falciparum* with microsatellites to complement routinely collected travel history to generate malaria prevalence and transmission estimates (chapter 5). Chapter 6 gives the overall conclusions from the study, specifically, this is the first report on the genetic structure of *P. falciparum* in Namibia, it showed that *P. falciparum* genetic

structure in Kavango East and Zambezi regions, Namibia do not follow the pre-described dogma for low transmission settings as a result of constant importation from Angola. The *P. falciparum* population structure also showed high levels of parasite connectivity in the Kavango East and Zambezi regions of Namibia providing new evidence that supports the need of simultaneous implementation of interventions in endemic regions and even across national borders. The study also showed high levels of *P. falciparum* importation from Angola to Kavango East region shown by similar distribution patterns of malaria cases across different age groups. Lastly, the study showed that *Plasmodium* population genetics matrices can be used as surrogate markers for measuring malaria transmission intensity and dynamics, including importation.

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CHAPTER 2: LITERATURE REVIEW

2.1 Malaria burden

In 2016, there were an estimated 216 million cases of malaria across 91 countries globally, an increase of 5 million cases over 2015 (Lover *et al.*, 2017). Malaria deaths reached 445 000 in 2016, a similar number (446 000) to 2015 (Tatem *et al.*, 2017). The WHO African Region carries a disproportionately high share of the global malaria burden as malaria is mostly spread in the tropical and subtropical regions were the conditions for transmission are optimal as shown in Figure 1 (Afoakwah, Deng & Onur, 2018). Above 30°C is the optimal temperature for the breeding and spread of the *Anopheles* spp. mosquito and below 25°C the life cycle of the *Anopheles* mosquito is interrupted or slow thereby reducing malaria transmission (Montosi *et al.*, 2012). Poverty in these African regions has also been reported as a major contributing factor (*Gueye et al.*, 2014). In 2016, the region was home to 90% of malaria cases and 91% of malaria deaths (WHO, 2017). In high malaria transmission areas, children under 5 are particularly susceptible to infection, illness and death; more than two thirds (70%) of all malaria deaths occur in this age group (World Health Organization, 2017a). Transmission intensity is clearly defined from high to zero as illustrated in Figure 2.

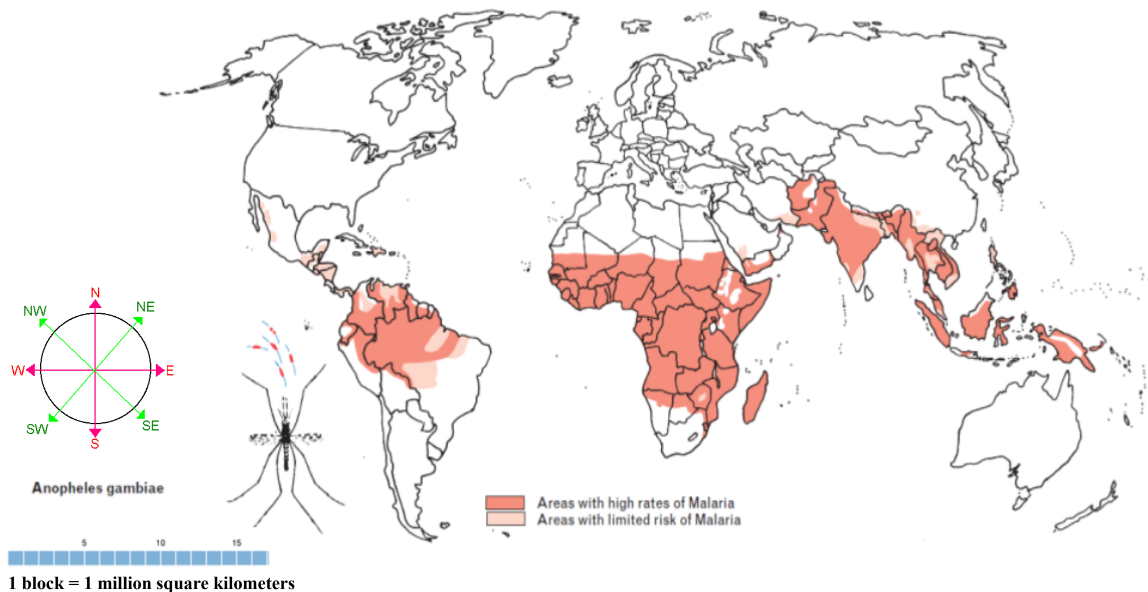

Figure 1: World malaria map showing the distribution of malaria cases 2017 (IAMAT, 2017; https://www.iamat.org/risks/malaria)

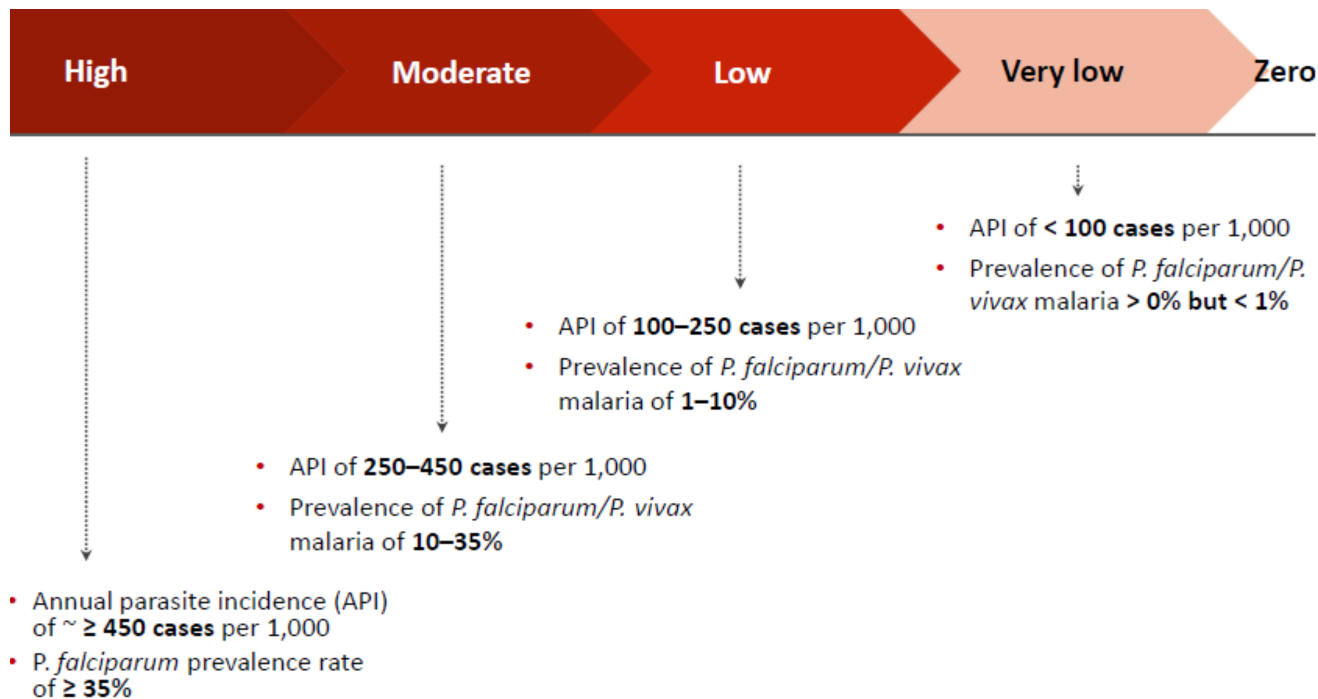

Figure 2: Transmission intensity defined from high to zero (WHO, 2017)

2.2 Malaria transmission and clinical presentation

Malaria is transmitted through bites by infected *Anopheles* (female) mosquitos, and there are currently five plus one species identified as causative agents which are *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale curtisi, Plasmodium ovale wallikeri* and *Plasmodium knowlesi.* The causative agents are five plus one because *P. knowlesi* is only transmitted from the mosquito to the human and not from the human host to the mosquito like in all other causative agents. The most virulent and prevalent of these species in Namibia is *P. falciparum*, accounting for a significant number of cases and deaths (Smith *et al*., 2017; Tambo *et al.*, 2018).

Malaria infections caused by *P. falciparum* and *P. vivax* can be asymptomatic or can develop into uncomplicated or severe malaria. In Namibia, up to 70% of the infections are estimated to be asymptomatic infections as it is a low transmission setting (Smith *et al*., 2017). In cases with *P. vivax* and *P. ovale*, relapses may occur weeks or months after being infected. The common nonspecific symptoms for uncomplicated malaria which overlap with other diseases like the flu are fatigue, fever, headaches and nausea. Severe malaria is often characterized by anaemia cerebral and respiratory distress, seizures and hypoglycaemia (Ndyomugyenyi, Magnussen & Clarke, 2007).

2.3 Malaria parasite life cycle

The malaria parasite life cycle is complex, requiring two different hosts; the vector (mosquitoes) and the vertebrate hosts as shown in figure 3. The parasite has two main stages in the vertebrate host which include the asymptomatic liver/ pre-erythrocytic stage

and the symptomatic erythrocytic (blood) stage (Gomes *et al.*, 2016). The *Plasmodium* parasites have specialized proteins, genetic changes that include allelic variation, biomolecular exposure of proteins, and intracellular replication that help the parasite to bypass and grow within multiple cell types and to evade host immune responses, these pose a challenge for vaccine development as well (Gomes *et al.*, 2016; Belachew, 2018). For example, the SPECT-1 (spororozoite microneme protein essential for cell traversal) and SPECT-2 (also called perforin-like protein 1 or PLP1) proteins are essential for cell traversal and for migration to the liver (Patarroyo, Alba & Curtidor, 2011). In addition, *Plasmodium* infected erythrocytes cluster with uninfected erythrocytes helping the parasites bind to red blood cell epitopes and avoid immune recognition, a phenomenon called rosettes (Gomes *et al.*, 2016). The *Plasmodium* parasites undergo several stages of development as illustrated in Figure 3.

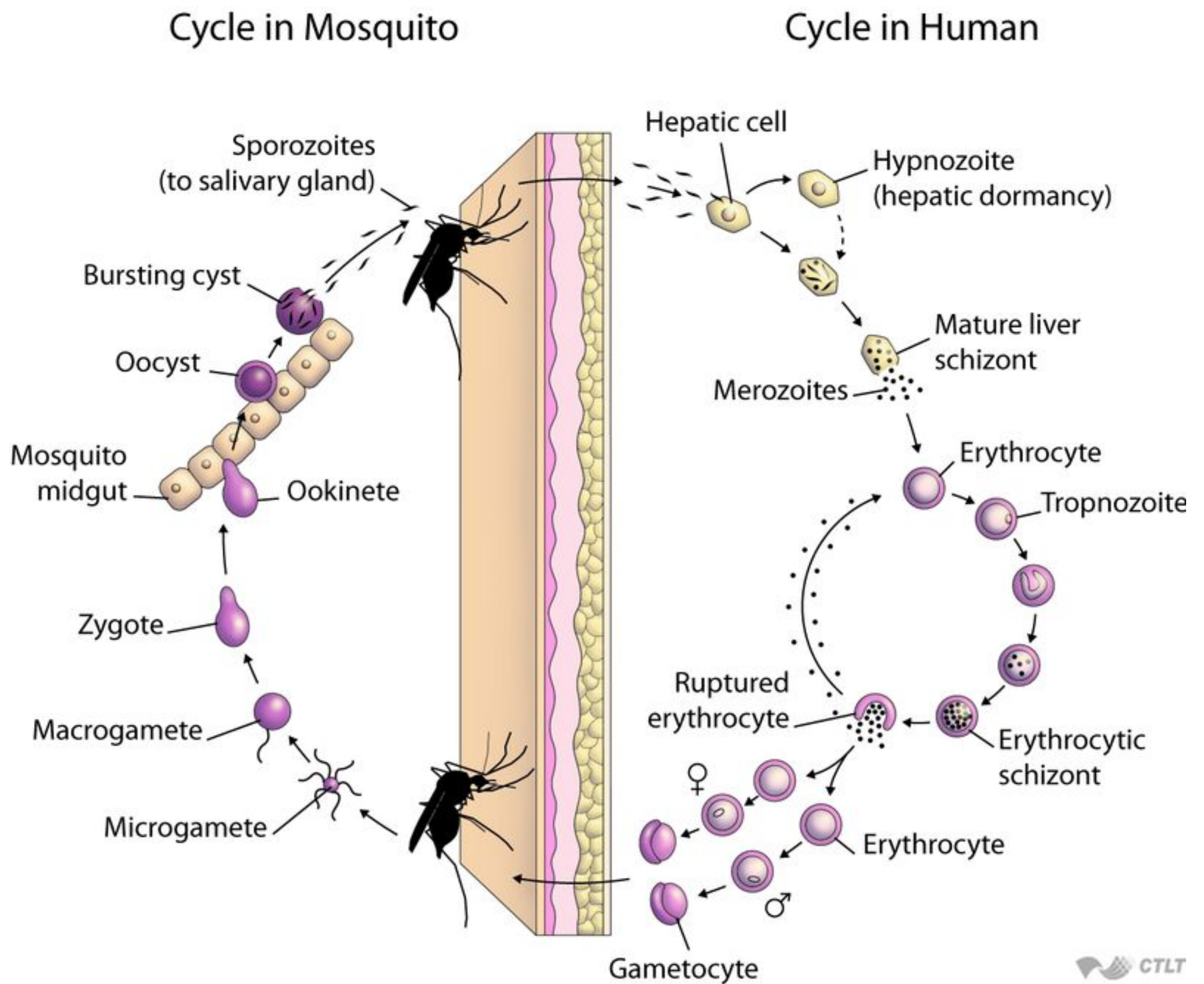

Figure 3: *Plasmodium* life cycle in the mosquito vector and vertebrate host. Source: http://ocw.jhsph.edu/index.cfm/go/imageLib:il.viewImageDetails/resourceID/438DCC50-FFE9-0B64-8515BF619797AA48/browseTopic/Malaria/topicID/16/

2.4 Global malaria trends

The global malaria eradication program (GMEP) in 1955 was the first to concert efforts to eradicate malaria off the face of the earth. During this period eradication was defined as the continued absence of transmission within a specified area and the elimination of the reservoir of infective cases (Cohen *et al.*, 2010). However, malaria eradication is now defined as a permanent global cessation of the disease prevalence to a point, where intervention measures are no longer necessary (Ferguson *et al.*, 2010; Hlongwana, Sartorius & Tsoka-Gwegweni, 2018) and malaria elimination is defined as the interruption of local transmission of a specified malaria parasite in a defined geographical area as a result of deliberate activities (WHO, 2016).

The GMEP managed to achieve significant reductions of malaria in certain geographical locations, however, this was in areas that already had effective malaria control programmes. However by 1969, it was acknowledged that global malaria eradication was not feasible with the then available tools in many areas, therefore, the campaign was stopped although the long term goal was kept (Roberts & Enserink, 2007; Cohen *et al.*, 2010; Nájera, González-Silva & Alonso, 2011). The goal for global malaria eradication was revived in 2007 as the number of malaria cases declined and the goal seemed attainable (Cohen *et al.*, 2010).

There have been general significant reductions in malaria globally following the introduction of RDTs and ACTs. Malaria RDTs are WHO recommended immunochromatographic tests that detect parasite antigens in whole blood samples,

specifically the histidine-rich protein 2 of *P. falciparum*, a pan-malarial *Plasmodium* aldolase, and the parasite specific lactate dehydrogenase (McMorrow, Aidoo & Kachur, 2011). This is as a result of the RDTs being a field user friendly point of care diagnostic tool, with a short turnaround time so less likely to have loss to follow up, and it is relatively cheap compared to other diagnostic tools such as microscopy and PCR. Ultimately, RDTs have improved access to malaria tests even in resource poor settings (Graz *et al.*, 2011).

Most malaria cases in 2017 were in the WHO African Region (92%), the WHO South-East Asia Region had 5% of the cases and the WHO Eastern Mediterranean Region with 2% of the malaria cases (WHO, 2018). Nigeria, Madagascar and the Democratic Republic of the Congo had the highest estimated increases whilst the WHO South-East Asia Region continued to see its incidence rate fall of 59% and Rwanda has also noted a reduction in its malaria burden. Sub-Saharan African countries, Namibia included experienced recent malaria outbreaks in the years 2016-2017 (Chanda *et al.,* 2018). These outbreaks were attributed to a number of factors which included flooding, low IRS coverage and low LLINs distribution (Chanda *et al.,* 2018, Nghipumbwa *et al.,* 2018).

In response to the steady decline in efficacy of mono-therapies for antimalarials globally as a result of drug resistance, artemisinin combination therapies (ACTs) were introduced in 2001 and are WHO recommended as first-line treatment for non-complicated malaria, now also used for severe malaria (Whitty *et al.*, 2008). These tools, RDTs and ACTs formed the basis of case-management of malaria through the early identification and treatment of those with mild disease with an effective antimalarial (Whitty *et al.*, 2008).

Additionally, the central dogma for *P. vivax* infections has been that the host Duffy antigen is essential to invade host red blood cells and thus establish a blood-stage infection (Aditya et al., 2015). Alarmingly, there have been recent reports of the emergence of strains able to invade Duffy-negative RBCs, raising concern over the potential spread of *P. vivax* into the large regions of sub-Saharan Africa, which were previously assumed to be protected by high proportions of Duffy negativity (Zimmerman *et al.,* 2013). There is a report of *P. vivax* asymptomatic infections in Botswana which suggests there could be P. vivax malaria previously neglected in Sub Saharan Africa (Haiyambo *et al.,* 2019). The emergence of strains able to invade Duffy-negative reticulocytes poses a major public health threat that can be avoided by active surveillance and implementation of appropriate *P. vivax* antimalarial policy.

2.5 Regional initiatives for malaria elimination

The WHO *Global Technical Strategy for Malaria 2016–2030* encourages the strengthening regional collaboration to achieve the goal towards elimination (WHO, 2015). There are a number of regional initiatives (RIs) such as Asia Pacific Leaders Malaria Alliance Secretariat (APLMA) an affiliation of 22 Asian and Pacific heads of government formed in 2013 to accelerate progress against malaria and to eliminate it in the entire region by 2030 (Lover *et al.*, 2017). Another RI in the Asia pacific region is the Asia Pacific Malaria Elimination Network (APMEN) formed in 2009 and comprises of 18 national malaria control programs and institutional partners working toward malaria elimination in the region (Lover *et al.*, 2017).

Africa has the bulk of the malaria burden, therefore, African countries have concerted efforts through RIs as well to eliminate malaria within their regions as malaria is transmitted across boundaries (Hall & Fauci, 2009). The African Leaders Malaria Alliance (ALMA) is a RI formed in 2009 and comprises of 49 heads of state and government working toward malaria elimination by 2030 (Lover *et al.*, 2017).

In Southern Africa, the Elimination 8 (E8) was formed in 2009 as an 8-country effort to achieve the overall goal of eliminating malaria by 2030 (Lover *et al.*, 2017). The 8 countries are classified into two groups which are frontline countries and second-line countries. The 4 frontline countries, namely, Botswana, Namibia, South Africa, and Eswatini (formerly Swaziland) are low transmission settings with the goal of eliminating by 2020 and to subsequently pave the way for elimination in the 4 second-line countries, namely, Angola, Mozambique, Zambia, and Zimbabwe to eliminate malaria by 2030 (Lover *et al.*, 2017). The reduction of malaria in the 8 countries is shown in Figure 4 with a distinction in malaria case burden between the frontline and second line countries. The elimination goal to eliminate malaria by 2020 of some of the frontline countries such as Namibia have however been revised to 2022 following recent outbreaks (Chanda *et al.*, 2018).

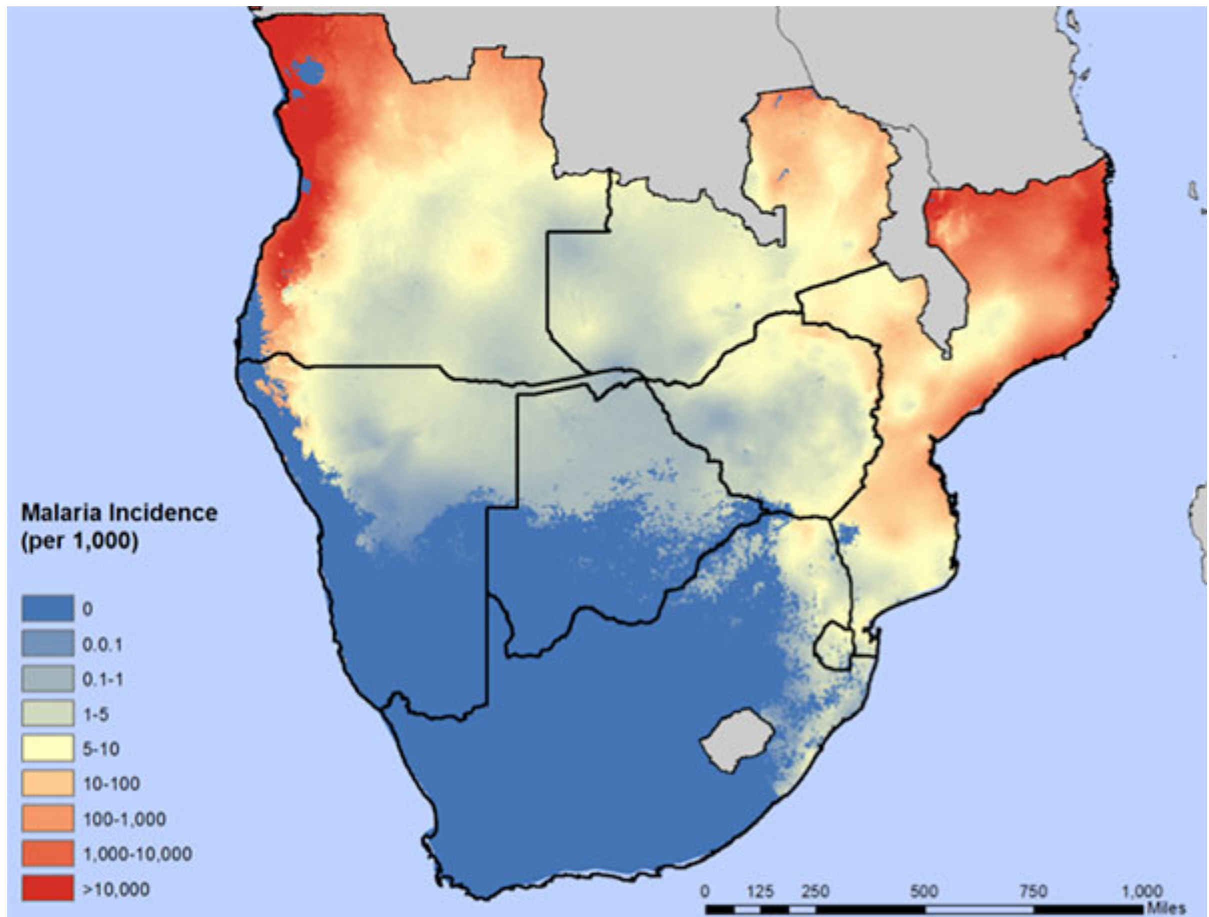

Figure 4: Varying levels of malaria incidence in the highly connected E8 region. Source: https://malariaelimination8.org/state-malaria-e8/

2.6 Malaria elimination

Malaria elimination has been defined as "interrupting local mosquito-borne malaria transmission in a defined geographical area, i.e. zero incidence of locally contracted cases, although imported cases will continue to occur." (Global malaria control and elimination: report of a technical review. Geneva: WHO 2009). Malaria progress towards elimination is classified and measured by the malaria elimination continuum. The 4 phases of the continuum are control, pre-elimination, elimination and prevention of reintroduction as illustrated in figure 6. The control phase involves vector control and decreasing prevalence

to 5cases/1000population and in Southern Africa, Angola and Zambia are examples of countries in the control phase (WHO, 2012). The pre-elimination phase focuses on health information systems, easy access to health facilities and effective coverage of health interventions on all transmission areas, and studies have shown improved adherence to interventions for malaria prevention with increased knowledge about malaria in the community (Ahmed, Hossain, & Kabir, 2014; WHO, 2017). Finally, during the elimination phase, identifying and treating all remaining malaria cases with efficacious antimalarial drugs are the focal points and the reduction of malaria cases in Namibia make the elimination goal feasible (Mumbengegwi *et al.*, 2018; Gueye *et al.*, 2014; Tambo *et al.*, 2018).

However, the current tools available to move towards elimination though useful, do not address some key elimination challenges such as accurate measurement of malaria transmission intensity, parasite diversity, parasite flow and connectivity of human populations carrying the parasite. Therefore, new methods are required to supplement the current tools for measurement of transmission intensity and importation.

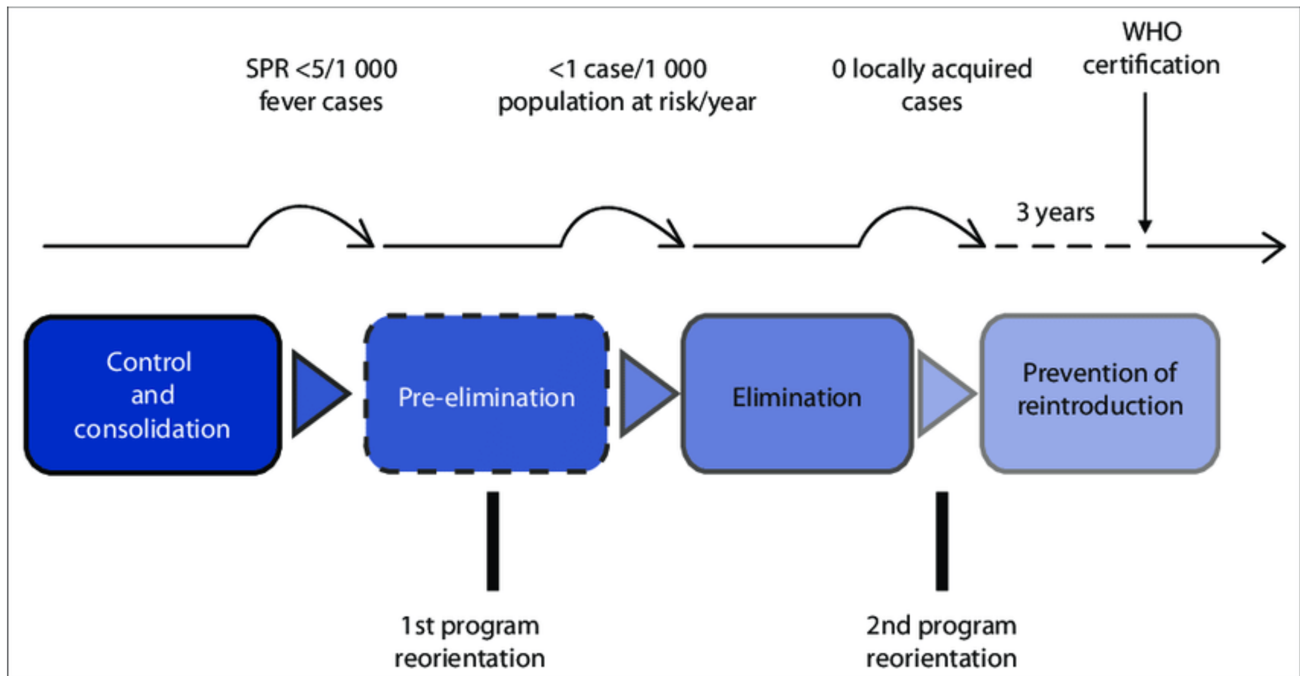

Figure 5: Malaria elimination continuum showing the 4 phases and their respective thresholds (Moonasar *et al.*, 2013)

2.7 Malaria elimination challenges and available tools

2.7.1 Diagnosis of malaria

2.7.1.1 Clinical diagnosis

Although it is no longer in use, clinical diagnosis was the most commonly used method for malaria diagnosis and the basis for self-treatment before the requirement of parasitological confirmation (Biritwum, Welbeck & Barnish, 2000). Clinical diagnosis of malaria is given based on the presenting symptoms only such as a fever, headache and fatigue (Stow, Torrens & Walker, 1999). However, the overlapping of malaria symptoms with other tropical diseases such as flu and Human Immunodeficiency Virus (HIV) impairs its specificity and therefore encourages the indiscriminate use of anti-malarials

for managing febrile conditions in endemic areas and this promotes the development of drug resistance (Mwangi *et al.*, 2005).

Therefore, WHO recommended tools for malaria diagnosis that confirm parasitological confirmation before treatment with appropriate drugs, the diagnostic tools are RDTs and microscopy (WHO, 2015). These tools allow for accurate confirmation of *Plasmodium* parasite presence to ensure appropriate use of drugs and thereby reducing the development of drug resistance.

2.7.1.2 Expert microscopy

Expert microscopic examination of Giemsa-stained blood smears is the gold standard of malaria diagnosis (Anchinmane & Shedge, 2011). Giemsa microscopy is considered the most appropriate recommended diagnostic instrument for malaria control because it is inexpensive to perform, allows visualization and differentiation of *Plasmodium* species, and quantification of *Plasmodium* parasites (Kahama-Maro *et al.*, 2011). Giemsa microscopy requires the examination of both thin and thick blood smears from the same individual. Microscopy can routinely detect parasitaemia levels as low as 50 parasites/ul (Moura *et al.*, 2014).

However, microscopy misses low parasite density infections (with a detection threshold of about 50 parasites/ul) that make up the majority of infections in low transmission settings, it is labour intensive, requires rigorous maintenance of functional infrastructures plus effective quality control (QC) and quality assurance (QA) (Moura *et al*., 2014; Tambo *et al*., 2018).

2.7.1.3 Rapid diagnostic Tests (RDTs)

Malaria RDTs are WHO recommended immuno-chromatographic tests that detect parasite antigens in a small amount of blood ranging from 5-15ul (McMorrow, Aidoo and Kachur, 2011). Currently, immuno-chromatographic tests target the histidine-rich protein 2 of *P. falciparum*, a pan-malarial *Plasmodium* aldolase, and the parasite specific lactate dehydrogenase enabling a distinction between *P. falciparum* and non- *P. falciparum* infections (Moura *et al.*, 2014). The RDTs are field user friendly, relatively cheap and accessible in most settings as they do not require additional infrastructure and have therefore improved access to malaria tests even in resource poor settings (Barber *et al.*, 2013; Cotter *et al.*, 2013).

The use of RDTs has replaced clinical diagnosis thereby obviating presumptive treatment and enabled test and treat as recommended by the WHO (Moura *et al.*, 2014). The use of RDTs unfortunately has shortcomings such as multiple reports on persistence of parasite antigens in the blood after parasite clearance for 4-8 weeks that gives rise to false positives and over use of drugs and they can miss malaria infections due to deletions in the *HRP2* gene in *P. falciparum* (Kyabayinze *et al.*, 2008; Harris *et al.*, 2010; Hemingway *et al.*, 2016). Another disadvantage that has been reported is the poor performance of RDTs at low parasite density below 100 parasites/ul, missing up to 60% of infections (Okell *et al*., 2012; Smith *et al*., 2017; Tambo, Mwinga & Mumbengegwi, 2018).

2.7.1.3 Ultra-sensitive Rapid diagnostic tests (uRDTs)

To overcome the current shortcomings of the RDTs, an ultrasensitive RDT (us-RDT) has been developed to facilitate the detection of asymptomatic *P. falciparum* infections in a

point-of-contact manner. However, the performance of the us-RDT in terms of sensitivity is statistically insignificant compared to the conventional RDT, the specificity is similar to that of the conventional RDT and as a result of parasite persistence the us-RDT shows positive for 8-12 weeks after *Plasmodium* parasite clearance and deletions in the *HRP2* gene in the *P. falciparum* genome (Hofmann, et al., 2018). There have been a few studies that have reported significantly increased sensitivity with the us-RDT but still significantly low compared to molecular diagnostic tools (Das *et al.*, 2018; Girma *et al.*, 2018; Landier *et al.*, 2018; Vásquez *et al.*, 2018).

2.7.2 Molecular diagnosis of malaria

In low transmission areas, a number of studies have reported that the majority of infections are asymptomatic with low parasite density that are frequently below the detection limit of microscopy and RDTs (Oriero *et al.*, 2015; Zheng & Cheng, 2017) Molecular methods such as loop-mediated isothermal amplification (LAMP) and the polymerase chain reaction (PCR) have been suggested to address the challenges with the conventional tools, the most sensitive diagnostic tools for malaria are based on the molecular detection of parasite DNA or RNA (Tambo *et al*., 2018).

2.7.2.1 Loop mediated isothermal amplification (LAMP)

Loop-mediated isothermal amplification uses nucleic acid amplification which uses a single temperature incubation thereby obviating the need for expensive thermal cyclers (Britton, Cheng & McCarthy, 2016). It employs strand displacement DNA synthesis performed with *Bst* (*Bacillus strearothemorphilus*) DNA polymerase from therefore isothermal conditions are used since there is no need for denaturation and has a robust

polymerase enzyme that is not as sensitive to PCR inhibitors thereby making it more suitable for use under field settings *(Lucchi et al.*, 2010; Mori & Notomi, 2009; Tambo *et al*., 2018). LAMP is highly sensitive (detecting low parasite densities as low as 1parasite/ul) and specific, gives results faster than PCR, requires minimal processing and instrumentation, and allows result detection with the naked eye (Han, 2013; Mori & Notomi, 2009; Tambo *et al*., 2018). In Uganda, LAMP was shown to detect low parasite density infections in clinical samples, with the same efficiency as Nested PCR in a reference lab in the United Kingdom (Polley *et al.*, 2013).

2.7.2.2 Polymerase Chain Reaction (PCR)

PCR also utilizes nucleic acid amplification to detect malaria infections in blood, it is highly sensitive (detecting low parasite densities as low as 1parasite/ul) as it amplifies the detection signal; therefore all the cases of malaria could be detected and treated (Pöschl *et al.*, 2010; Hopkins *et al.*, 2013). Specifically, PCR has a sensitivity and specificity of 100 per cent with a detection limit of just 1 *P. falciparum* or 3 *P. Vivax* parasites/µl of blood when compared with the microscopy and has been credited to detect mixed infections with ease (Mahende *et al.*, 2016; Ranford-Cartwright & Ciuffreda, 2016). However, PCR, is a highly sophisticated technique, requires infrastructural support, expensive to set up and more time consuming than microscopy and immunochromatography (Tambo *et al.*, 2018). However, these molecular tools are limited to reference laboratories and research teams as they are not easy to set up, require skilled personnel and are more expensive compared to the conventional tools: most of the malaria

cases are in resource poor settings and molecular tools are limited to tests in reference laboratories (Tambo *et al.*, 2018).

2.7.3 Alternative diagnostic methods

Alternative methods for malaria diagnosis are available, these include the detection of malaria antibodies by indirect immunofluorescence antibody assay [IFA] and enzyme-linked immunosorbent assays [ELISA]), DNA probes, fluorescent staining like the quantitative buffy coat [QBC] analysis, interference filter system for acridine orange-stained thin blood smear, and flow cytometry (Demirev *et al.*, 2002; Moura *et al.*, 2014). However, these alternatives have shown limited success in malaria diagnosis (Moura *et al.*, 2014).

2.7.4 Vector control

Malaria is transmitted by mosquito vectors only, therefore vector control is essential for reducing malaria transmission (Raghavendra *et al.*, 2011, 2017; Noor *et al.*, 2013). The control phase in the malaria elimination continuum focuses on vector control across the population and decreasing prevalence to 5cases/1000population (WHO, 2015). However, even as transmission is reduced and countries move to elimination, vector control remains an important element in transmission interruption, but it becomes more targeted (Russell *et al.*, 2013). There are 3 main tools used for vector control which are Indoor Residual Spraying (IRS), long lasting insecticide treated nets (LLINs) and larviciding (Russell *et al.*, 2013; Benelli and Beier, 2017; Protopopoff *et al.*, 2018).

The most successful and widely used of all the vector control interventions are insecticide treated nets (ITNs) including long lasting ITNs and indoor residual spraying (IRS) using chemicals such as actelic and Dichlorodiphenyltrichloroethane (DDT) (Raghavendra *et al.*, 2011; Russell *et al.*, 2013; Kleinschmidt *et al.*, 2015; Benelli and Beier, 2017). The use of IRS kills mosquitoes as they rest on walls within sleeping structures or repels them before they feed whilst LLINs prevents the mosquito from biting individuals under the net (Mumbengegwi *et al.*, 2018). Other alternative vector control methods such as larviciding are difficult to implement as vectors can use a diverse and extensive range of breeding sites that vary by species (Russell *et al.*, 2013).

Namibia has adopted the WHO recommended implementation of integrated vector management (IVM) along the five key strategic elements and the global plan for insecticide resistance management (GPIRM) along its five pillars as a means of preventing the development and spread of resistance (WHO, 2004, 2012; WHO, 2015). The IVM is defined as "A rational decision-making process for the optimal use of resources for vector control, i.e. a management approach in which: decisions are based on evidence and surveillance data; there may be several vector control methods addressing single or multiple diseases; and there is broad participation across sectors and within the community" (WHO, 2004). Importantly, IVM promotes capacity-building at all levels to implement, making it a sustainable approach. Extensive coverage with IRS above 80% has contributed to the reduction in the number of malaria cases in Namibia (MoHSS, 2010b).

The LLINs are designed as an effective vector control measure for malaria and they are mostly used in combination with IRS in order to achieve greater health benefits (Okumu *et al.*, 2013). In Namibia, more than 500 000 LLINs were distributed with pregnant women and children under the age of 5 being the focal points as they are the most vulnerable (MoHSS, 2010a). The combination of LLINs and IRS is wide spread in malaria endemic regions in Namibia as an effort to keep reducing the number of malaria cases.

2.8 Malaria treatment

To reduce the malaria burden globally, appropriate antimalarial chemotherapy is essential (Noor *et al.*, 2013). The key challenge in Africa is improving access to effective malaria diagnosis and treatment which is critical to proper management of the disease and improvement of health outcomes (Gharbi *et al.*, 2013). The WHO recommends early diagnosis, appropriate treatment following parasitological diagnosis, Test, Treat, Track, launched by Margret Chen in Namibia (WHO, 2015a). However recently a major challenge to malaria elimination is the development and spread of resistance in parasites particularly the *P. falciparum* (Sharma *et al.*, 2015). *P. falciparum* has been reported to be the most virulent parasite and it has developed drug resistance to almost all the currently used antimalarial drugs (Petersen, Eastman and Lanzer, 2011) namely, amodiaquine, chloroquine (CQ), mefloquine (MQ), quinine and sulfadoxine–pyrimethamine (SP) (WHO, 2015). Surveillance and monitoring of emerging drug resistance and reduced drug efficacy is important as Namibia moves towards malaria elimination by 2023.

Artemisinin combination therapy (ACTs) was introduced as first-line treatment for uncomplicated *falciparum* malaria (Mace, Arguin, & Tan, 2018; WHO, 2015). However, reports of reduced efficacy and treatment failure first reported artemisinin efficacy was reported in South East Asia along the Thailand-Cambodia boarder and are now emerging from northern Angola raising concerns around the efficacy of ACTs in Namibia (Veiga et al., 2016), this was partly due to the use of counterfeit drugs in Thailand (Nayyar *et al.*, 2012). Drug efficacy and resistance is monitored by molecular assessment of putative genes at specific positions that have been identified as molecular indicators of resistance (Petersen *et al.*, 2011; WHO, 2015) but therapeutic efficacy studies are difficult in low transmission settings as the number of cases is low and there are challenges with the diagnosis tools (Warsame *et al.*, 2015).

Mutations in the *Plasmodium falciparum*, propeller region *Pfk-13* gene have been associated with artemisinin resistance (Miotto *et al.*, 2015). In low transmission settings like South East Asia, there is generally reduced competition between parasites as mosquitoes carry a single genotype (Murray *et al.*, 2016). This makes Asia a suitable setting for *kelch13* mutations to maintain association and propagate with a favourable genetic background and spread via natural selection (Cerqueira *et al.*, 2017). The WHO recommends taking antimalarial drugs, chemoprophylaxis before travelling to a malaria endemic region, however, chemoprophylaxis does not give complete protection against malaria, it significantly reduces the chances of acquiring a malaria infection (WHO 2017). For example, 2-3 weeks before departure, weekly doses of mefloquine should be taken and a weekly dose of chloroquine should be started a week before departure (WHO, 2017).

2.9 Heterogeneous and cross boarder transmission

Heterogeneous transmission has been reported to be a typical characteristic of infectious diseases where infection and disease are unevenly distributed; condensed in certain geographical locations and populations (Woolhouse *et al.*, 1997; Reiner *et al.*, 2015; Baidjoe *et al.*, 2016). In malaria, heterogeneous transmission is exhibited in small groups of households (hotspots) and populations (hot pops), within malaria endemic communities that are at a substantially increased risk of malaria transmission compared to surrounding households as illustrated in figure 7 where there are high and low transmission settings for neighbouring communities (Cui *et al.*, 2012; Reiner *et al*., 2015; Smith *et al.*, 2017).

The relevance of the non-uniform decrease of malaria cases illustrated in figure 7 is that there is high risk of continuous importation of malaria into Namibia from Angola which could frustrate malaria elimination efforts in Namibia. Heterogeneity occurs across all transmission settings, but are most easily detected at low transmission and could be sources of outbreaks (Bousema & Baidjoe, 2013; Cotter *et al.*, 2013). Malaria transmission heterogeneity has been reported for years but its importance has only been highlighted recently, previously assumptions where made for uniform transmission which led to inaccurate conclusions (Males, Gaye & Garcia, 2008; Schwarz *et al.*, 2008; Bousema, Kreuels & Gosling, 2011). For successful malaria elimination, national malaria control programs (NMCPs) must locate and keep track of both local and imported infections, conduct contact tracing, and ensure that onward transmission resulting from importation events are detected and treated rapidly (Gueye *et al.*, 2014).

A variety of approaches have been suggested and used to characterize the spatial dynamics of malaria and to allocate resources effectively (Auburn & Barry, 2017). In Namibia, reactive case detection (RACD) has been adopted as policy where malaria cases are counted and recorded where they are diagnosed and traced to their place of residence where individuals around the reported case are tested and treated ('WHO, 2015). It is assumed without evidence that a case originates where it is reported but cases can be imported from other countries or different malaria endemic countries within a country (Galappaththy, Fernando & Abeyasinghe, 2013; Sturrock *et al.*, 2015). This does not provide sufficient information on origin of infections and transmission, especially in the elimination setting, since infections may be acquired at locations other than where they ultimately present and asymptomatic cases that make up the majority of cases in low transmission setting do not present at health facilities (Reiner *et al.*, 2015; Tambo *et al.*, 2018).

Additional surveillance tools, including data on travel history and human population movement, have been suggested to fill this gap, but the self-reported travel history and human population movement data are inaccurate and incomplete (Auburn & Barry, 2017; Pacheco et al., 2015; Reiner *et al*., 2015). This leads to suboptimal allocation and use of resources which is essential in elimination settings where interventions need to be targeted; ultimately frustrating elimination efforts.

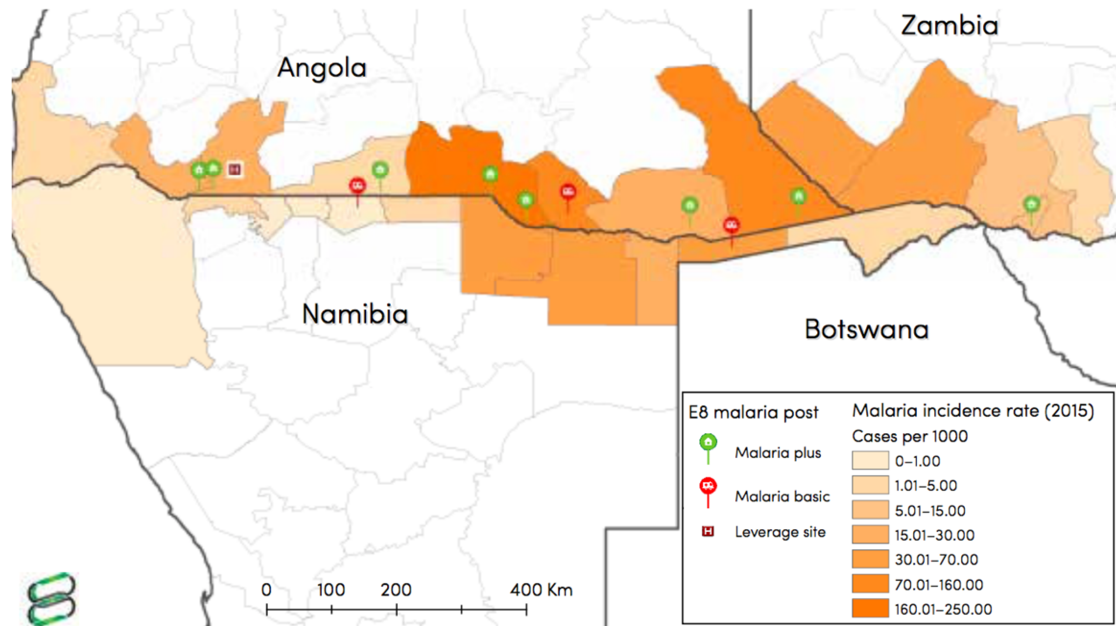

Figure 6: Non-uniform decrease of malaria across boarders; a challenge for elimination. Source: https://malariaelimination8.org/namibia/

2.10 Population genetics of *Plasmodium* (Hardy-Weinberg theorem)

Population genetics is based on a fundamental principle, the Hardy- Weinberg theory, that describes a non-evolving population. The theorem is defined as population gene frequencies and genotype frequencies remain constant from generation to generation if mating is random and if mutation, selection, immigration, and emigration do not occur (Marantz, 2009). The Hardy-Weinberg equilibrium can be affected by factors that include mutations, natural selection, nonrandom mating, genetic drift, and gene flow (Baily, 2015).

For instance, mutations in the *Plasmodium* genome disrupt the equilibrium of allele frequencies by introducing new alleles into a population and could lead to drug resistance (Sharma *et al.*, 2015). Similarly, natural selection and nonrandom mating disrupt the Hardy-Weinberg equilibrium because they result in changes in gene frequencies by promoting the unequal propagation of genetically favoured *Plasmodium* genotypes (genetic vigour) (Auburn & Barry, 2017). This occurs because certain alleles help or harm the reproductive success of the organisms that carry them. Another factor that can upset this equilibrium is genetic drift, which occurs when allele frequencies grow higher or lower by chance and typically takes place in small populations as *Plasmodium* populations have been shown to cluster in "hotspots" around reported symptomatic cases (Schultz *et al.,* 2010). Gene flow, which occurs when breeding between two populations transfers new alleles into a population, can also alter the Hardy-Weinberg equilibrium and occurs frequently as human mobility increases within regions and across boarders from areas of high malaria transmission, to areas of low malaria transmission (Greenhouse & Smith, 2015).

Because all of these disruptive forces commonly occur in nature, the Hardy-Weinberg equilibrium rarely applies in reality (Marantz, 2009). The Hardy-Weinberg equilibrium is therefore an idealized model, and genetic variations in nature can be measured as deviations from this equilibrium state.

2.10.1 *Plasmodium* genome

The appreciation of the molecular basis for disease development and propagation led to a number of genome projects to understand the genetic composition of humans, parasites and vectors (Gardner *et al.*, 2002; Greenhouse *et al.*, 2006; Greenhouse & Smith, 2015). In 1995 the malaria genome project commenced with a focus on *P. falciparum* which is the most lethal of all malaria causing parasites in humans (Murray *et al.*, 2016; Auburn & Barry, 2017). The genome of its mitochondrion was the first to be sequenced and reported in 1995, and the entire genome was reported on 3 October 2002 (Gardner *et al.*, 2002; Bernardes, Vaquero & Carbone, 2017). The *P. falciparum* genome is composed of 22.8 mega bases (Mb) and the nuclear genome is composed of 14 chromosomes ranging in size from 0.643M b to 3.29Mb and encodes about 5 300 protein coding genes (Bushell *et al.*, 2017) as illustrated in Figure 7. Extensive chromosome length variation has been observed as a result of recombination and it is concentrated in the subtelomeric regions. The genome has on average 1 gene per 4 338 base pairs (bp) and is the most (A+T) - rich genome to date, making up to 80% of genome (Escalante, Lal & Ayala, 1998; Greenhouse *et al.*, 2006; Pava *et al.*, 2017).

The *Plasmodium* genome in general is highly adapted for rapid host colonization, highly polymorphic genes that encode surface antigenic proteins have been observed (Anderson *et al.*, 2000; Barry *et al*., 2013; Fola *et al.*, 2017). The identification of consequential genes and pathways is important for drug and vaccine development (Gardner *et al.*, 2002; Razakandrainibe *et al.*, 2005; Bernardes, Vaquero and Carbone, 2017; Bushell *et al.*, 2017; Amambua-Ngwa *et al.*, 2018).

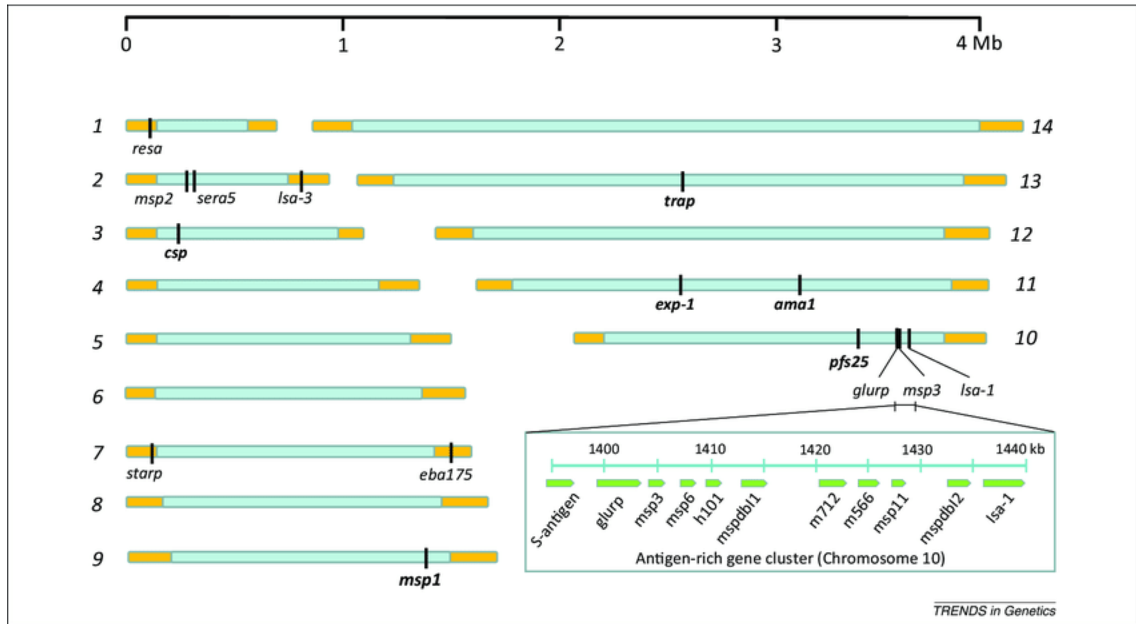

Figure 7: Haploid *P. falciparum* genome with 14 chromosomes. Source: https://www.researchgate.net/figure/Haploid-Plasmodium-falciparum-genome-containing-14-chromosomes-The-whole-genome-of-23-Mb_fig1_271225415 [accessed 21 Apr, 2019]

As malaria transmission declines, the focus of malaria programs shifts from measuring prevalence to monitoring changes in incidence (Bhatt *et al.*, 2015). The prevalence of malaria is defined as the proportion of cases in the population at a given time and incidence is the rate of occurrence of new cases (Cameron *et al.*, 2015). Thus measuring incidence gives information on the risk of contracting the disease whereas prevalence indicates how wide spread the disease is (Bhatt *et al.*, 2015; Boncy *et al.*, 2015).

However, malaria epidemiology is multi-dimensional; it requires measurement of more than transmission intensity but should also include the incidence of certain parasite species, direction of parasite flow, transmission linkages, onset of natural immunity, spread of antimalarial drug resistance, vector diversity among others that vary among

endemic areas (Escalante *et al.*, 2015; Pacheco et al., 2015). Therefore, molecular methods such as genotyping have found increased utility in the last two decades and have been enriched by including population biology and population genetics (Conway, 2007).

210.2. Size polymorphisms

Traditional malaria genotyping is based on the size polymorphism of genes encoding surface antigens with variable numbers of tandem repeats such as, *msp2* in *P. falciparum*, *msp3α* in *P. vivax* and *msp1* in both *P. vivax* and *P. falciparum* (Auburn & Barry, 2017). The primary use of this method is to determine the number of parasite genotypes infecting an individual at the same time or multiplicity of infection (MOI) (Escalante *et al.*, 2015). This method is however limited and cannot be used to interpret other aspects of molecular epidemiology such as population structure by geographical location (Bruce *et al.*, 2000; Bozdech *et al.*, 2003; Conway, 2007).

2.10.3 Partial sequencing

Another approach to genotyping malaria parasites is based on sequencing partial or complete genes from nuclear or organellar which has good utility in determining diversity of genes that carry markers or mutations linked to drug resistance and for genes under consideration as vaccine candidates (Auburn & Barry, 2017; Duffy *et al.*, 2017). This method has the advantage of being comparable across different geographical locations and transmission settings but it is costly and time consuming (Conway, 2007; Escalante *et al.*, 2015; Auburn & Barry, 2017).

2.10.4 Multilocus genotyping

The most popular approach to malaria parasite genotyping is multi-locus genotyping that targets non-antigenic loci (Pacheco et al., 2015; Schultz et al., 2010). The most commonly used markers with this approach are microsatellite markers and single nucleotide polymorphisms (SNPs) which both allow a wide range of epidemiological aspects to be determined such as MOI, population structure, diversity and linkage disequilibrium (Collins and Jeffery, 2005; Vardo-Zalik *et al.*, 2013; Auburn & Barry, 2017). Microsatellites have the advantage of higher mutation rates and can therefore be used to detect recent events over SNPs (Cramer *et al.*, 2004). In addition, microsatellite makers are abundant with an average of 1 microsatellite marker per 2-3kb. Most of the population genetics to date of *P. falciparum* is based on a set of consensus markers described in 1999 and most studies use 8-12 of these markers (Anderson *et al.*, 2000). There are no consensus markers for *P. vivax* although it is the more ubiquitous species (Anderson, Su, Bockarie, Lagog, & Day, 1999; Auburn & Barry, 2017; Pacheco et al., 2015).

2.10.5 Whole genome sequencing

Whole genome sequencing is the most advanced technique for genotyping as it provides the highest resolution in differentiation of genotypes (Pacheco et al., 2015). Whole genome sequencing involves determining the genetic makeup of the *Plasmodium* parasites and base by base comparison of the *Plasmodium* (Nabet *et al.*, 2016). Studies have reported extensive microsatellite polymorphisms and this information led to the developed multiple polymorphic and easy to score microsatellite loci that can be used in epidemiological investigations (Winter et al., 2015; Nabet et al., 2016). The use of whole

genome sequencing has led to effective surveillance of drug resistance, accurate evaluation of outbreaks and intervention and tracking of genetic fitness traits (Auburn & Barry, 2017; Schultz *et al.,* 2010). The investigation of adaptive molecular changes in natural populations of Plasmodium is of importance in prevention of disease and implementing interventions informed by evidence. However, whole genome sequencing is inaccessible for routine use because of the cost and technical expertise required for processing , especially in resource limited regions where malaria is endemic (Conway, 2007; Vardo-Zalik *et al.*, 2013; Auburn & Barry, 2017).

2.11 Plasmodium population diversity variation dogma

In the population genetics studies to date, there is general consensus on what is expected when malaria is declining and the differences between high and low transmission settings as shown in Table 1 (Koepfli, Waltmann, & Ome-kaius, 2018; Pacheco *et al*., 2015). In high transmission settings (mostly in Africa), high levels of MOI have been reported and the *P. falciparum* populations are panmictic; meaning that they are highly mixed (Greenhouse & Smith, 2015). In addition, the *P. falciparum* populations show high levels of genetic diversity and linkage equilibrium as reported in Angola, Zambia, Zimbabwe and other high transmission settings (Auburn & Barry, 2017; Cheeseman et al., 2009; Conway, 2007;Koepfli et al., 2015; Rebaudet *et al.*, 2010; Vardo-Zalik *et al*., 2013).

The high genetic diversity and frequency of heterozygous forms could in fact show that random mating events probably occurred within mosquito bloodmeals between gametes

belonging to different parasite clones (Babiker *et al*., 1994). The random mating in the mosquito leads to high genetic diversity in the blood stage of the parasite where it is haploid; every unique allele represents a unique genotype (Nabet *et al.,* 2016). The practical significance of high level of genetic diversity and linkage equilibrium in terms of control is that with high genetic diversity and linkage equilibrium there is a high risk of complicated malaria as the clinical presentation, high risk for the development of drug resistance and a high risk of the spread of drug resistance (Schultz et al., 2010). Additionally, polymorphisms in the host could also lead to different clinical presentations of *P. falciparum* malaria (clinically asymptomatic parasitism, acute uncomplicated malaria and severe malaria as defined by WHO criteria) and counteract interventions such as anti-malarial therapy; therefore polymorphism surveillance in the host is of importance as well (Omodu *et al.,* 2005). Omodu et al., (2005) genotyped genes coding for four human adhesion molecules at six different loci (ICAM-1 exons 2, 4 and 6, E-selectin exon 2, CD36 exon 10, and PECAM exon 3) and the other loci were at Hardy-Weinberg equilibrium (HWE); the results showed that genetic polymorphisms at host adhesion molecules loci are an important variable in the susceptibility to severe malaria.

On the other hand, in lower transmission settings such as South America and parts of Southeast Asia such as Comoros Archipelago, Shandong province in China and the Solomon Islands, MOI is generally low, there is high linkage disequilibrium, low diversity which could be an indication of interruption of transmission (Razakandrainibe *et al.*, 2005; Pacheco *et al.*, 2015). In *P. vivax* the differences in low and high transmission settings are not as pronounced as in *P. falciparum* (Assefa *et al.*, 2015; Auburn & Barry, 2017;

Azas *et al.,* 2002; Conway, 2007; Duffy, 2012; Escalante *et al.*, 2015; Pacheco *et al.*, 2015; Waltmann *et al.*, 2018).

Table 1: Molecular criteria used to characterize epidemiology in low and high transmission malaria settings and expected outcomes (Anderson *et al.*, 1999; Auburn & Barry, 2017; Escalante *et al*., 2015; Mobegi *et al.*, 2012; Vardo-Zalik *et al.*, 2013)

	High transmission	**Low transmission**
Polyclonal infections	common	less common
Linkage disequilibrium	low	high
Genetic diversity	high	low
Parasite populations	highly admixed	fragmented

2.12 Manuscripts addressing specific objectives

To address objective A (To determine *P. falciparum* genetic diversity across district health facilities in the Kavango East and Zambezi regions of Namibia through determining complexity of infections as a measure of within host *P. falciparum* genetic diversity calculating the population level genetic diversity, determining *P. falciparum* random or nonrandom mating patterns through calculation of linkage disequilibrium and describing the *P. falciparum* clustering patterns geographically) in chapter 3 the complexity of infections (within host diversity), population level genetic diversity, linkage disequilibrium and *P. falciparum* clustering patterns geographically were calculated and determined. This manuscript addresses the challenge that there are no data on *P. falciparum* population genetic structure which gives information on the risk of clinical presentations as complicated malaria, risk of development of drug resistance, risk of the spread of drug resistance, parasite flow, parasite origin and connectivity of malaria endemic regions.

To address objective B (To compare the malaria case distribution in Angolan and Namibian residents in the Kavango East region in Namibia by age and district residence using primary residency as a proxy for residency) chapter 4 compared the distribution of malaria cases by age in both the Namibian and Angolan malaria infections. In addition to this, the risk to travel to Angola and acquire a malaria infection was calculated by residence in Namibia. This manuscript addresses the challenge that there are no accurate data on importation from Angola to Namibia and the level of contribution of imported infections to local transmission. The distribution of malaria cases by age is expected to

differ significantly by age and if there are similarities in the distribution it will be an indication of importation and the contribution of importation to local malaria transmission

To address objective C (To investigate the usefulness of *P. falciparum* genetic diversity as a measure of malaria transmission intensity) chapter 5 determined the use of population genetic matrices including complexity of infections, population level genetic diversity, allelic richness to measure transmission intensity. These population genetic matrices were plotted against the number of reported cases to determine if there is a correlation. This manuscript addresses the challenge that current tools miss asymptomatic infections and underestimate malaria transmission intensity as genetic tool only require a representative small sample to accurately determine transmission intensity. In addition, travel to Angola was plotted against the number of reported cases to determine if there is a correlation between the incidence of malaria and travel to a high malaria transmission setting, Angola.

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CHAPTER 3: GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* IN THE KAVANGO EAST AND ZAMBEZI REGIONS OF NAMIBIA

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3.1 Abstract

Accurate information on malaria transmission is required for determining malaria elimination strategy, monitoring progress towards zero local malaria transmission, and verifying and maintaining elimination status. Therefore in this study, malaria positive samples and the corresponding epidemiological data were collected from 4 health districts in the Kavango East and Zambezi regions of Namibia. DNA was extracted and the parasites genotyped with capillary electrophoresis using a 26 microsatellite marker set. The study showed that (1) microsatellite genotyping resolve differences between parasites within the host and at health facility level (2) There is substantial connectivity amongst health districts; that is there is high importation and mixing of *P. falciparum* parasites amongst the health districts and (3) Namibia in particular does not follow the pre-described population genetics pattern for low transmission settings as a result of connectivity amongst high and low transmission countries. Genotyping has the potential to address unique elimination challenges by accurately informing the elimination strategy on origins of infections, level of connectivity and parasite flow.

3.2 Background

Over 200 million cases of malaria are reported annually across 91 countries globally with over 90% of the malaria-related deaths being reported in Sub-Saharan Africa with children under the age of 5 at highest risk (Gething *et al.*, 2011; Gbalégba *et al.*, 2018). Following the revitalized interest in global eradication of malaria in 2007 with support from the Bill and Melinda Gates foundation, a number of countries formed regional initiatives in order to eliminate malaria within endemic regions with concerted efforts which has resulted in some significant declines (Hall and Fauci, 2009; Lover *et al.*, 2017).

Namibia, which is part of the Southern Africa's Malaria Elimination 8 regional initiative declared a goal in 2009 to eliminate malaria within its borders by 2020 (Namibia malaria indicator survey, 2009) and has revised the goal to 2022. Malaria elimination is a worthy goal however it has several challenges with the main ones being emerging drug resistance to antimalarial medicines (Fairhurst, 2015; Olasehinde *et al.*, 2014), failure to diagnose low parasite density infections by the widely used rapid diagnostic tests and microscopy (Tambo *et al.*, 2018), a lack of resources (Smith Gueye *et al.*, 2014), heterogeneous transmission (Baidjoe *et al.*, 2016), high genetic diversity, incomplete and inaccurate data whose underlying assumptions are unclear (Reiner *et al*., 2015; Schultz et al., 2010; Sharma *et al.*, 2015).

Currently, malaria cases in Namibia are recorded and reported at health facilities where they present and are classified as local or imported based on travel history. This is not always the case as patients exhibit a preference in selecting and visiting health facilities

based on perceptions of care given. Moreover , asymptomatic cases do not present at health facilities and travel history which is self-reported and can be incorrect and incomplete (Galappaththy et al., 2013; Rulisa et al., 2013; Smith et al., 2017). In addition, accurate measurement of transmission intensity, parasite flow and monitoring progress towards zero which cannot be determined by incidence data are required (Escalante *et al.*, 2015; Greenhouse & Smith, 2015). The levels of genetic diversity are also indicators of the potential of drug resistance emergence (Schultz *et al.*, 2010). Therefore the current study was conducted to use molecular genotyping of *Plasmodium falciparum* (*P. falciparum*), the most prevalent species in Namibia, to address some of these key elimination challenges.

Multi-locus genotyping of non-antigenic loci is the widely used approach in malaria genotyping, with microsatellite markers and SNPs being the most utilized (Pacheco et al., 2015). The use of these markers allows a wide range of epidemiological aspects to be determined such as multiplicity of infection (MOI), population structure, diversity, linkage and parasite flow among others (Vardo-Zalik *et al.*, 2013; Auburn & Barry, 2017). In the population genetics of *P. falciparum,* in high transmission settings in Africa such as Angola, Congo and parts of Zambia and Zimbabwe, high levels of MOI, highly panmictic populations, high levels of genetic diversity and linkage equilibrium have been reported (Anderson *et al.*, 2000; Schultz *et al.*, 2010) In lower transmission settings such as South America and parts of Southeast Asia, such as Thailand, the Shandong province in China, Colombia and Brazil there is low MOI, with high linkage disequilibrium, low diversity and more evidence of population structure which could be an indication of interruption of transmission. (Anderson *et al.*, 1999; Auburn & Barry, 2017; Murray *et*

al., 2016; Pacheco *et al.,* 2015; Rebaudet *et al*., 2010; Schultz *et al.*, 2010).

In Namibia, a low transmission setting, malaria is confined to the North-Eastern regions of the country. The Kavango East and Zambezi regions account for about 90% of malaria cases in Namibia (Nghipumbwa *et al*., 2018). Genetically, the malaria infections in Namibia would be expected to have a low MOI, high linkage disequilibrium as there is less random mating of *P. falciparum* parasites with fewer cases, low genetic diversity and fragmented populations as a result of transmission interruption. Populations are fragmented as transmission is interrupted in some geographical areas and *Plasmodium* parasites in one geographical area evolve without mixing with other parasite populations as they would if the number of cases were high and widespread. However, malaria transmission has been reported to be heterogonous; influenced by geography, behavior and socio-economic status of the population in the malaria endemic region (Escalante *et al.*, 2015; Greenhouse & Smith, 2015; Reiner *et al.*, 2015). Therefore, the current study aimed to describe transmission of malaria in these regions using genetic data to supplement the incidence data in order to inform the elimination strategy. This study employed novel approaches such as using all alleles for population genetics analysis and novel validated markers for the Sub Saharan settings.

3.3 Methods

3.3.1 Study sites and *P. falciparum* isolates

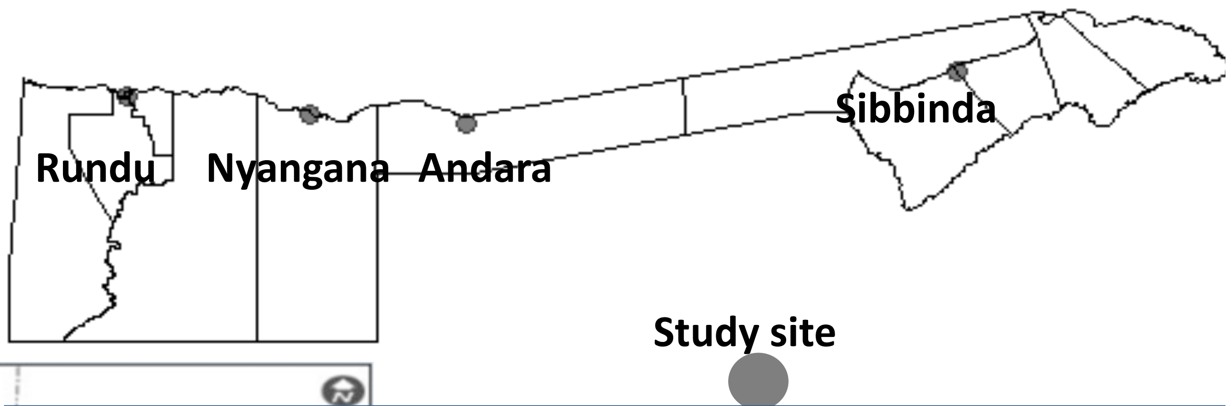
Inclusion criteria used was all RDT confirmed malaria cases at Heath Facilities that consented (>=18 years) to participating in the study and assent was given for minors (<

18 years). All age groups were included, participation was voluntary, participants could drop out of the study at any time without consequences and their personal information was treated confidentially.

The study sites were selected in Kavango East and Zambezi regions as illustrated in Figure 8, which account for the majority of malaria cases in Namibia (Mumbengegwi *et al.*, 2018; Nghipumbwa *et al.,* 2018). In the Kavango East region, positive rapid diagnostic tests (RDTs) from all reported symptomatic cases were collected by Health care workers from 3 district health facilities, namely, Rundu, Andara and Nyangana from March to June 2016. Simultaneously, epidemiological data such as age, residence, local and international travel history were collected on the RDT as illustrated in Fig. 10. The RDTs were then stored in 2 zip-lock bags with desiccant and kept at -20°C. In Zambezi region, Dried Blood Spots (DBS) were collected from all reported symptomatic cases from Sibbinda district health facility. Blood was collected on filter paper and left to air dry for 20 minutes to make four DBS, the DBS card were stored at ambient temperature in zip lock bags with desiccant until laboratory analysis. Epidemiological data such as age, residence, local and international travel history were collected as well.

The health facilities in this study are district hospitals; therefore, they are the main catchment facilities and have a minimum distance of 80km between the nearest health facilities. The distance between the health facilities was regarded as enough to detect changes in transmission, diversity of parasites and connectivity as shown in Fig. 9 and Table 1. The district hospitals Rundu and Nyangana have the largest catchment radius and are at least 80km apart, which is sufficient to observe different underlying transmission

dynamics. In a previous study, samples were collected from villages 2-10km apart and this was enough distance in some instances to observe different transmission dynamics (Schultz *et al.,* 2010).In addition, the district health facilities have different proximities to the border posts with Angola and Zambia that are high transmission areas as illustrated in Fig. 9; this could influence transmission dynamics in the different catchment areas.



e study sites, namely, Rundu, Nyangana, Andara and

Table 2: Showing the physical distance in kilometers between health facilities, sufficient distance to detect genetic differences in *P. falciparum* population genetics

Health facility one	Health facility two	physical distance between health facilities in KM
Nyangana DH	Rundu DH	95.40
Andara DH	Rundu DH	176.68
Sibbinda HC	Rundu DH	429.73
Andara DH	Nyangana DH	81.28
Sibbinda HC	Nyangana DH	335.30
Sibbinda HC	Andara DH	255.11

3.3.2 DNA extraction

DBS and RDT positive samples were stored at -20°C in double ziplocked bags with desiccant until DNA extraction. The DNA was extracted from the DBS using the chelex DNA extraction protocol as described previously (Tambo *et al.*, 2018). Briefly, 6mm filter paper discs punched out of DBS's were incubated overnight in 1mL of 1% saponin and 1x Phosphate Buffered Saline (PBS) in a 1.5mL tube. The samples were then washed with 1xPBS and heated at 98°C for 10 mins in 75ul of deionized water and 25ul of 20% chelex solution. The chelex beads were removed and the DNA was stored at -20°C until further analysis. For RDTs, the cassettes were opened using a thin metal spatula and DNA was extracted from the nitrocellulose strip in accordance with the worldwide antimalarial resistance network (WWARN) guidelines (WWARN, 2011), with the exception that DNA extraction was performed in deep-well plates as opposed to 1.5mL tubes (Wesolowski *et al.*, 2018). DNA samples for extraction were chosen randomly by code from a spreadsheet in R software to avoid bias by region or health facility.

3.3.3 Genotyping

Genotyping was carried out according to methods previously described (Wesolowski *et al.*, 2018). Briefly, extracted DNA from the DBS and RDT samples stored at -20°C was used for genotyping. Firstly, parasite density was quantified using the varATS ultra-sensitive qPCR protocol as described previously (Hofmann *et al.*, 2015). Briefly, *plasmodium* parasite DNA was amplified with primers from the varATS region targeting multi-copy genes as shown in table 2. The varATS protocol consistently detects low density infections down to 1 parasite/uL (Hofmann *et al*., 2015).

Parasite density was determined on all DBS samples and only a fragment of RDT extracted DNA; as RDT positive samples usually have a parasite density above 50 parasites/µL (Smith *et al.*, 2017). All DBS samples with a parasite density of 10 parasites/ul or more were included in the study. A total of 612 samples were genotyped using 26 putatively neutral microsatellite markers as described previously (Wesolowski *et al.,* 2018). PCR was performed on 612 samples with primers that target the 26 microsatellite markers spread across the parasite genome as illustrated in Figure 9. The primers are multiplexed in 4 groups according to melting temperatures and primer sequences and there are 2 PCR cycling conditions for the groups of primers as illustrated in Table 3.

Table 3: Primer, probe sequences and cycling conditions for varATS qPCR to determine parasite density

Primers	**Sequence**
varATS Primer-fw (5'-3')	Cccatacacaaccaaytgga
varATS Primer-rev (5'-3')	Ttcgcacatatctctatgtctatct
Probe (5'-3')	6-FAM-trttccataaatggt-NFQ-MGB
qPCR cycling conditions	**varATS**
Pre-incubation	2 min - 50°C
Initial denaturation	10 min -95°C
Denaturation	15 sec - 95°C
Annealing & Elongation	1 min - 55°C (Data Collection Step)
Number of cycles	60

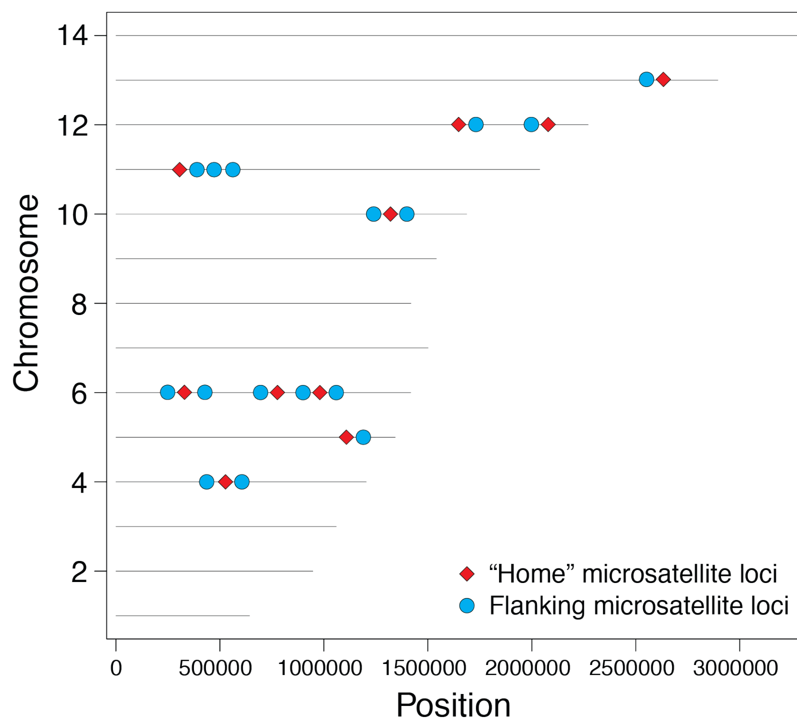


Figure 9: Distribution and location of the 26 microsatellite markers on the parasite genome (Liu et al., unpublished data).

Table 4: Master mix for the 1st round of PCR and 10X Primer Mixture for Groups 1-4

Group 1-10x Primer Mixture (100uL)				
Primer	**Concentration (µM)**	**Volume (µL)**	**Multiple**	
AS1	1.25	1.25		12.5
AS11	1.25	1.25		12.5
AS12	2.5	2.5		25
AS20	2	2	10	20
AS3	1.5	1.5		15
AS31	1.25	1.25		12.5
AS32	2	2		20
AS34	2	2		20
TA1	3	3		30
TA109	3	3		30
TE Buffer		60.5		605
Group 2-10x Primer Mixture (100uL)				
Primer	**Concentration (µM)**	**Volume (µL)**	**Multiple**	
TA60RevNewII + TA60NewForT	3	3		30
AS25	1.5	1.5		15
PFG377	2	2		20
AS19	3	3	10	30
Ara2New	3	3		30
AS21	1.25	1.25		12.5
AS8	2	2		20
TE Buffer		65.5		625
Group 3- 10x Primer Mixture (100uL)				
Primer	**Concentration (µM)**	**Volume (µL)**	**Multiple**	
TA81	3	3		30
AS7	2	2		20
AS15	1.25	1.25	10	12.5
TA40	3	3		30
PolyA New	3	3		30
PfPk2 New	3	3		30

TE Buffer		65.5		675
Group 4-10x Primer Mixture (100uL)				
Primer	**Concentration (µM)**	**Volume (µL)**	**Multiple**	
AS14	2	2		20
TA87	3	3		30
B7M19	2	2		20
AS2	2	2	10	20
TE Buffer		82		820
Master Mix 1st round	**Volume (µL)**	**Multiple**	**Final Volume (µL)**	
2x Type-It Multiplex Master Mix	12.5	110	1375	
10x Primer Mix	2.5		275	
Water	5		550	
Template DNA	5			
Reaction Volume			25	

Table 5: Master mix for the 2nd round of PCR

Microsatellite Master Mix 5µl scale (1µl temp) Phusion PCR 2nd round				
	Final Conc.	**Volume (µl)**	**Multiple**	**Total (µl)**
H2O		1.95		214.5
P1 10uM	500nM	0.25		27.5
P2 10uM	500nM	0.25		27.5
5X HF Phusion Buffer	1X	1	110	110
dNTP 2mM	200uM	0.5		55
Phusion II Hot Start (1U/uL)	0.4u (0.04u/µl)	0.05		5.5
		4		Mix / Well
THEN ADD template DNA		1		

The products from the 26 microsatellite loci were then diluted and sized by denaturing capillary electrophoresis on an ABI 3730XL analyzer with GeneScan™ 400HD ROX™ size standard (Thermo Fisher Scientific). The resulting electropherograms were analyzed using microSPAT software to automate identification of true alleles and reduce artifacts (Murphy *et al., in preparation*).

The MicroSPAT software allows a plate view for quality checking, automated bin generation using a clustering algorithm, automated artifact estimator generation, automated quantification bias estimation, and automated genotyping of samples with the

option of manual curation. A total of 586 (96%) samples as illustrated in Fig. 11 with data in at least 15 or more loci were included in the data analyses to obtain at least 50% of the targeted loci genetic data as previously described (Roh et al., 2019). It is a published standard to do population genetics analysis on at least 50% or more of the targeted loci (Wesolowski et al., 2018). Genotyping data from all samples were combined and processed with similar software settings to avoid variability in allele calling and any other machine errors.

Figure 10: Used positive RDTs and epidemiological data on the RDT that were collected and stored at -20℃ until further genetic analysis

3.4 Population genetics analysis

3.4.1 Within-host diversity

Multiplicity of infection (MOI) is a measure of within host diversity. The MOI was calculated as the second highest number of alleles detected at any of the 26 loci to avoid inclusion of false alleles in the data analysis. In addition, the F_{WS} metric was calculated for each infection;

$$F_{WS} = 1 - \frac{H_w}{H_s}$$

Where; H_w=heterozygosity of the individual and H_s=heterozygosity of the local parasite population as previously described by Mobegi *et al.,* (2014). The Fws metric measures the genetic differentiation of parasites infecting a single individual at the same time. Mean F_{WS} was calculated for each individual by taking the mean across all loci as described previously (Roh et al., 2019).

3.4.2 Population genetic diversity

Genetic diversity at population level is the number of alleles in a population at a given time. Genetic diversity at population level was determined by calculating expected heterozygosity (H_E) using all alleles data;

$H_E = [n/(n - 1)][1 - \sum p_i^2]$,

Where n is the number of genotyped samples and p_i is the frequency of the ith allele in the population (Roh *et al.,* 2019).

In addition, multilocus Linkage Disequilibrium (LD) which is the non-random association of alleles was determined; the standardized index of association (ISA) was calculated

using the program LIAN version 3.5 for the whole dataset using only the dominant alleles data (Schultz *et al.*, 2010). Only one locus per chromosome was used to calculate multilocus LD to avoid false linkage signals based on proximity of the loci. Multilocus LD is used to determine if there is random mating within and amongst the populations.

3.4.3 Population structure and Genetic differentiation

MavericK software was used to investigate whether haplotypes clustered by distinct genetic populations (K) according to defined geographical origins; district health facilities for the purposes of this study. Individual multilocus haplotypes are assigned by probability to one cluster (K) or to multiple clusters (a condition termed admixture) based on allele frequency per locus (L Schultz *et al.*, 2010). The analysis was run for K=1:5, burnin = 1e4, converge_test = 1000, samples = 2e3, rungs = 15 using the R software (Verity & Nichols 2015).

For all health facility pair combinations, G_{ST} was used to estimate genetic differentiation. The G_{ST} metric is used to measure how much a sub population contributes or differs from the overall genetic diversity and it was calculated using all alleles data as follows; $G_{ST} = (H_T - H_S)/H_T$. Where H_T is the overall/total population heterozygosity, H_S is the sub population heterozygosity. The G_{ST} metric values range from 0 that represents complete genetic similarity to 1 which indicates complete genetics differentiation between populations.

3.4.4 Ethical approval

Ethical clearance for this study was obtained from the University of Namibia, the Ministry of Health and Social Services of Namibia and the University of California San Francisco.

3.5 Results and discussion

Descriptive population genetics analysis can be employed to measure efficacy of the interventions in use and to identify if there is need to adapt new strategies to reduce malaria and move towards zero (Daniels *et al.*, 2015; Auburn & Barry, 2017). However, to date, the population genetic structure for Sub Saharan Africa, Namibia in particular has not been described and transmission is known to differ geographically. Therefore, this study employed the use of parasite genetic data to describe key malaria transmission dynamics in the Kavango East and Zambezi regions of Namibia, as well as to complement information from incidence data and travel history.

MOI can be a useful indicator of the intensity of transmission and has been reported to influence clinical manifestation of malaria (Vafa, Troye-blomberg, *et al.*, 2008). The parasite genetic data was aggregated by district health facility, namely Rundu, Nyangana, Andara (Kavango East region bordering Angola) and Sibbinda (Zambezi region bordering Zambia and Angola). There was generally a low to moderate within host diversity in the Kavango East and Zambezi regions of Namibia with a mean MOI of 2.54. Low MOI was expected in the Kavango East and Zambezi regions of Namibia based on the reported low and decreasing incidence data (Mumbengegwi et al., 2018; Nghipumbwa et al., 2018). Moderate within host diversity across was observed across all the populations with a mean MOI of 2.5. Rundu has a significantly higher within host diversity compared to Nyangana, Sibbinda and Rundu. This could be as a result of frequent population movement to and from Angola. Variability could be observed across all 4 populations suggesting fine-scale heterogeneity of malaria transmission in the study areas as illustrated in figure 4.

In particular, Rundu had a high within host diversity which is not expected in a low transmission. However, the high within host *P. falciparum* genetic diversity could due to proximity to porous borders with high transmission Angola in both the Kavango East and Zambezi regions. Similar results were reported in Eswatini with importation from Mozambique (Roh *et al.,* 2019). In addition, Rundu is the commercial center for Kavango, therefore there is a lot of movement of people between Rundu, other regions and surrounding countries with high transmission. Hence, continuous importation of *P. falciparum* malaria could be a contributing factor to the high within host diversity.

The correlation between MOI and prevalence is not always a clear indication of population level transmission (Koepfli, Waltmann & Ome-kaius, 2018). A few households might be at high risk of infection, and several clones might be transmitted among them despite being a low transmission setting. In addition, there was fine scale variability of within host diversity amongst the populations as illustrated in Fig 11. Nyangana and Sibbinda had a lower within host diversity compared to Rundu and Andara as illustrated in Fig. 11. This could be as a result of the differences in levels of *P. falciparum* importation due to differences in proximity to the boarder with Angola and receipt ability of *P. falciparum* amongst the populations (Conway, 2007; Pacheco *et al.*, 2015; Schultz *et al.,* 2010); receipt ability depends on the vector population, climate and levels of immunity against malaria in the sink. Rundu and Andara have more human movement and in comparison with Nyangana and Sibbinda as a result of Rundu being the commercial capital of the Kavango region and Andara's proximity to Rundu.

These results suggest that there could be moderate to high local transmission in Kavango

East and the Zambezi regions sustained by continuous importation (Pacheco et al., 2015). There is a higher probability of developing resistance with high within host diversity (Auburn & Barry, 2017). A similar pattern was observed with the residence data as was with the health facility data. As expected, there was a high genetic differentiation of plasmodium parasites in individuals who have high within host diversity (Auburn & Barry, 2017; Pacheco *et al.*, 2015).

Figure 11: Increase in genetic differences with an increase in MOI (F_{WS}: p-value < 2.2e-16 at 95%CI)

In the Kavango East and Zambezi regions of Namibia, a low H_E is expected because of

the decrease in the number of reported malaria cases. However, moderate to high heterozygosity was observed with a mean H_E of 7.5 across all 4 district health facilities. This suggests on going local transmission and high levels of importation across all populations. Levels of diversity are an indication of the fitness of the parasite population and thus how difficult it may be to target with drugs or vaccines (Auburn & Barry, 2017; Nabet *et al.*, 2016). Therefore, this could present a challenge for the national malaria control programs. The high levels of within host diversity, MOI, corresponded with the high population level diversity of *P. falciparum;* Rundu and Andara had the highest MOI and corresponding highest H_E. Rundu had the highest H_E, indicating high levels of importation and ongoing local transmission as a result of continuous importation both from other regions and countries. Populations mirror heterozygosity across the boarders in high transmission settings (Nabet *et al.*, 2016), therefore the current model for descriptive population genetics does not fit in Sub Saharan Africa.

There was also fine scale variability of population diversity amongst the 4 populations. This is an indication of heterogeneous transmission across the populations as a result of geographical differences, differences in human behavior and economic status (Reiner *et al.*, 2015; Smith et al., 2017). Malaria transmission varies by geographical location and is usually focal, hence the observed fine-scale differences in H_E as illustrated in Fig. 12.

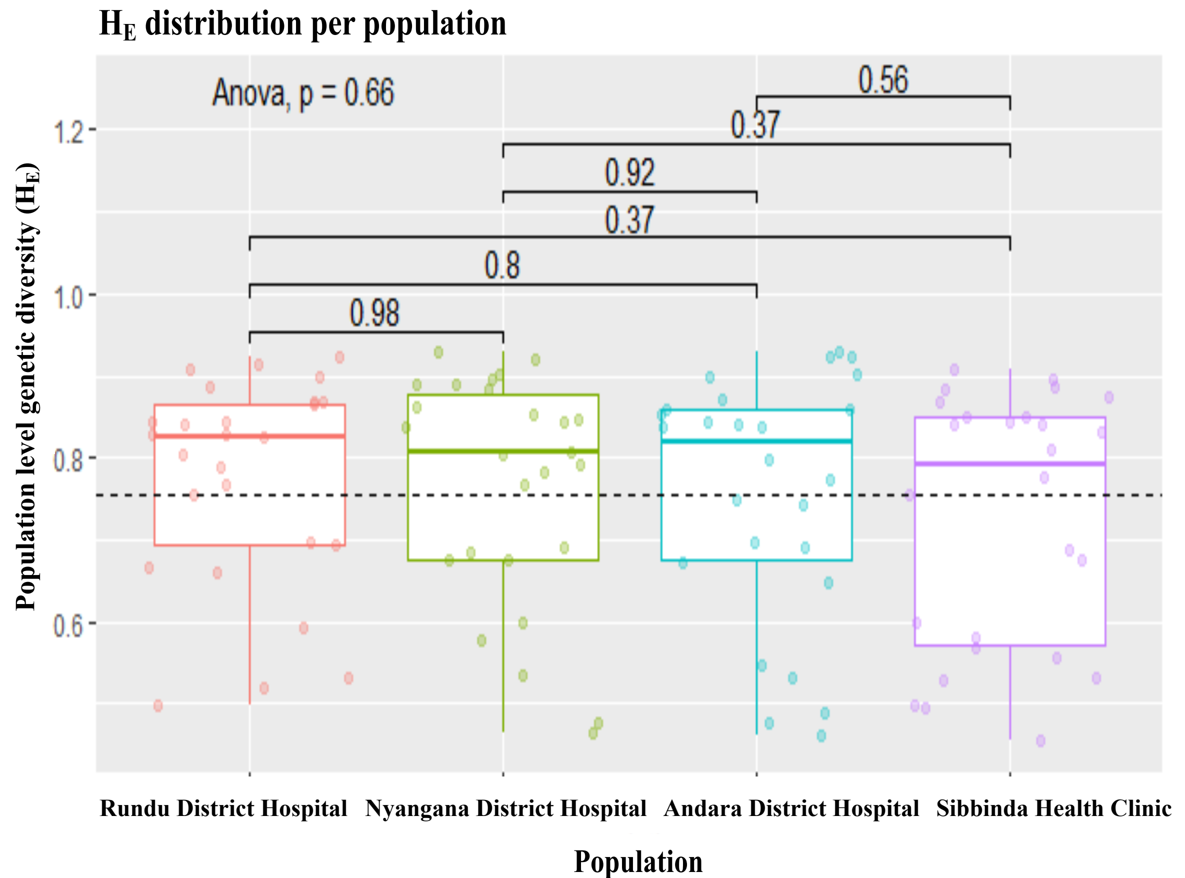


Figure 12: Distribution of population level genetic diversity across the 4 populations

Multilocus linkage disequilibrium is defined as the non-random association of alleles (Nabet et al., 2016). Multilocus LD has important implications on the spread and emergence of multilocus drug resistance haplotypes, inbreeding is conducive for the dispersal of these haplotypes (Schultz et al., 2010). A high multilocus LD is expected for a low transmission setting (Pumpaibool *et al.*, 2009), however, low to moderate but significant multilocus linkage disequilibrium ($p < 0.001$) was observed, showing random association of alleles consistent with high heterozygosity and an indicator of ongoing local transmission as illustrated in Fig. 13.

A high multilocus LD was observed for Nyangana and Sibbinda which had lower within host and population level genetic diversity. There were low genetic differences observed across the populations measured by the G_{ST} metric. This could be an indicator of a strong connection amongst the human populations in the health districts. The multilocus linkage disequilibrium was calculated based on loci on different chromosomes to avoid “false positive” linkage from loci on the same chromosome.

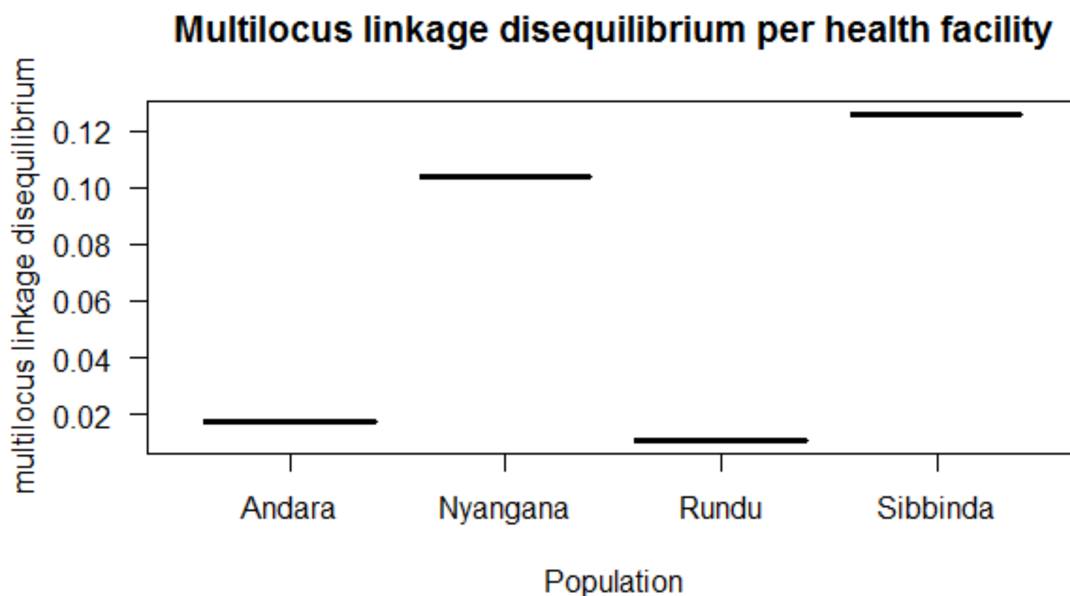


Figure 13: Analysis of the association of alleles across the 4 populations

The calculated G_{ST} metric showed low to no genetic differentiation ranging from 0.001783 to 0.012503 as illustrated in Table 5. This suggests strong connectivity among all 4 district health facilities. The G_{ST} metric measures genetic differentiation between populations by pairwise comparison and ranges from 0 (no genetic difference) to 1 (complete genetic differentiation) (Auburn & Barry, 2017).

Table 6: G_{ST} metric analysis of genetic differentiation between populations

	Rundu	**Nyangana**	**Andara**	**Sibbinda**
Rundu	-	-		
Nyangana	0.005984	-		
Andara	0.008338	0.001783		0.009173
Sibbinda	0.012503	0.006057		

As the level of malaria transmission decreases, the parasite populations are expected to cluster geographically in a fragmented fashion (Iwagami *et al.*, 2009; Auburn & Barry, 2017). Population cluster analysis was performed for *P. falciparum* microsatellite haplotypes from dominant alleles (Wesolowski *et al.*, 2018) in the Kavango East and Zambezi regions of Namibia as illustrated in Fig. 14.

The clustering analysis is used to identify groups of similar genotypes in a multivariate data set which contains several genotypes in a clearly defined geographical area or areas. Where "K" represents the optimal number of clusters in the given geographical population (Schultz et al., 2010). In the case of fragmentation as would be expected in Namibia, distinct clusters represented by single colours would be observed on the x-axis and where populations are highly admixed, a mixture of colours would be observed on the x-axis. The malaria parasite populations in the current study show no fragmentation, which suggests that there is random mating which would explain the low linkage disequilibrium. A similar pattern was observed with the residence data as was with the health facility data. The populations were highly admixed and conventional methods failed to differentiate the

populations as illustrated in Fig. 14. This could suggest high levels of connectivity with parasites moving to and from the villages in Kavango East and the Zambezi regions for trade and other reasons.

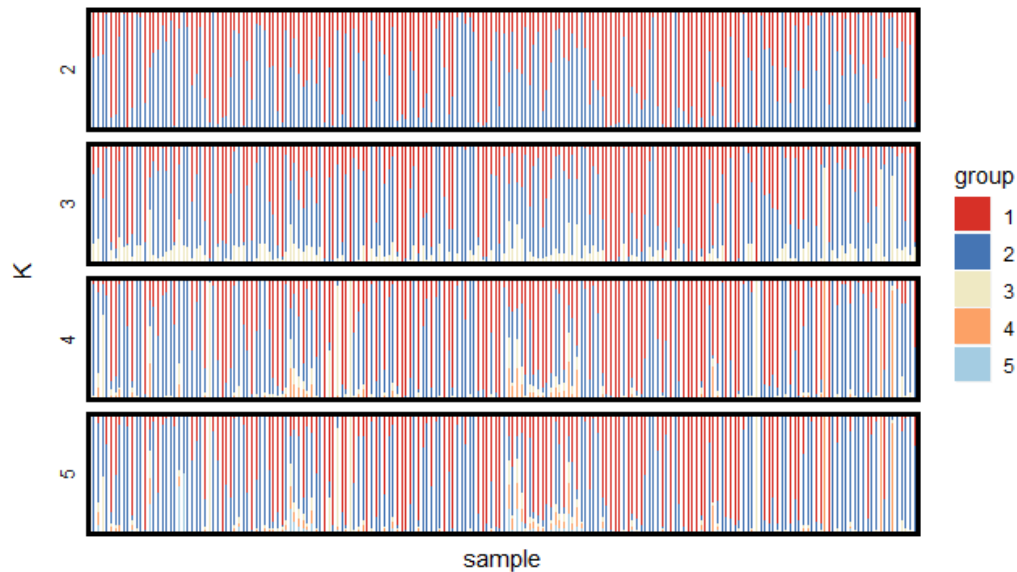


Figure 14: Highly admixed populations showing no structure with the maverick software.

3.5 Conclusion

There was high genetic diversity in Rundu, Andara, Nyangana and Sibbinda which are connected with Angola, a high transmission setting. There were high levels of importation contributing to the observed high genetic diversity in the low transmission setting. It is therefore important to coordinate elimination efforts between regions and between neighboring countries as there is connectivity across porous borders and regions. Namibia in particular does not follow the pre-described population genetics pattern for low transmission settings. There is need for re-working the *P. falciparum* population genetics dogma to describe the population genetics structure for P. falciparum in Namibia to

account for importation. There was high within host and population level *P. falciparum* genetic diversity of the *P. falciparum* parasite as opposed to the expected low to moderate *P. falciparum* genetic diversity. This poses a high risk of the development of drug resistance and its subsequent spread in addition to increased parasite fitness. In addition, there was low multilocus LD and highly admixed populations indicating nonrandom association of alleles and panmictic populations despite the decreasing malaria burden. The highly admixed *P. falciparum* populations in Namibia in the current study could not be described by conventional methods, there is no clustering geographically by genotype. These particular transmission dynamics point to ongoing imported malaria cases from Angola that contribute to local malaria transmission in Kavango East region, Namibia, as the infected populations are located in porous border areas and well connected; therefore, the population genetics structure mirrors that of a high transmission setting. Genotyping could complement current data and address key elimination challenges such as identification of the parasite flow (connectivity), multiplicity of infections which have known implications on clinical presentation of malaria infections and the development and spread of drug resistance. These data inform the elimination strategy on targeted interventions.

3.6 References

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CHAPTER 4: A COMPARISON OF MALARIA CASE DISTRIBUTION AND TRANSMISSION DYNAMICS IN ANGOLAN AND NAMIBIAN RESIDENTS IN THE KAVANGO EAST REGION OF NAMIBIA

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4.1 Abstract

This study compared the risk and distribution of malaria infection based on age and residence and within host genetic diversity between two bordering countries, Angola a high transmission setting and Namibia a low transmission setting. The study reported 1) The malaria burden was significantly higher in Angola than it is in Namibia regardless of importation as indicated by the differences of malaria burden in the <5years age group. 2) The malaria case distribution in the Kavango East region of Namibia mirrors the case distribution in Southern Angola. 3) Individuals with a primary residence in Rundu and individuals in the age range 45-59 years are at highest risk to travel to a malarious region. Therefore the proposed transmission dynamics dogma by transmission setting needs to be redefined for Sub Saharan Africa where there is strong connectivity amongst countries.

4.2 Background

Malaria incidence in Namibia has significantly declined over the past decade (Mumbengegwi *et al.*, 2018; Nghipumbwa *et al.*, 2018; Tambo *et al.*, 2018). As the number of malaria cases declined, Namibia declared a goal to eliminate malaria within its borders by the year 2020 (Gueye *et al.*, 2014). However, moving towards the malaria elimination goal requires accurate and relevant data in order to inform the elimination strategy.

Malaria transmission is heterogeneous and focal, meaning infection and disease are concentrated in a small proportion of individuals and not distributed evenly across the population as seen in many infectious diseases (Stresman *et al.*, 2015). With regards to malaria specifically, heterogeneity occurs at household, village or district level, or hotspots, within malaria endemic communities that have a significantly increased risk of malaria transmission compared to surrounding households (Bousema, Kreuels & Gosling, 2011). These hotspots exist in all transmission settings, but are most easily detected at low transmission (Bousema & Baidjoe, 2013; Cotter *et al.*, 2013; Reiner *et al.*, 2015). The heterogeneous nature of malaria transmission is due to a number of factors which include meteorological conditions, connectedness with other malarious regions, immunity, socio-economic status, behavior, malaria importation and age (Oesterholt *et al.*, 2006; Silva & Marshall, 2012; Baidjoe *et al.*, 2016).

In high transmission settings of malaria and increasing transmission settings the peak age of infection shifts towards children under the age of 5 (Carneiro *et al.*, 2010), this has been

shown in high transmission settings such as Mozambique and Ghana (Griffin, Ferguson & Ghani, 2014). This shift is observed as a result of a lack of immunity in the age range 0-5 years, the older individuals develop immunity against malaria. In contrast, low malaria transmission settings and areas with decreasing transmission, the peak age of malaria cases shifts toward older children ages of 5-15 years and young adults, this has been shown in low transmission settings such as parts of Senegal and Tanzania (Griffin, Ferguson & Ghani, 2014).

Other factors that could affect the peak age of malaria infections are behavioral patterns of individuals in malaria endemic regions and frequent travel to malarias regions or countries by young adults in search of employment and trade (Pemberton-Ross *et al.*, 2015). In addition to peak age infection, within host genetic diversity of the *Plasmodium* parasite measured as multiplicity of infection (MOI) is another indicator of malaria transmission intensity as it is influenced by the entomological inoculation rate (EIR) which is the number of bites by an infected mosquito in a given time frame (Vafa *et al.*, 2008). There are more mosquitos and bites by infected mosquitos in high transmission settings (Bousema & Baidjoe, 2013). A high MOI is expected in high transmission settings and a low MOI is expected at low transmission (Barry et al., 2013; Schultz *et al*., 2010).

Although there is a general decrease in malaria transmission in Angola, it is a high transmission setting. *P. falciparum* accounts for over 90% of malaria cases in Angola which had a civil war for 3 decades (1985-2002) and this crippled the health systems and destroyed infrastructure (Lima, 2017). Angola has however put in place measures to reduce malaria mainly through prevention by vector control, prompt diagnosis and

appropriate treatment of cases (Fançony *et al.*, 2013). Therefore, the highest proportion of the malaria cases whose primary residence is in Angola are expected to be individuals under the age of 5 years (Carneiro *et al*., 2010) because individuals in this age range are the ones who have not yet developed immunity. In addition, a high MOI is expected in malaria cases whose primary residence is in Angola (Mueller *et al.*, 2009).

However, Namibia is a moderate to low transmission setting and *P. falciparum* accounts for over 90% of reported malaria cases (Nghipumbwa *et al.*, 2018; Tambo *et al.*, 2018). Malaria in Namibia is generally confined to the North Eastern regions of the country and is seasonal (Mumbengegwi *et al*., 2018). Therefore the highest proportion of malaria cases in whose primary residence is in Namibia is expected to be individuals in the age groups 5-15 years and young adults. In addition, a low MOI is expected in malaria cases whose primary residence is in Namibia.

Malaria importation presents a challenge moving towards malaria elimination in Namibia (Sturrock *et al.*, 2015; Nghipumbwa *et al.*, 2018). Specifically, the challenge is that Angola focuses resources in the northern parts of the country where the malaria burden is highest and lacks in southern Angola where it shares a border with Namibia (Gueye *et al.*, 2014). It has been reported that about 25% of the malaria cases reported and recorded in the Kavango region of Namibia are imported from Angola (Nghipumbwa *et al.*, 2018). In addition, there is no health facility in Calai (Southern Angola) therefore there is movement to Kavango East in Namibia to seek medical attention and for trade (Nghipumbwa *et al.*, 2018). These migrant populations from high transmission areas could be sustaining malaria transmission in the Kavango East region and there is need to determine if case

distribution and transmission dynamics differ across the border (Zahrani *et al.,* 2018; Moss *et al*., 2012; Raman *et al.,* 2016; Sturrock *et al*., 2015).

The connectivity and geography of Sub Saharan Africa is unique and the transmission dynamics hypothesis needs to be tested. Therefore in this study, the unknown malaria indicators which include transmission intensity, malaria case distribution, travel related risk of acquiring a malaria infection and level of connectivity between the Kavango East region of Namibia and Southern Angola were investigated. This was done using primary residence of malaria cases in Kavango East presenting at health facilities as a proxy for the population in the region and imported cases (primary residency in Angola) at facilities in Kavango East as a proxy for population in Southern Angola. This manuscript reports on the risk of malaria infection based on age and residence, in addition, within host genetic diversity calculated as multiplicity of infection (MOI) was performed. The MOI was used to determine transmission intensity differences between the Angolan residents and Namibian residents in the Kavango East region of Namibia.

4.3 Methods

4.3.1 Study sites and sample collection.

Inclusion criteria was stated as all RDT confirmed malaria cases at Heath Facilities that consented (>=18 years) to participating in the study and assent was given for minors (< 18 years). All age groups were included, participation was voluntary, participants could drop out of the study at any time without consequences and their personal information was treated confidentially.

A total of 3869 positive rapid diagnostic tests (RDTs) from all passively detected symptomatic cases were collected from 24 health facilities from the Rundu, Nyangana and Andara districts in the Kavango East region as illustrated in Figure 15, Namibia bordering Angola between March and June 2016. This period is part of the malaria peak season in Namibia, toward the end of the rain season. The study was health facility based in the Kavango East region of Namibia bordering Angola. Study participants were recruited after obtaining consent to participate part in the study. Participating adults (18years or older) gave written assent by signature or fingerprint whilst for minors below the age of 18years, parents or guardians gave written consent or fingerprint. All the RDTs were stored in zip lock bags with desiccant at room temperature until further laboratory analysis in the form of genotyping was performed. In addition, epidemiological data which comprised of age, residence and travel history was collected and recorded. One of the 24 health facilities, Takawasa clinic, had only 3 patients enrolled during the course of the study and was excluded from the data analyses.

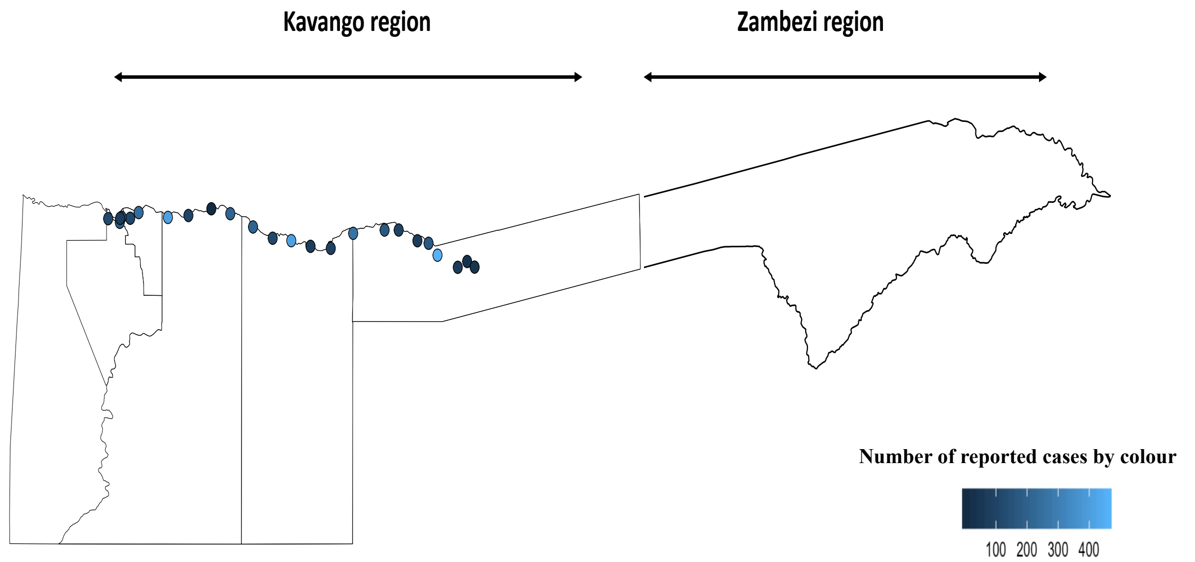

Figure 15: Study site showing the 24 health facilities in the Kavango East region where the samples were collected and the specific clinics

4.3.2 Malaria case distribution

Data was collected on malaria cases that included age, district residence, primary residence (village) and travel history in the past 4 weeks (destination) taking into account the incubation period of the infection. Primary residency was defined as the main place of residence that an individual has slept in the previous 6 months for this study. The epidemiological data was used to compare malaria cases with individuals with primary residency in Angola as a proxy for the Angolan population and individuals with Primary residence in Namibia as a proxy for the Namibian Kavango East population. Comparison of malaria distribution between the Namibian and Angolan populations was done through calculation of the proportion of the reported malaria cases by age (Carneiro *et al.*, 2010) and primary residence (Kittichai *et al.*, 2017).

Travel history was used to calculate relative risk (OR) of travel to a malarias region by age and residence in individuals with a primary residence in Namibia. Briefly, the proportion of travelers to a malarious region was calculated and multiplied by the incidence of malaria in the primary residence. Potential risk factors were assessed by logistic regression using generalized estimating equations (GEE) in R. An adjusted logistic regression model was built by adding factors one at a time, based on the unadjusted associations.

4.3.3 DNA extraction and genotyping

The DNA was extracted using the chelex DNA extraction protocol as previously described (Plowe *et al.*, 1995) with the exception that 96 well plates were used instead of individual tubes. Briefly, the RDT cassette was opened by a metal spatula and DNA was extracted from the nitrocellulose strip according to the worldwide antimalarial resistance network (WWARN) guidelines. The chelex extraction has been reported to give a good DNA yield (Mharakurwa *et al.*, 2006). Quantitative PCR using the ultra-sensitive varATS protocol as previously described was performed on a subset of the RDTs to confirm that there is a parasite density of at least 10parasites/ul (Hofmann *et al.*, 2015) . On all plates, the samples were randomly and not sequentially placed by date or health facility, and positive and negative controls were used on all plates. A control with a pre-determined parasite density was prepared for all plates analyzed (Meirmans, 2015).

All samples were genotyped across 26 putatively neutral microsatellite markers across 7 chromosomes. In order to minimize genotyping errors, strict thresholds were used to call alleles using the semi-automated microSPAT software as previously described. Nested

PCR was performed to amplify the 26 microsatellite loci (Wesolowski *et al.*, 2018). The PCR primers were multiplexed in 4 groups. The resulting PCR amplicons were then diluted and sized by denaturing capillary electrophoresis on an ABI 3730XL analyzer with GeneScan™ 400HD ROX™ size standard (Thermo Fisher Scientific). Electropherograms from the capillary electrophoresis were analyzed using microSPAT software to automate identification of true alleles and reduction of artifact (Murphy *et al., in preparation*). The peak heights were corrected for amplification bias using a standardized locus specific correction factor calculated using mixtures of parasites of known relative proportion. Only samples with data for 15 or more loci were included in the downstream analysis (Roh *et al.,* 2019).

4.3.4 Multiplicity of infection

Only samples with data on 15 or more loci of the 26 putatively neutral microsatellite markers were included in the population genetics analysis. Multiplicity of infection (MOI), also referred to as complexity of infection (COI), is defined as the number of genetically distinct parasite strains co-infecting a single host. The MOI measures within host genetic diversity. The *P. falciparum* parasite is haploid in the human host stage of its life cycle, therefore, multiple alleles correspond to an infection with multiple genotypes (Nabet *et al.*, 2016; Schultz *et al.*, 2010). For this study, the MOI for each sample was scored as the second maximum number of alleles observed when taking into account all analyzed loci (n= ≥ 15). MOI was calculated for each individual and analyzed by age and travel history. The mean MOI was calculated for the total number of individuals from each population and has been used as a measure of malaria transmission intensity previously.

4.4 Ethical approval

Ethical clearance for this study was obtained from the University of Namibia, the Ministry of Health and Social Services of Namibia and the University of California San Francisco.

4.5 Results and Discussion

4.5.1 Malaria case distribution by age

Individuals with primary residency in Angola had a significantly higher proportion of cases (p-value = $<2.2e^{-16}$) in the <5 years age group as illustrated in Fig. 16. This shows that the malaria burden is significantly higher in Angolan residents compared to Namibian residents. In addition, the < 5 years age group was the least likely to travel to a malarious region as reported previously (Smith *et al.*, 2017) and therefore it was expected that there would be a difference in this particular age group.

In age groups 5-14 years, 15-29 years, 30-44 years and 45-59 years there were no significant differences in the proportion of malaria cases between Namibian and Angolan residents as illustrated in Fig. 17. Namibia is a low transmission setting and Angola is a high transmission setting, significant differences in proportion of malaria cases were expected between the two populations (Bousema & Baidjoe, 2013; Pemberton-Ross *et al.*, 2015). Namibia and Angola are well connected, there is no need for a passport, no fence/wall on most parts, porous border and this is compounded by the poor access to health facilities and infrastructure in Southern Angola (Moura *et al.*, 2014) which is a motive to move from Southern Angola a high transmission setting to Namibia, a low transmission setting. This connectivity could lead to continuous and consistent

importation of malaria cases from Southern Angola to Namibia; which could be a major contributing factor to similarities in proportions of malaria cases in these age groups (Sriwichai *et al.*, 2017; Tatem *et al.*, 2017).

There is a tail off seen with malaria cases in Namibian residents in the age group >= 60 years as illustrated in Fig. 16. This observation could be as a result of the differences in life expectancy; Angola has a life expectancy of 51years whilst Namibia has a life expectancy of 63.7 years (WHO, 2015). In addition, the sampling was done at health facilities in the Kavango East region in Namibia therefore the age group >= 60 years from Angola might not have been represented as the age group is less likely to travel to a malarious region.

The peak age group of malaria case distribution in both Namibian and Angolan residents based on individuals tested by RDT at health facilities was 5-14 years as illustrated in Fig. 2 shows no significant difference between the two populations (p value = 0.48). The finding for the Namibian population is consistent with previous studies in areas of declining malaria cases that reported the peak age for malaria infections to be around 8-15 years and sometimes in young adults over the age of 15 years (Schwartz *et al.*, 2001; Mueller *et al.*, 2009; Pemberton-Ross *et al.*, 2015). In low transmission settings, children in this age group would not have developed immunity against malaria and are an additional risk due to social behavior such as playing outside at night, therefore, there is a shift in the peak age of malaria transmission (Carneiro et al., 2010). However, malaria case distribution by age is expected to have a peak in children under the age of 5 years in high transmission settings (Griffin, Ferguson & Ghani, 2014) such as Southern Angola.

This deviation from the expected peak age group (< 5 years), could be an indication of overall reduction in malaria transmission although the burden is still high (Carneiro *et al.*, 2010; Naidoo *et al.*, 2011). The fight against malaria in Angola has been affected by a 3 decade long civil war which crippled the health infrastructure, but there have been efforts to reduce malaria and join the move toward malaria elimination (Fançony *et al.*, 2013; Lima, 2017).

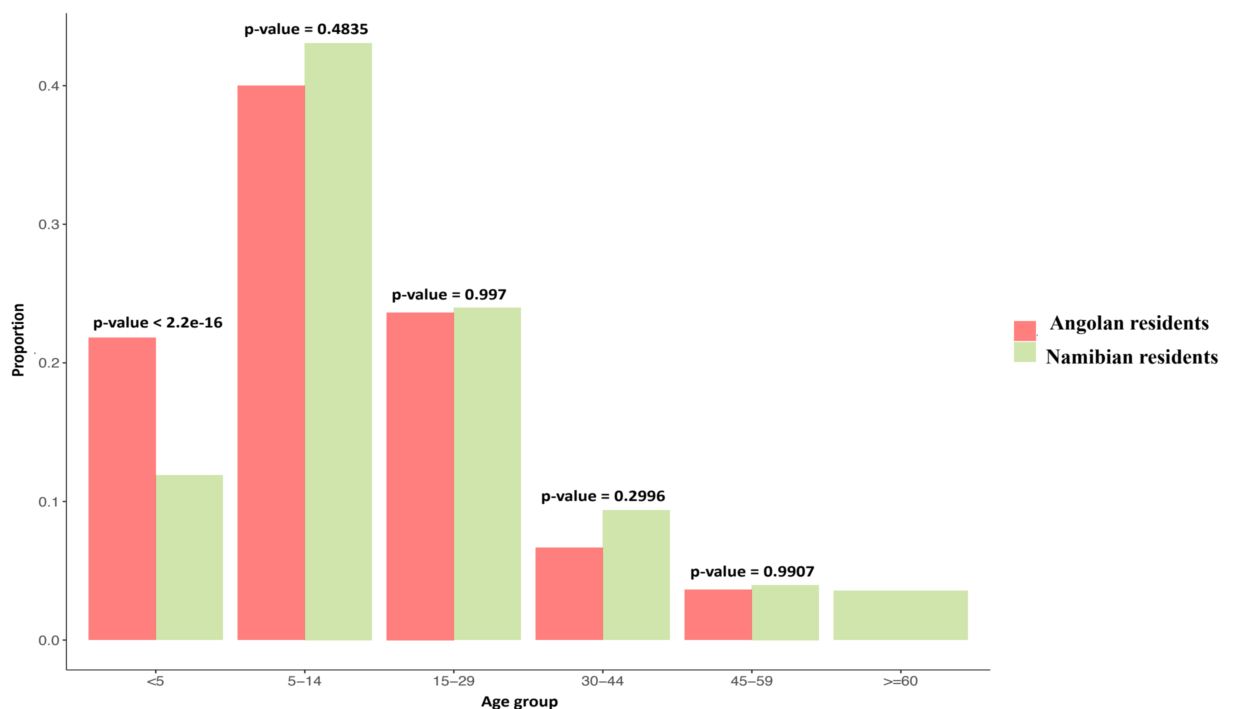

Figure 16: Proportion of malaria case distribution by age between Namibian and Angolan residents

4.5.2 Within host diversity in Namibian and Angolan residents

Within host diversity (MOI) has been employed as a measure of the level of malaria transmission (Chenet *et al.,* 2012; Koepfli *et al.*, 2018; Pacheco *et al.*, 2015). In this study, children under the age of 5 years had a high median MOI of 3 in both Angolan and

Namibian residents indicating high levels of transmission in both populations as illustrated in Table 7 although there is a significant difference in proportion of malaria cases in this age group (< 5 years). However, a low MOI is expected in pre-elimination settings (Nabet *et al*., 2016) such as Namibia given that most infections have been reported to be monoclonal in these settings (Wesolowski *et al.*, 2018) meaning that there is only a single genotype of *P. falciparum* infecting an individual at a given time.

This finding is consistent with a recent study which reported that MOI is not a good indicator of transmission levels (Koepfli, Waltmann & Ome-kaius, 2018), however, this result could be significantly influenced by continuous importation from Southern Angola to the Kavango East region of Namibia (Reiner *et al.*, 2015; Sturrock *et al.*, 2015). In high transmission settings such as Southern Angola, high MOI is expected (Pacheco *et al.*, 2015) therefore this finding is consistent with previous studies (Pemberton-Ross *et al.*, 2015).

There was no difference in MOI in the 15-29, 30-44 and 45-59 years age groups as shown in Table 7. These similarities are an indication of consistent importation from Southern Angola that is contributing to local transmission in the Kavango East region of Namibia (Sturrock *et al.*, 2015).

Table 7: A comparison of within host diversity by age group between Namibian and Angolan residents

Age group (years)	n (Namibian residents)	median MOI [IQR: 25% & 75%]	n (Angolan residents)	median MOI [IQR: 25% & 75%]
<5	226	3 [2-4]	33	3 [2-4]
5-14	833	2 [2-4]	60	2 [1-4]
15-29	487	3 [1-4]	34	2 [2-4]
30-44	186	2 [2-4]	9	3 [2-4]
45-59	75	3 [2-4]	6	2 [1.25-3.5]
>=60	82	2 [1.25-3]	0	-

Overall there is no significant difference in MOI in both Angolan and Namibian residents with a p value of 0.6843 as illustrated in Fig. 17. This could be as a result of political borders being different from biological borders (Meirmans, 2015). In addition, these results do not fit into the previously described model that states that a higher MOI is expected in a high transmission setting as a result of more exposure (higher entomological inoculation rate) compared to a low transmission setting where monoclonal infections are expected (Waltmann *et al.*, 2018). Therefore, molecular transmission dynamics of the *P. falciparum* parasite needs to be redefined in pre-elimination settings to account for proximity and connectivity to high transmission settings.

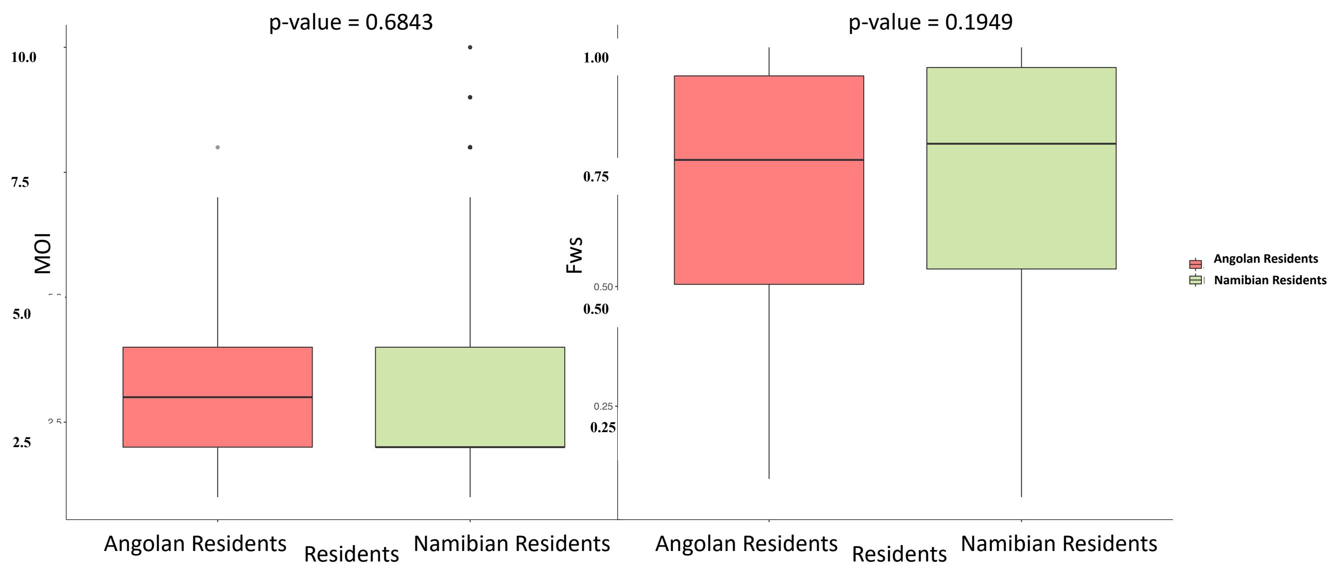

Figure 17: An overall comparison of within host diversity between Namibian and Angolan residents

4.5.3 Relative risk to travel to a malarious region by age and primary district residence

Individuals in Rundu were 5.48 times more likely to travel to a malarious region than individuals in Andara and are therefore at highest risk to travel. Adjusted for age, individuals in Rundu were 5.14 times more likely to travel to a malarious region compared to individuals in Andara as illustrated in Table 8. In addition, there is a significant difference in risk to travel to a malarious region between individuals in Andara and individuals in Rundu. Rundu is the commercial capital of the Kavango East region, therefore there is a more population movement from Angola for commercial reasons and to seek medical attention which could contribute to higher levels of importation and

further local transmission (Lover *et al.*, 2017; Nghipumbwa *et al.*, 2018; Gueye *et al.*, 2014). Andara was used as the reference in calculation of risk to travel by district residence, it has the lowest levels of transmission and was therefore used as the baseline (Smith *et al.*, 2017) in comparison to Nyangana and Rundu which had the higher number of malaria cases reported.

Table 8: Relative risk to travel by age and residents between Namibian and Angolan residents

District	Travel to Angola (n)	Namibia, no travel (n)	Unadjusted OR of travel [95% CI]	P value	Adjusted OR of travel [95% CI]	P value
Andara (intercept)	8	1030	0.0078 (0.0035 - 0.015)	$<2e^{-16}$		
Nyangana	19	1325	1.85 (0.83 - 4.49)	0.148	1.77 (0.80 - 4.30)	0.1803
Rundu	54	1268	5.48 (2.75 - 12.52)	$8.02e^{-06}$	5.14 (2.57 - 11.77)	$1.96e^{-05}$
Age group (years)						
<5 (Intercept)	8	431	0.019 (0.0084 - 0.035)	$<2e^{-16}$	0.0059 (0.0020 - 0.015)	$< 2e^{-16}$
5-14	32	1562	1.10 (0.53 - 2.59)	0.8047	1.30 (0.62 - 3.06)	0.5146
15-29	18	869	1.12(0.50 - 2.74)	0.7982	1.30(0.58 - 3.21)	0.5374
30-44	12	340	1.90 (0.78 - 4.90)	0.1644	2.18(0.89 - 5.64)	0.0943
45-59	7	144	2.62 (0.90 - 7.42)	0.0674	3.21 (1.10 - 9.20)	0.0282
>=60	1	129	0.42 (0.022 - 2.31)	0.4124	0.54 (0.029 - 3.04)	0.5693

There was no significant difference in malaria risk associated with travel between individuals in Andara and individuals in Nyangana as illustrated in table 2. This observation was expected as there was no significant difference in the malaria transmission intensity between Nyangana and Andara (Tambo *et al,* unpublished data).

In this study, the age groups 5-14 years, 15-29 years, 30-44 years and >= 60 years all have

no significant difference in risk to travel to a malarious region compared to the < 5 years age group as illustrated in Table 8. Individuals in the age group < 5 years are the least likely to travel and therefore were used as the reference group to calculate risk to acquire a malaria infection due to travel (Smith *et al.*, 2017). In a previous study in the Ohangwena region of Namibia, the age group 15-29 years was reported to be at the highest risk of acquiring a malaria infection due to travel (Smith *et al.,* 2016). However, adjusted for residence by district, individuals in the 45-59 years age group were 3.2 times more likely to travel to Angola and acquire a malaria infection and at significantly more risk to travel compared to the < 5 years age group. This could be as a result of travel to Angola for economic reasons (Nghipumbwa *et al.*, 2018).

4.6 Conclusion

Malaria transmission is decreasing in the Kavango East region of Namibia as well as Southern Angola as shown by the peak age of infection and corresponding within host diversity in the 5-14 years age range in both populations. There is strong connectivity between the Kavango East region of Namibia and Southern Angola for health and economic reasons that results in continuous importation of malaria from Southern Angola to the Kavango east region of Namibia. The continuous importation of malaria contributes to local transmission dynamics, therefore the distribution of malaria cases in the Kavango East region of Namibia and Southern Angola is similar as shown by the similar distribution of malaria cases across the age range 6-59 years in both the Kavango East region of Namibia and Southern Angola. However, sampling was done in the Kavango East region of Namibia therefore it is recommended that future studies collect samples

from both the Kavango East region of Namibia and Southern Angola to get a better resolution of malaria case distribution in both populations.

Malaria transmission is heterogeneous in the Kavango East region as shown by the uneven distribution of malaria cases by residence and age. In addition, individuals with a primary residence in Rundu and individuals in the 45-59 years are at highest risk to travel associated with acquiring a malaria infection. In Namibia there is strong connectivity with surrounding countries, therefore the proposed population genetics dogma and malaria case distribution by transmission setting needs to be redefined for pre-elimination settings to account for proximity and connectivity with high transmission countries.

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CHAPTER 5: GENETIC DIVERSITY OF *PLASMODIUM FALCIPARUM* IN THE ZAMBEZI REGION OF NAMIBIA AS A MEASURE OF TRANSMISSION INTENSITY AND IMPORTATION

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5.1 Abstract

This study was conducted to characterize the *Plasmodium falciparum* population structure using population genetics analysis of diversity against the number of reported cases in the Zambezi region. The multiplicity of infection (MOI), heterozygosity (H_E), proportion of genetically related infections and proportion of travelers to malarious regions were calculated and plotted against the reported number of malaria cases. The MOI and H_E showed a significant positive correlation (r = 0.83, p value = 0.04; 0.84, p value = 0.04 respectively) with the reported number of malaria cases whilst the F_{WS} metric was inversely proportional to the reported number of cases. This study shows that population genetics matrices have potential use as surrogate markers for malaria transmission intensity.

5.2 Background

Namibia is part of the malaria elimination 8 (E8) formed in 2009 to concert efforts and share best practices moving toward malaria elimination by 8 countries in Sub Saharan Africa. These include Mozambique, Angola, Zambia, Zimbabwe which make up the second line countries and South Africa, Botswana, Eswatini and Namibia which make up the front line countries (Lover *et al.*, 2017). The frontline line countries are in the pre-elimination phase for malaria with a goal to eliminate malaria in 2020, revised to 2022 for Namibia (Chanda *et al.*, 2018) whilst the second line countries are still in the control phase of malaria with a high number of reported cases and are expected to have eliminated malaria by the year 2030 (Lover *et al.*, 2017; WHO, 2017).

Malaria elimination requires targeted interventions; however, current transmission matrices that include prevalence and incidence data provide insufficient information for malaria elimination as they do not account for key populations such as imported malaria infections (Reiner *et al*., 2015; Smith *et al.*, 2017). In addition, malaria like other infectious diseases is heterogeneously transmitted, that is high levels of transmission are confined to a few households, health facilities or villages whilst other household have a few to no cases of malaria (Woolhouse *et al.*, 1997; Bousema, Kreuels & Gosling, 2011). Measures from health facility detected malaria cases can indicate local outbreaks but do not accurately identify focal malaria transmission areas known as hotspots (Koepfli, Waltmann & Ome-kaius, 2018). The malaria hotspots would need to be identified by extensive cross sectional surveillance with molecular diagnostic tools. Additionally, these measures do not account for connectivity of malarias regions both in country and across

different countries (Galappaththy, Fernando & Abeyasinghe, 2013).

Alternatives such as highly sensitive molecular tools such as PCR and LAMP with population wide surveys have been suggested to fill in this gap as they can accurately determine hotspots and asymptomatic infections (Auburn & Barry, 2017; Koepfli, Waltmann and Ome-kaius, 2018). However, these surveys are expensive for resource limited settings where the malaria burden is highest, they are labor intensive, require highly skilled personnel and are time consuming (Escalante *et al.*, 2015). Therefore, new alternative methods such as molecular genotyping of the *Plasmodium falciparum* parasite on a representative sample could be employed to complement the current data and give more accurate information on connectivity of malarias regions, identification of at risk populations and transmission intensity (Greenhouse & Smith, 2015; Koepfli, Waltmann & Ome-kaius, 2018). This would obviate the need and cost of population wide surveys. Additionally, use of molecular genotyping could allow quick responses and predictions of malaria transmission.

In *Plasmodium* genotyping, high and low transmission areas are expected to follow distinct previously described *Plasmodium* genetic diversity patterns (Campino *et al.*, 2011; Auburn & Barry, 2017). In high transmission settings such as Angola and Zambia, a high MOI, H_E, and lower genetic relatedness are expected (Anderson *et al.*, 2000; Cerqueira et al., 2017). (Chauhan, Pande & Das, 2013). Whilst in low transmission settings such as Columbia, Bolivia and Brazil, low MOI, H_E, and a low population level genetic diversity have been reported (Anderson et al., 2000; Auburn & Barry, 2017). These matrices have therefore been suggested as measures of malaria transmission

intensity (Greenhouse & Smith, 2015).

In Namibia in particular, there is no genetic evidence for the *P. falciparum* transmission dynamics to supplement the data collected from the health facilities and reactive case detection. Moving toward elimination, accurate data is required to effectively target interventions on key populations (Sakuntabhai *et al.*, 2008; Reiner *et al.*, 2015). Therefore, this study employed molecular genotyping for population genetics analysis of the *P. falciparum* parasite in the Zambezi region of Namibia to complement current data and accurately measure transmission and connectivity to allow effective targeting of interventions.

5.3 Methods

5.3.1 Study sites and sample collection.

Inclusion criteria was stated as all RDT confirmed malaria cases at Heath Facilities that consented (>=18 years) to participating in the study and assent was given for minors (< 18 years). All age groups were included, participation was voluntary, participants could drop out of the study at any time without consequences and their personal information was treated confidentially.

A total of 772 dried blood spots (DBS) and epidemiology data including travel history from all passively detected symptomatic cases were collected from 6 health facilities namely Sibbinda health clinic, Choi clinic, Kasheshe clinic, Sesheke clinic, Chinchimani clinic and Kanono clinic as illustrated in Fig. 18. The health facilities are in the Zambezi region of Namibia bordering Angola and Zambia (high transmission settings) and were

collected between March and June 2015. The participating adults (18 years or older) gave written consent by signature or fingerprint whilst for minors below the age of 18 years, parents or guardians gave written consent or fingerprint for recruitment. All the DBS were stored in double zip lock bags with desiccant at room temperature until further laboratory analysis was performed.

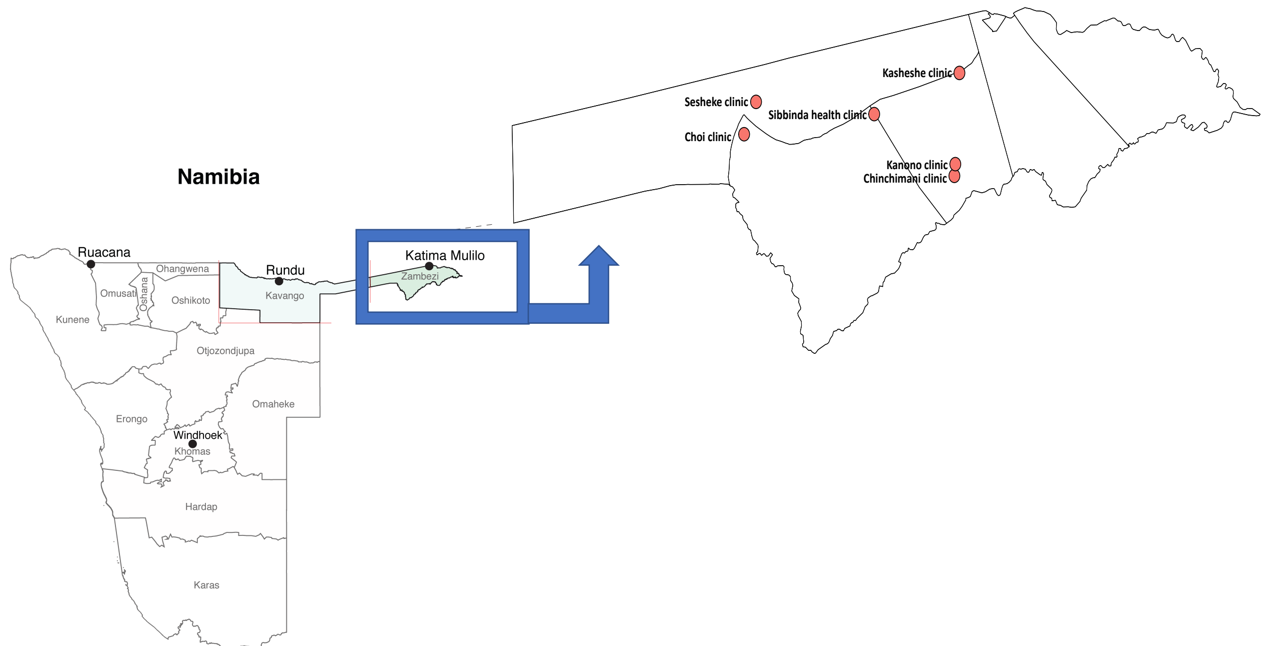

Figure 18: Map of the Zambezi region showing the locations of the 6 health facilities in this study

5.3.2 Microsatellite genotyping of P. falciparum

The parasite density was first quantified using the var-ATS ultra-sensitive qPCR protocol on all DBS samples as described previously (Hofmann *et al*., 2015) following DNA extraction with the chelex extraction method (Polski *et al*., 1998). Only samples with a parasite density of at least 10parasites/ul were used in further analysis. A total of 772 samples were genotyped using microsatellite markers at 26 loci distributed across 7

chromosomes the *P. falciparum* genome (Anderson, Paul, Donnelly, & Day, 2000), the DNA was sized by denaturing capillary electrophoresis on an ABI 3730XL analyzer with GeneScan™ 400HD ROX™ size standard (Thermo Fisher Scientific). The MicroSPAT software was used with the same settings for all samples to semi-automate the calling of alleles, thereby reducing human error. Samples that were genotyped on at least 15 loci were used for further analysis.

5.3.3 Within-host diversity

To increase the accuracy of true allele identification in analysis, the within host genetic diversity was calculated as the second highest number alleles across all loci. The mean MOI was calculated at health facility level against the number of malaria reported cases. In addition, the mean F_{WS} metric was also calculated at health facility level against the number of reported cases;

$$F_{WS} = 1 - \frac{H_w}{H_s}$$

Where; H_w=heterozygosity of the individual and H_s=heterozygosity of the local parasite population as previously described by Mobegi et al., (2014). The F_{WS} metric is a measure of genetic similarity of *Plasmodium* parasites co-infecting an individual.

5.3.4 Population genetic diversity

Genetic diversity at population level was determined by calculating mean expected heterozygosity (H_E) at health facility level against the number of reported cases;

$H_E = [n/(n - 1)][1 - \sum p_i^2]$,

Where n is the number of genotyped samples and p_i is the frequency of the ith allele in the

population as previously described by Roh et al., (2019). In addition to heterozygosity, the mean number of alleles was calculated from 12 samples from each health facility against the number of reported cases. The mean number of alleles is significantly influenced by the number of samples used in analysis, therefore the health facility with the least number of samples determined the total number of samples used for analysis from each health facility (n=12).

5.3.5 Genetic relatedness of *P. falciparum* infections

The proportion of highly related *P. falciparum* infections was calculated within each health facility with a cutoff point of 0.7 (70% genetic similarity) (Wesolowski *et al.*, 2018) and plotted against the number of reported cases. That is, only samples with genetic similarity on at least 70% of their loci were considered to be highly related through pairwise comparisons. In addition, the proportion of travelers was calculated at health facility level and plotted against the number of reported cases

5.4 Results and Discussion

The calculated MOI for Zambezi region showed a significant positive correlation (r = 0.83, p value = 0.04) with the reported number of malaria cases as illustrated in Fig. 19 panel A. Similar result have been observed across different transmission settings where there was a low MOI in regions with a low number of reported cases such as Columbia and parts of Senegal (Cerqueira et al., 2017) and a high MOI within regions with a high number of cases such as Uganda and the Democratic Republic of Congo (Anderson et al., 2000; Greenhouse et al., 2006). (Barry *et al.*, 2013; Razak *et al.*, 2016). Therefore, MOI has been suggested as a surrogate indicator for malaria transmission intensity using microsatellite markers for genotyping (Koepfli *et al.*, 2018; Schultz *et al.*, 2010) to

supplement current transmission estimates with genetic evidence.

However, there was a deviation from the expected increase in MOI with the increasing number of reported cases in Chinchimani clinic which had a mean MOI similar to Sesheke clinic with about double the number of reported cases. This could be as a result of high continuous importation spreading polyclonal infections acquired from malarious regions (Reiner *et al.*, 2015; Strano *et al.*, 2018). In Chinchimani, up to 60% of the reported cases are highly related to cases from Choi clinic which has a higher number of reported cases. In this study therefore, MOI was shown to be a good indicator of malaria transmission intensity and importation as shown in previous studies (Razak *et al*., 2016; Soe *et al*., 2017; Vafa, *et al*., 2008).

The F_{WS} metric was generally inversely proportional to the reported number of cases as would be expected as illustrated in Fig. 19 panel B given findings in Eswatini (Roh *et al.,* 2019). However, in Kanono clinic, although there were a low number of reported cases the *Plasmodium* parasites had low genetic similarity. This finding could be a result of importation from different sources, it has been reported that as the imported cases in Kanono were acquired from Angola, Zambia and Choi, all which have a much higher number of reported cases compared to Kanono (Gueye *et al.*, 2014).

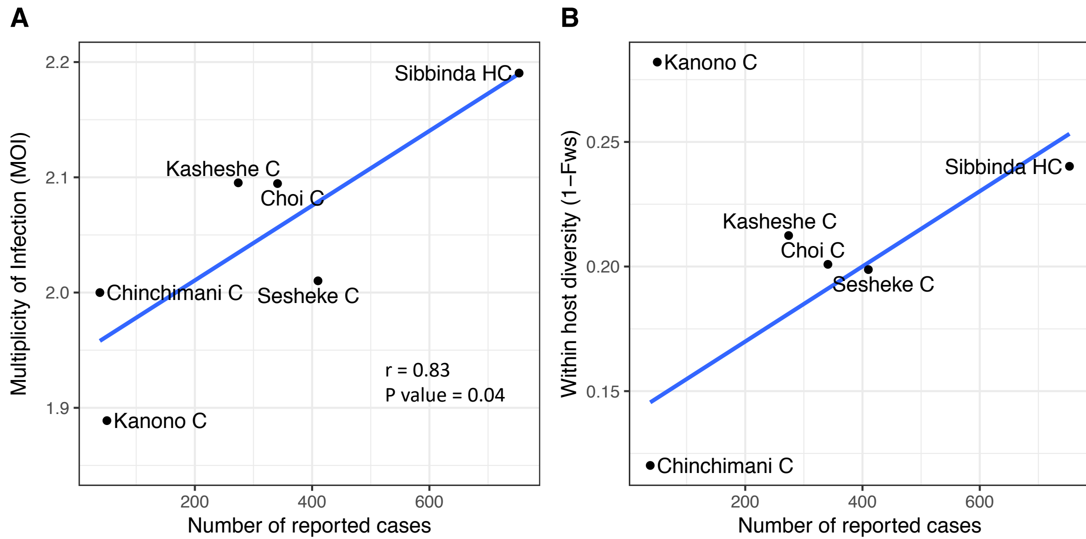

Figure 19: Panel A showing positive correlation between multiplicity of infections and reported number of cases. Panel B shows an inverse correlation between Fws and the reported number of cases

The H_E was observed to increase with the increasing number of cases with a significant positive correlation (r = 0.84, p value = 0.04) as illustrated in Fig. 20 panel A. This finding corresponds with previous studies in Columbia and Brazil that showed a low H_E in regions with a low number of reported malaria cases as well studies in Zambia and Angola that had a high H_E in regions with a high number of reported cases (Anderson *et al.*, 2000; Escalante *et al.*, 2015). However, the observed increasing H_E plateaus (stops increasing and remains constant) as the number of reported cases continues to increase. The mean number of unique alleles showed a trend similar to H_E with an increasing number of cases as illustrated in Fig. 20 panel B. The mean number of alleles was calculated from the same number of samples from each health facility (N = 12) and therefore the trend was not influenced by the number of samples (Meirmans, 2015).

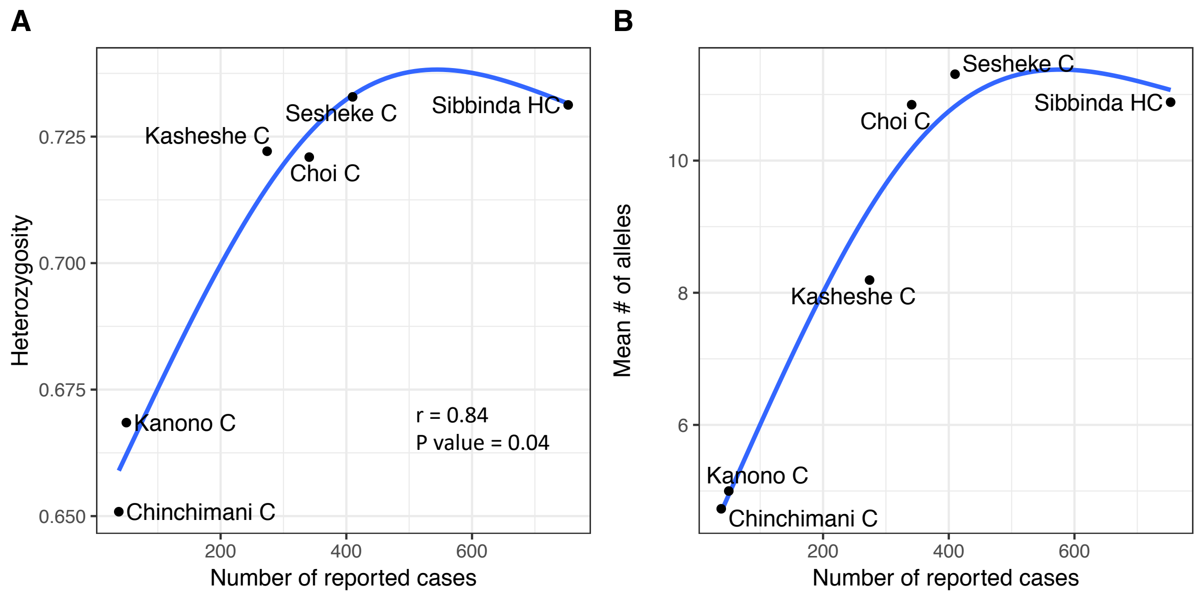

Figure 20: Panels A and B show a positive correlation between population level genetic diversity with reported number of malaria cases

The observed proportion of international travel to malarious countries (Angola) was inversely proportional to the number of reported malaria cases at health facility level as illustrated in Fig. 21 panel A. This suggests that in areas of lower transmission, the majority of malaria cases are imported as the number of reported travel cases make up a significant proportion of the cases and in areas of higher transmission there are fewer reported cases of importation. Malaria importation is a potential significant barrier for eliminating malaria in many Sub Saharan countries as a result of significant connectivity amongst the countries (Sriwichai *et al.*, 2017; Tatem *et al.*, 2017). This indicates that there is more local transmission in areas with a high number of reported cases and where local transmission has been reduced, importation poses a great risk of reintroduction of transmission (Sturrock *et al.*, 2015, Wesolowski *et al.*, 2018).

As illustrated in Fig. 21 panel B, the proportion of related cases within a health facility is inversely correlated with increasing number of reported malaria cases. This means that malaria infections within a health facility are more related if there is a low number of reported cases and the genetic relatedness of *P. falciparum* decreases as the number of reported cases increases due to random mating of *P. falciparum*. Similar findings were observed in Mali (Nabet *et al*., 2016b). However, there was a deviation in Kanono clinic where the proportion of highly related infections was low although there were a low number of reported malaria cases. This could have been influenced by importation (Auburn & Barry, 2017) from different sources as imported cases in Kanono were from both Angola and Zambia.

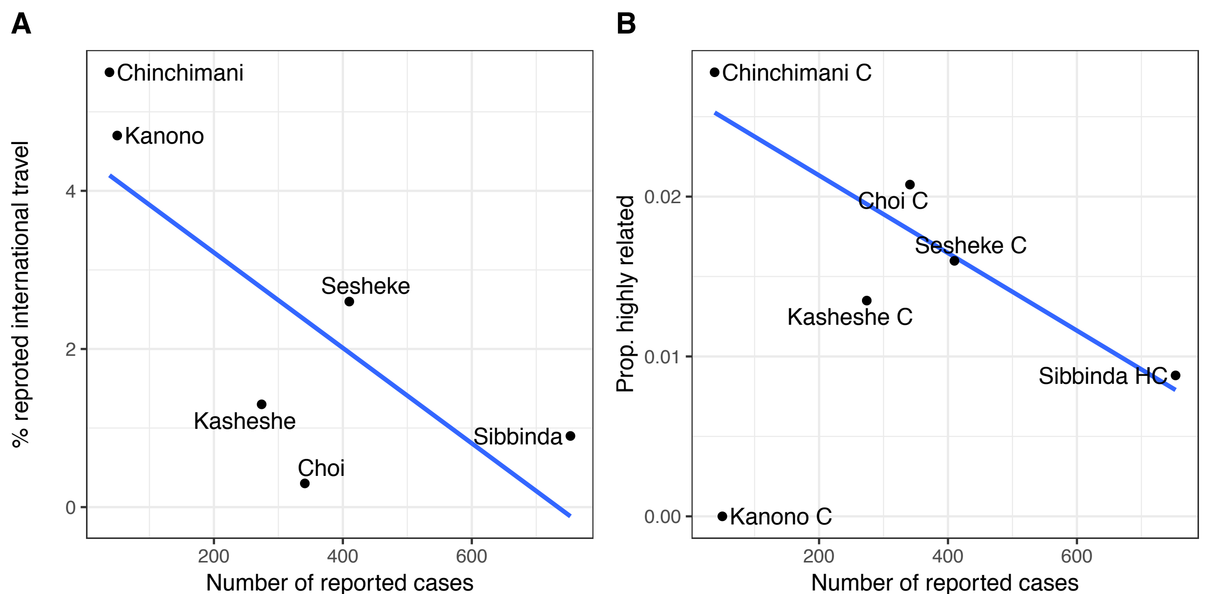

Figure 21: Panels A and B show the correlation of proportion of travel and genetic relatedness with the reported number of cases respectively

5.5 Conclusion and recommendations

In conclusion, this study showed that genetic diversity measures, MOI, F_{WS}, H_E and proportion of highly related infections are good indicators of malaria transmission intensity. The MOI and H_E both increase with an increase of the reported number of cases thereby indicating the level of transmission intensity. However, the MOI and H_E measures are sensitive to importation, therefore deviations when interpreted with epidemiological data could be attributed to *P. falciparum* importation from a high transmission setting, Southern Angola. Therefore, MOI and H_E can both be used as measures of importation levels and transmission intensity. The genetic relatedness of P. falciparum parasites within a single host decreases with an increasing reported number of cases and importation from high transmission settings. Therefore Fws can be used to estimate transmission intensity levels and levels of importation, there is reduced genetic relatedness of *P. falciparum* in imported malaria infections from Angola and in high local transmission areas. The transmission intensity and importation level results using MOI, H_E and Fws were achieved with a relatively small number of representative samples (as low as 12 samples per health facility). However, these results were obtained from one malaria season therefore it is recommended that genetic analysis be done over different malaria seasons to investigate the accuracy of the prediction potential, identify reservoirs of malaria infection from one season to the next and evaluating interventions through the identification of the hotspots and corresponding interventions. Additionally, these matrices are influenced by importation and have to be interpreted with epidemiological data to evaluate interventions. Lastly, it is recommended that a regional genotype database for the elimination 8 region be constructed in order to accurately identify and quantify importation from one region or

country to another and further local transmission seeded by importation.

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CHAPTER 6: OVERALL CONCLUSIONS

The current malaria transmission estimates and classification of cases as local or imported based on inaccurate and incomplete self-reported travel history and reactive case detection from cases that present at health facilities is insufficient. Accurate and evidence based malaria transmission estimates are of importance moving toward malaria elimination as they inform the malaria elimination strategy. Therefore, 1) population genetics analysis was carried out in the Zambezi and Kavango East regions of Namibia that account for the majority of malaria cases in Namibia, 2) The distribution of malaria cases in the Namibian and Angolan populations was compared to estimate the levels of importation and 3) The usefulness of population genetics matrices as transmission intensity surrogate markers was determined in order to fill these critical information gaps.

Firstly, this study investigated the *P. falciparum* genetic diversity with 26 putatively neutral microsatellite markers across the 7 chromosomes of the *P. falciparum* parasite in the Kavango East and Zambezi region of Namibia. The mean MOI across the health facilities was 2.54 and the highest was observed in Rundu. A moderate to high mean MOI across the populations poses a risk for clinical presentation of malaria infections as complicated malaria and the development of drug resistance traits. The higher the MOI, the less genetically related the parasites within each individual. Despite the Kavango East and Zambezi regions being low to moderate transmission settings, mostly polyclonal infections were observed as opposed to the expected monoclonal infections observed in similar transmission settings. A similar trend was observed with population level

heterozygosity that was high with a mean of 7.5 and very low genetic relatedness which is in contrast with what is expected in a pre-elimination setting.

The high genetic diversity poses a threat for development of drug resistance and better genetic fitness for the *P. falciparum* parasite in Namibia. Additionally, the genetic population structure of *P. falciparum* showed no fragmentation of parasite populations indicating that the parasite populations are highly admixed as a result of continuous importation in the regions. This poses a high risk of the spread of unfavourable genetic traits such as drug resistance and frustrates targeted interventions as all the endemic regions are strongly connected.

These findings infer that the population diversity of *P. falciparum* parasites in the Kavango East and Zambezi regions in Namibia does not fit the current model for pre-elimination settings. This is due to importation of malaria from high transmission settings such as Angola, in addition, there is within country importation across the regions. High levels of parasite genetic diversity need efficient surveillance systems as the potential for outbreaks and potential resistance to antimalarial drugs increases with an increase in parasite diversity. The *P. falciparum* diversity in Namibia and neighboring Sub-Saharan countries in the E8 regional initiative need to be investigated as the transmission dynamics in this region are not fully understood. The investigation of the *P. falciparum* genetic diversity and connectivity of regions was the first of its kind in Namibia.

Secondly, as a result of porous borders between Angola and Namibia in the Kavango East region, this study compared the malaria case distribution in Angolan and Namibian

populations with samples from 23 health facilities in the Kavango East region using primary residence as a proxy for both the Angolan and Namibian population. The age groups 6-59 years showed no significant difference in the proportion of malaria case distribution. This is evidence for continuous importation of malaria from Angola to the Kavango East region. Furthermore, malaria cases in the > 60 years age group was only observed in Namibia, this could be as a result of the difference in life expectancy between the two countries. In addition the study found that in the Kavango East region, individuals in the age group 45-59 years residing in Rundu were at the highest risk of travelling to a malarious region. A high mean MOI of 2.5 with a p value of 0.6843 was observed in both the Angolan and Namibian populations with no significant difference indicating continuous importation that further contributes to local transmission of malaria. Importation from high transmission neighboring countries poses a threat to malaria elimination efforts in Namibia.

Lastly, *P. falciparum* genetic diversity matrices were investigated as potential measures of malaria transmission intensity in the Zambezi region with samples from 6 health facilities. The MOI showed a positive correlation with an increasing number of reported malaria cases ($r = 0.83$, p value $= 0.04$) and an inverse relationship between genetic similarity in *P. falciparum* within a host and the increasing number of reported malaria cases. The results show that population genetics matrices are good surrogate markers for transmission intensity and levels of importation in Namibia. However, there was a deviation in Chinchimani clinic where there was a high MOI regardless of a low number of reported malaria cases and low genetic similarity in Kanono clinic with a low number of reported cases. Both deviations were caused by high levels of importation in

Chinchimani clinic from Choi clinic which has a high number of reported cases and high levels of importation from Angola in Kanono clinic. Similar trends were observed with population level heterozygosity and genetic relatedness were the matrices showed clear patterns with the increasing number of cases.

There is also predictive potential in the use of *P. falciparum* genetic diversity matrices as they require a small representative number of samples to accurately report transmission intensity. The *P. falciparum* genetic diversity is sensitive to importation and as such has to be interpreted with epidemiological data. The findings from this study show the importance and usefulness of *Plasmodium* genotyping to supplement current data to accurately measure transmission, determine parasite flow, to measure importation and to determine the levels of connectivity with other malarias regions. This provides useful information for the National Malaria Control programs in the E8 region to efficiently allocate resources and target interventions.

CHAPTER 7: CONTRIBUTION TO NEW KNOWLEDGE

This study is the first to provide the genetic structure of *P. falciparum* in Namibia to inform the national malaria control programme on interventions to implement. The *P. falciparum* genetic structure in Namibia does not follow the pre-described dogma for low transmission settings, instead, it follows the high transmission pattern as a result of constant importation from Angola (a high malaria transmission setting) that contributes to further local transmission and observed high genetic diversity. Furthermore, the *P. falciparum* population structure shows high levels of parasite connectivity amongst malaria endemic regions although Namibia is moving towards malaria elimination. This finding is new evidence for the need of simultaneous implementation of interventions in endemic regions as opposed to targeted interventions in one region as previously recommended for low transmission settings. In addition, this study shows high levels of *P. falciparum* importation from Angola to Namibia shown by similar distribution patterns of malaria cases across different age groups. Furthermore, this study shows that individuals who with primary residence in Rundu are at the highest risk of acquiring a malaria infection in the Kavango East and Zambezi regions due to travel to Angola. Lastly, the study shows that *Plasmodium* population genetics matrices can be used as surrogate markers for measuring malaria transmission intensity and dynamics, including importation. This finding obviates the need for extensive population wide surveys that are both costly and time consuming as population genetics matrices can provide the same information with a significantly smaller number of samples.

CHAPTER 8: RECOMMENDATIONS

It is recommended that the findings from this study be used as a basis and evidence for the need of population genetics analysis in all malarious regions in Namibia to supplement current data on malaria transmission intensity. The E8 region is peculiar in its connectivity and the *Plasmodium* genetic diversity in Namibia and Eswatini has been shown to deviate from the *Plasmodium* genetic diversity theory because of importation, therefore, it is recommended that *Plasmodium* genetic diversity is investigated and characterized for this region. In addition, it is recommended that drug resistance surveillance be strengthened in the E8 pre-elimination settings as a result of the observed high *Plasmodium* genetic diversity regardless of low numbers of reported malaria cases. Furthermore, it is recommended that *Plasmodium* genetic diversity be studied over time to explore its potential use in malaria transmission intensity prediction. Lastly, it is recommended that a regional genotype database for *Plasmodium* parasites in the elimination 8 region be constructed in order to accurately identify and quantify importation and further local transmission seeded by importation.

CHAPTER 9: APPENDICES

9.1 Appendix A: Population genetics analysis code chapter 3

```
library(tidyverse)
library(ggplot2)
library(dplyr)
library(ggpubr)
#Set working directory
setwd("/Users/gp-admin/Dropbox/Munya.Genotyping/Script from ST/Data/Paper 3 data")
#Read incidence data
df_p3<-read.csv("/Users/gp-admin/Dropbox/Munya.Genotyping/Script from ST/Data/Paper 3 data/CleanP3data copy.csv")
#Read popperclinic
popclinic<-read.csv("PopPerClinic.csv")
#Rename columns to join by "Facility"
colnames(df_p3)[colnames(df_p3) == "facility"] <- "Facility"
#count number of cases per village
df_p3<-as.data.frame(table(df_p3$Facility))
#Change column names
colnames(df_p3) <- c("Facility", "ncases")
#Join with population data at health facility level
df_p3<-merge(df_p3, popclinic, by = "Facility")
#Annual population growth by year to 2017 (2%) source:http://worldpopulationreview.com/countries/namibia-population/
df_p3<- transform(df_p3, POP2016 = (POP2015*0.02)+POP2015)
df_p3<- transform(df_p3, POP2017 = (POP2016*0.02)+POP2016)
#Calculate incidence at healthfacility level
df_p3<- transform(df_p3, incidence = (ncases/POP2017)*1000)
#Save the incidence file
#write.csv(df_p3, "incidence_HF.csv")
incid_HF<-read.csv("incidence_HF.csv")
incid_HF$Facility<- factor(incid_HF$Facility, levels = c("Choi_Clinic", "Katima_District_Hospital", "Sachona_Clinic", "Sangwali_HC", "Sibbinda_HC", "Batubaja_Clinic", "Linyanti_Clinic", "Chinchimani_Clinic", "Kanono_Clinic", "Kasheshe_Clinic", "Masokotwane_Clinic", "Mafuta_Clinic", "Sesheke_Clinic"))
#Incidence per health facility orderd from west to east representation
```

```
ggplot(data= incid_HF, aes(Facility, incidence2014, fill = Facility)) + guides(fill=FALSE) +
  geom_bar(stat = "identity") + labs(title = "Malaria incidence across health facilities in the Zambezi region")
+
    xlab("Health facility") + ylab("Incidence") + theme(axis.text.x = element_text(angle=-45, hjust=.1))
#Calculate MOI
## MOI
library(tidyverse)
library(ggplot2)
library(dplyr)
all_alleles <- read.csv('MERFAT-all-alleles-27102017.csv') # The column name for sample ID should be
namesd as "Sample"
head(all_alleles)
samples <- dplyr::filter(all_alleles, !grepl(pattern = 'NTC|POS', x = barcode))
negatives <- dplyr::filter(all_alleles, grepl(pattern = 'NTC', x = barcode))
positives <- dplyr::filter(all_alleles, grepl(pattern = 'POS', x = barcode))
loci <- colnames(samples)[-c(1)]
sample_alleles = data.frame(barcode=c(), Allele=c(), Locus=c())
for(i in 1:length(loci)) {
  locus = loci[i]
  res <- cbind(tidyr::separate_rows_(samples, `locus`)[, c('barcode', `locus`)], c(`locus`))
  colnames(res) <- c('barcode', 'Allele', 'Locus')
  res <- dplyr::filter(res, Allele != "")
  sample_alleles <- rbind(sample_alleles, res)
}
by_sample <- dplyr::group_by(sample_alleles, barcode)
by_locus <- dplyr::group_by(sample_alleles, Locus)
head(by_sample)
moi_offset = 2 # takes the second highest
sample_moi <- dplyr::summarise(by_sample[complete.cases(by_sample$Allele),],
                               moi = sort(table(Locus), decreasing = T)[moi_offset])
#Save MOI results by sample
write.csv(sample_moi, "Sample_moi_p3.csv")
#Merge data with facility data
HF_data<-read.csv("MERFAT-genotyping-key.csv")
sample_moi<-merge(sample_moi, HF_data, by = "barcode")
#Save sample_moi_HF data
write.csv(sample_moi, "Sample_moi_HF_p3.csv")
#Order Healthfacilities from west to East
```

```
sample_moi$healthfacility<- factor(sample_moi$healthfacility, levels = c("Choi C", "Sibbinda HC", "Chinchimani C", "Kanono C", "Kasheshe C", "Sesheke C"))
#Presentation
ggplot(data= sample_moi, aes(healthfacility, moi, colour = healthfacility)) +
 geom_boxplot() + geom_jitter(alpha=0.3) + labs(title = "MOI per healthfacility") +
   xlab("Health facility") + ylab("MOI")
# Heterozygosity
all_alleles<-merge(HF_data,all_alleles, by = 'barcode')
head(all_alleles)
cleaned = all_alleles[,c(1,3, 19:44)] # id, pop, and alleles
names (cleaned)
cleaned = dplyr::rename(cleaned, pop = healthfacility) # rename healthfacility to pop
loci <- colnames(cleaned)
loci <- loci[loci != 'barcode' & loci != 'pop']
loci # lsit of loci
long_form_results <- data.frame("barcode"=integer(0), "locus"=character(0), "allele"=integer(0), "population"=character(0));
for(i in 1:(dim(cleaned)[1])) {
  entry = cleaned[i,]
  sample_id = entry["barcode"]
  population = as.character(entry["pop"]$pop)
  print(sample_id[1,]);
  print(i)
  for(j in 1:(length(loci))) {
    locus = loci[j];
    alleles_list = entry[locus];
    #print(alleles_list);
    alleles <- unlist(strsplit(gsub(" ", "", unlist(alleles_list)), ";"));
    for(k in 1:(length(alleles))) {
      allele <- as.integer(alleles[k]);
      if(length(allele) > 0) {
        long_form_results <- rbind(long_form_results, data.frame("barcode" = sample_id, "locus" = locus, "allele" = allele, "population" = population));  } } }}
dim(long_form_results)#13927
head(long_form_results)
is.na (long_form_results)
long_form_results<- na.omit(long_form_results)# removes NA
dim(long_form_results) # 13259
```

```
str(long_form_results)#104534 including NA
#convert allele to numeric
hist (long_form_results$allele)##Check if there are zeros
#rename to allforpopfws
allforpopfws  <-  long_form_results[complete.cases(long_form_results),]
#Omit if samples have less than 10 number of successful loci ----#
# Number of successful genotyping per unique barcode - will exclude if >10 missing
SuccessCountPerSample <- function(x){
  SuccessCount<-length(unique(allforpopfws$locus[allforpopfws$barcode==x]))
  return(SuccessCount)
}
barcodelist<-c(unique(allforpopfws$barcode)) # List of unique barcodes
gtsuccesscount.barcode<-lapply(barcodelist, FUN=SuccessCountPerSample)
gtsuccesscount.barcode<-cbind(barcodelist, gtsuccesscount.barcode)
gtsuccesscount.barcode<-as.data.frame(gtsuccesscount.barcode)  #  row  =  230  barcodes,  col  =  barcode,
successful genotype count
gtsuccesscount.barcode$num.locipresent <- gtsuccesscount.barcode$gtsuccesscount.barcode
gtsuccesscount.barcode$gtsuccesscount.barcode<- NULL
gtsuccesscount.barcode <- as.data.frame(lapply(gtsuccesscount.barcode, unlist))
gtsuccesscount.barcode$num.locimissing <- 26 - gtsuccesscount.barcode$num.locipresent
head(gtsuccesscount.barcode)
names(gtsuccesscount.barcode)[names(gtsuccesscount.barcode)=="barcodelist"]<-"barcode"
hist(gtsuccesscount.barcode$num.locimissing) # Histogram shows that only a small number of
abline(v=11, col="red") # not substed as I already remove based on the home MS
allforpopfws.locimissing<-(merge(gtsuccesscount.barcode, allforpopfws, by="barcode"))
allforpopfws<-as.data.frame(allforpopfws.locimissing)
allforpopfws <-subset(allforpopfws, num.locimissing<=11)
head(allforpopfws)
#---------------------------------------------------------------------------#
# Calculate heterozygosity
library(dplyr)
str(allforpopfws)
pop.list <- as.character(unique(allforpopfws$population))
pop.stats <- list()
for(i in 1:length(pop.list)){
  pop <- pop.list[i]
  pop.data <- list()
  allforpop.sub <- allforpopfws[allforpopfws$population==pop,]
```

```
  call.rate.perloci <- function(x) {
    #missing<-1-length(unique(gtdb$barcode[x]))/length(unique(gtdb$barcode))
    call.rate<-
length(unique(allforpop.sub$barcode[allforpop.sub$locus==x]))/length(unique(allforpop.sub$barcode))
    return(call.rate)
  }
  numofcalls.perloci <- function(x){
    numberofsuccess<-length(unique(allforpop.sub$barcode[allforpop.sub$locus==x]))
    return(numberofsuccess)
  }
  loci.list <- as.character(unique(allforpop.sub$locus))
  call.rate <- lapply(loci.list, FUN=call.rate.perloci)
  loci.call.rate <- cbind(loci.list, call.rate)
  call.perloci <- lapply(loci.list, FUN=numofcalls.perloci)
  loci.call.rate <- cbind(loci.list, call.rate)
  call.perloci <- lapply(loci.list, FUN=numofcalls.perloci)
  numofcall.perloci<- cbind(loci.list, call.perloci)
  loci.callrate <- as.data.frame(cbind(loci.list, call.rate, call.perloci))
  loci.callrate <- as.data.frame(lapply(loci.callrate, unlist))
  loci.callrate<-loci.callrate[order(loci.callrate$call.rate),]
  loci.callrate$row.names<-NULL
  names(loci.callrate)[names(loci.callrate)=="loci.list"]<-"locus"
  pop.data$loci.callrate <- loci.callrate
  funSubsetCount <- function(x) {
    subset.int <-subset(allforpop.sub, locus==x)
    myfreq <- as.data.frame(table(subset.int$allele))
    names(myfreq) <- c("allele", "Freq")
    locusid <- rep(x,nrow(myfreq))
    output.df<-cbind(myfreq, locusid)
    #return(fun.data.frame)
    return(output.df)
  }
  ?count
  loci.allele <- lapply(loci.list, FUN=funSubsetCount)
  lociallele <-do.call(rbind, loci.allele) # Table of all allele types present in pop.
  lociallele <- as.data.frame (lociallele)
  funallelefreq <- function(x){    # Function to determine sum of all samples that were successful at specific
loci
```

```
    alleledenominator<-sum(lociallele$Freq[lociallele$locusid==x])
    return(alleledenominator)
  }
  sum.allelesperloci<-lapply(loci.list, FUN=funallelefreq)
  allelefreq.denom <-cbind(loci.list, sum.allelesperloci) # col of 27 markers, each row contains how many samples were genotyped at each loci
  allelefreq<-merge(lociallele, allelefreq.denom, by.x="locusid", by.y="loci.list", all=FALSE)
  allelefreq$sum.allelesperloci<-as.numeric(allelefreq$sum.allelesperloci)
  allelefreq$allelefreq<-(allelefreq$Freq)/(allelefreq$sum.allelesperloci) # Calculate allele frequency at each ith allele
  funHe <- function(x){
    He <- ((length(unique(allforpopfws$barcode)))/(((length(unique(allforpopfws$barcode)))-1))*(1-sum((allelefreq$allelefreq[allelefreq$locusid==x]^2))))
    return(He)
  }
  He.loci.list <- lapply(loci.list, FUN=funHe)
  He.perloci<- cbind(loci.list, sum.allelesperloci, He.loci.list)
  He.perloci<- as.data.frame(He.perloci)
  allelecount <- as.data.frame(table(allelefreq$locusid))
  He.perloci<-cbind(He.perloci, allelecount)
  He.perloci$x<-NULL
  names(He.perloci)[names(He.perloci)=="He.loci.list"]<- "He"
  names(He.perloci)[names(He.perloci)=="sum.allelesperloci"]<- "TotalNo.Samples"
  names(He.perloci)[names(He.perloci)=="Freq"]<- "Freq.Uniquealleles"
  names(He.perloci)[names(He.perloci)=="loci.list"]<- "Loci"
  He.perloci$He<-as.numeric(He.perloci$He)
  He.perloci$TotalNo.Samples<-as.numeric(He.perloci$TotalNo.Samples)
  He.perloci$Loci<-as.character(He.perloci$Loci)
  pop.data$He.perloci <- He.perloci
  pop.data$allele.freq <- allelefreq
  pop.stats[[pop]] <- pop.data
}
head(pop.stats)
# summary of HE
Choi_C  = pop.stats$`Choi C`$He.perloci[, -4]
Choi_C$pop = "Choi_C"
head(Choi_C)
Sibbinda_HC  = pop.stats$`Sibbinda HC`$He.perloci[, -4]
```

```
Sibbinda_HC$pop = "Sibbinda_HC"
Chinchimani_C  = pop.stats$`Chinchimani C`$He.perloci[, -4]
Chinchimani_C$pop = "Chinchimani_C"
Kanono_C  = pop.stats$`Kanono C`$He.perloci[, -4]
Kanono_C$pop = "Kanono_C"
Kasheshe_C  = pop.stats$`Kasheshe C`$He.perloci[, -4]
Kasheshe_C$pop = "Kasheshe_C"
Sesheke_C  = pop.stats$`Sesheke C`$He.perloci[, -4]
Sesheke_C$pop = "Sesheke_C"
# combine all data
all.he <- rbind(Choi_C, Sibbinda_HC, Chinchimani_C, Kanono_C,Kasheshe_C,Sesheke_C)
head(all.he)
#Save unique alleles data
write.csv(allelefreq, "Unique_alleles_p3.csv")
#mean and se by popualtion
het <- all.he %>% dplyr::group_by(pop)
dd.he = het %>% dplyr::summarise_each(funs(mean,sd,se=sd(.)/sqrt(n())), He)
dd.he %>% group_by(pop) %>% summarise(median(mean))
pairwise.t.test(all.he$He, all.he$pop, p.adjust.method ="none")
#Save mean he per healthfacility
write.csv(dd.he, "Mean_he_per_HF.csv")
# visualize
het$pop<- factor(het$pop, levels = c("Choi_C", "Sibbinda_HC", "Chinchimani_C", "Kanono_C", "Kasheshe_C", "Sesheke_C"))
library(ggpubr)
compare_means(method= "t.test", He ~ pop,  data = het)
my_comparisons <- list( c("Choi_C", "Sibbinda_HC"), c("Choi_C", "Chinchimani_C"), c("Choi_C", "Kanono_C"), c("Choi_C", "Kasheshe_C"), c("Choi_C", "Sesheke_C"), c("Sibbinda_HC", "Chinchimani_C"), c("Sibbinda_HC", "Kanono_C"), c("Sibbinda_HC", "Kasheshe_C"), c("Sibbinda_HC", "Sesheke_C"), c("Chinchimani_C", "Kanono_C"), c("Chinchimani_C", "Kasheshe_C"), c("Chinchimani_C", "Sesheke_C"), c("Kanono_C", "Kasheshe_C"), c("Kanono_C", "Sesheke_C"), c("Kasheshe_C", "Sesheke_C"))
#Without p-value
ggplot(data=het, aes(pop, He, colour = pop)) +
  geom_boxplot() + geom_jitter(alpha=0.3)+
  labs(title = "He distribution per population") +
  xlab("population") +
  guides(col=FALSE)+
```

```
  ylab("He")+
  theme(axis.text.x = element_text(angle=-45, hjust=.1))
#With p-value
ggplot(data=het, aes(pop, He, colour=pop)) +
  geom_boxplot() + geom_jitter(alpha=0.3) +geom_hline(yintercept = mean(het$He), linetype = 2)  + stat_compare_means(comparisons = my_comparisons) + stat_compare_means(method = "anova", label.y = 2.0) +
  labs(title = "He distribution per population") +
  xlab("population") +
  guides(col=FALSE)+
  ylab("He")
#|-----------------------------------|-------------------------------|----------------------------------|#
## Fws
head(allforpopfws)
funSubsetAlleleCount <- function(x) {
  subset.int <-subset(allforpopfws, barcode==x)
  countlocus <- dplyr::count(subset.int, locus)
  barcode <- rep(x,nrow(countlocus))
  output.df<-cbind(countlocus, barcode)
  #return(fun.data.frame)
  return(output.df)
}
barcodelist<-c(unique(allforpopfws$barcode))
allele.count<- lapply(barcodelist, FUN=funSubsetAlleleCount)
AlleleCount.table <-do.call(rbind, allele.count)
addrows1 <-expand.grid(id = unique(AlleleCount.table$barcode),
                       locus = unique(AlleleCount.table$locus))
names(addrows1)
allelecount.loci<-merge(addrows1, AlleleCount.table, by.x=c("id","locus"), by.y=c("barcode","locus"), all=FALSE)
# Dataframe where row = barcodes and col = allele count per loci
allelecount<-reshape(allelecount.loci, dir="wide", idvar="id", timevar="locus")
names(allelecount)<-gsub("freq.", "", names(allelecount), fixed=TRUE) # Remove "freq." from all locus markers
names(allelecount)[names(allelecount)=="id"]<-"barcode"
allelecount[is.na(allelecount)]<-0
names (allelecount)
```

```
## Sum of number of alleles present at each locus, disregarding if they are unique or not.
sum.numalleles.perloci<- as.data.frame(apply(allelecount[,2:27], 2, sum, na.rm=TRUE))
names(sum.numalleles.perloci)[names(sum.numalleles.perloci)=="apply(allelecount[, 2:27], 2, sum, na.rm = TRUE)"]<-"allele.sum"
library(data.table)
setDT(sum.numalleles.perloci, keep.rownames = TRUE)[]
names(sum.numalleles.perloci)[names(sum.numalleles.perloci)=="rn"]<-"locus"
allforpopfws.sort <- allforpopfws[order(allforpopfws$locus,allforpopfws$allele), ]
allelefreq$locus<-allelefreq$locusid
allelefreq.sort<-allelefreq[order(allelefreq$locus, allelefreq$allele), ]
allforpopfws.allele.freq<-merge(allforpopfws.sort, allelefreq.sort, by=c("locus", "allele"), all=FALSE)
allforpopfws.allele.freq$locusid<-NULL
allforpopfws.allele.freq$allelefreq2<-(allforpopfws.allele.freq$allelefreq)^2
fws <- matrix(0, nrow = length(barcodelist), ncol = length(loci.list))
rownames(fws) <- barcodelist
colnames(fws) <- loci.list
for(i in 1:(length(barcodelist))) {
  barcode <- barcodelist[i]
  for (j in 1:(length(loci.list))){
    locus <- loci.list[j]
    Hs<-1-sum(allforpopfws.allele.freq$allelefreq2[allforpopfws.allele.freq$barcode==barcode & allforpopfws.allele.freq$locus==locus])
    Hw<-1-(length(allforpopfws.allele.freq$allele[allforpopfws.allele.freq$barcode==barcode & allforpopfws.allele.freq$locus==locus]))*(1/length(allforpopfws.allele.freq$allele[allforpopfws.allele.freq$barcode==barcode & allforpopfws.allele.freq$locus==locus])^2)
    fws[i,j]<-1-Hw/Hs
  }
}
## fws[i,j] will not work if the rows or columns are not in the same order as the matrix.
## fws[barcode, locus] = will find that specific barcode row and locus column and stick that calculation into that cell.
Fws.barcode<-as.data.frame(fws)
#Fws.barcode[is.na(Fws.barcode)]<-1 ## For all missing alleles at loci, Fws = 1
setDT(Fws.barcode, keep.rownames = TRUE)[]
names(Fws.barcode)[names(Fws.barcode)=="rn"]<-"barcode"
Fws.barcode<-as.data.frame(Fws.barcode)
Fws.barcode$mean.Fws<- rowMeans(Fws.barcode[,2:24], na.rm=TRUE)
Fws.barcode = Fws.barcode[, c(1, 28)]
```

```
head(Fws.barcode)
summary(Fws.barcode$mean.Fws)
#|-----------------------------------|-------------------------------|----------------------------------|#
# merge with popualtion and determine the mean fws, calcualte p-value
Fws.pop <- merge(Fws.barcode,sample_moi, by="barcode")
plot(Fws.pop$mean.Fws, Fws.pop$moi)
#Order HF
Fws.pop$healthfacility<- factor(Fws.pop$healthfacility, levels = c("Choi C", "Sibbinda HC", "Chinchimani C", "Kanono C", "Kasheshe C", "Sesheke C"))
#Presentation of data
ggplot(data=Fws.pop, aes(healthfacility, mean.Fws, colour=healthfacility)) +
  geom_boxplot() + geom_jitter(alpha = 0.3) +
  labs(title = "Fws distribution per healthfacility") +
  xlab("Health facility") +
  ylab("Fws")
#Create data for LD
ld_df<-read.csv("MERFAT_all_27102017_Dominant_Peaks.csv")
ld_df<-merge(ld_df, HF_data[,c(1,3)], by = "barcode")
write.csv(ld_df, "LD_p3_main.csv")
#Allelic richness
unique_alleles<-read.csv("Unique_alleles_p3.csv")
unique_alleles<-as.data.frame(table(unique_alleles$locusid))
colnames(unique_alleles)[colnames(unique_alleles) == "Var1"] <- "Loci"
colnames(unique_alleles)[colnames(unique_alleles) == "Freq"] <- "count"
ggplot(data=unique_alleles, aes(x=loci, y=count, fill = loci)) +
  geom_bar(stat="identity") + guides(fill=FALSE) +
  theme(axis.text.x = element_text(angle=-45, hjust=.1)) +
  labs(title = "Allelic richness") + xlab("Loci") + ylab("Unique allele count")
```

9.2 Appendix B: Data analysis Chapter 4

```
#set working directory
setwd("~/Dropbox/Munya.Genotyping/Script from ST/Data")
#Read csv files
all_alleles_p2<-read.csv("Kavango_all_alleles.csv")
#MOI calculation
library(tidyverse)
library(ggplot2)
library(dplyr)
library(ggpubr)
## MOI
all_alleles <- read.csv('Kavango_all_alleles.csv', as.is=T, row.names = 1) # The column name for sample
ID should be namesd as "Sample"
head(all_alleles)
samples <- dplyr::filter(all_alleles, !grepl(pattern = 'NTC|POS', x = barcode))
negatives <- dplyr::filter(all_alleles, grepl(pattern = 'NTC', x = barcode))
positives <- dplyr::filter(all_alleles, grepl(pattern = 'POS', x = barcode))
loci <- colnames(samples)[-c(1)]
sample_alleles = data.frame(barcode=c(), Allele=c(), Locus=c())
for(i in 1:length(loci)) {
  locus = loci[i]
  res <- cbind(tidyr::separate_rows_(samples, `locus`)[, c('barcode', `locus`)], c(`locus`))
  colnames(res) <- c('barcode', 'Allele', 'Locus')
  res <- dplyr::filter(res, Allele != "")
  sample_alleles <- rbind(sample_alleles, res)
}
by_sample <- dplyr::group_by(sample_alleles, barcode)
by_locus <- dplyr::group_by(sample_alleles, Locus)
head(by_sample)
moi_offset = 2 # takes the second highest
sample_moi <- dplyr::summarise(by_sample[complete.cases(by_sample$Allele),],
                        moi = sort(table(Locus), decreasing = T)[moi_offset])
#merge MOI result with health facility, residence, travel and age data
Age_res_data<-read.csv("KavangoTS04092018.csv",as.is=T, row.names = 1)
sample_moi<-merge(sample_moi, Age_res_data, by = "barcode")
colnames(sample_moi)[8]<- "healthfacility"
```

```
sample_moi<-sample_moi[,-c(3, 5, 7, 9, 10)]
colnames(sample_moi)[9]<- "Travel_history"
write.csv(sample_moi, "sample_moi_p2.csv")
colnames(Age_res_data)[13]<- "Travel_history"
# Distribution of cases by age
diag1_p2<-ggplot(Age_res_data, aes(x=Age, fill = Travel_history))+ geom_bar()+ stat_bin(binwidth = 1)
+ stat_bin(breaks = seq(0, 80, by = 1)) + ggtitle(ylab("count")) + ylab("count") + ggtitle("Malaria case
distribution by age and travel history")
# Distribution of cases by Age andPrimary residence
diag2_p2<-ggplot(Age_res_data, aes(x=PrimaryResidence, fill = PrimaryResidence))+ geom_bar() +
stat_count() + ggtitle(ylab("count")) + ylab("count") + ggtitle("Malaria case distribution by Primary
residence") + theme(axis.text.x = element_text(angle=-90, hjust=.1))
library(gridExtra)
grid.arrange(diag1_p2, diag2_p2, nrow = 2)
#presentation of MOI by primary residence
diag3_p2<-ggplot(data= sample_moi, aes(PrimaryResidence, sample_moi$moi,
colour=PrimaryResidence)) +
          geom_boxplot() + geom_jitter(alpha = 0.3) + geom_hline(yintercept =
        mean(sample_moi$moi), linetype = 2)+
          stat_compare_means(method = "anova", label.y = 10.2)+ ggtitle("MOI distribution by primary
        residence")+ xlab("Primary residence")+ ylab("MOI") + theme(axis.text.x =
        element_text(angle=90, hjust=.1))
        #presentation of MOI by age
        library(ggpubr)
        diag4_p2<-ggplot(data= sample_moi, aes(Age, sample_moi$moi, colour=Age)) +
          geom_count() + ggtitle("MOI distribution by Age")+ xlab("Age")+ ylab("MOI") +
        theme(axis.text.x = element_text(angle=90, hjust=.1))
        #presentation of MOI by travel
        diag5_p2<-ggplot(data= sample_moi, aes(Travel_history, sample_moi$moi, fill =
        Travel_history), colours(distinct = TRUE)) +
          geom_boxplot() + geom_jitter(alpha = 0.2) + geom_hline(yintercept =
        mean(sample_moi$moi), linetype = 2)+
          stat_compare_means(method = "t.test", label.y = 10.2)+
          stat_compare_means(label = "p.signif", method = "t.test",
                    ref.group = ".all.") + labs(title = "MOI distribution by Travel") +
          xlab("Age") +
          guides(col=FALSE)+
          ylab("MOI")
```

```
#He distribution
all_alleles <- read.csv("Kavango_all_alleles.csv", as.is=T, row.names = 1) # The column name
for sample ID should be namesd as "Sample"
head(all_alleles)
all_alleles<-merge(sample_moi, all_alleles, by = "barcode")
colnames(all_alleles)[9]<- "travel"
cleaned = all_alleles[,c(1,9, 10:35)] # id, pop, and alleles
names (cleaned)
cleaned = dplyr::rename(cleaned, pop = travel) # rename healthfacility to travel
loci <- colnames(cleaned)
loci <- loci[loci != 'barcode' & loci != 'pop']
loci # lsit of loci
long_form_results <- data.frame("barcode"=integer(0), "locus"=character(0), "allele"=integer(0),
"population"=character(0));
for(i in 1:(dim(cleaned)[1])) {
  entry = cleaned[i,]
  sample_id = entry["barcode"]
  population = as.character(entry["pop"]$pop)
  print(sample_id[1,]);
  print(i)
  for(j in 1:(length(loci))) {
    locus = loci[j];
    alleles_list = entry[locus];
    #print(alleles_list);
    alleles <- unlist(strsplit(gsub(" ", "", unlist(alleles_list)), ";"));
    for(k in 1:(length(alleles))) {
      allele <- as.integer(alleles[k]);
      if(length(allele) > 0) {
        long_form_results <- rbind(long_form_results, data.frame("barcode" = sample_id, "locus"
= locus, "allele" = allele, "population" = population));
      }
    }
  }
}
dim(long_form_results)#13927
head(long_form_results)
is.na (long_form_results)
long_form_results<- na.omit(long_form_results)# removes NA
```

```
dim(long_form_results) # 13259
str(long_form_results)#104534 including NA
#convert allele to numeric
hist (long_form_results$allele)##Check if there are zeros
#rename to allforpopfws
allforpopfws  <-  long_form_results[complete.cases(long_form_results),]
#Omit if samples have less than 10 number of successful loci ----#
# Number of successful genotyping per unique barcode - will exclude if >10 missing
SuccessCountPerSample <- function(x){
  SuccessCount<-length(unique(allforpopfws$locus[allforpopfws$barcode==x]))
  return(SuccessCount)
}
barcodelist<-c(unique(allforpopfws$barcode)) # List of unique barcodes
gtsuccesscount.barcode<-lapply(barcodelist, FUN=SuccessCountPerSample)
gtsuccesscount.barcode<-cbind(barcodelist, gtsuccesscount.barcode)
gtsuccesscount.barcode<-as.data.frame(gtsuccesscount.barcode) # row = 230 barcodes, col =
barcode, successful genotype count
gtsuccesscount.barcode$num.locipresent <- gtsuccesscount.barcode$gtsuccesscount.barcode
gtsuccesscount.barcode$gtsuccesscount.barcode<- NULL
gtsuccesscount.barcode <- as.data.frame(lapply(gtsuccesscount.barcode, unlist))
gtsuccesscount.barcode$num.locimissing <- 26 - gtsuccesscount.barcode$num.locipresent
head(gtsuccesscount.barcode)
names(gtsuccesscount.barcode)[names(gtsuccesscount.barcode)=="barcodelist"]<-"barcode"
hist(gtsuccesscount.barcode$num.locimissing) # Histogram shows that only a small number of
abline(v=11, col="red") # not substed as I already remove based on the home MS
allforpopfws.locimissing<-(merge(gtsuccesscount.barcode, allforpopfws, by="barcode"))
allforpopfws<-as.data.frame(allforpopfws.locimissing)
allforpopfws <-subset(allforpopfws, num.locimissing<=11)
head(allforpopfws)
#-------------------------------------------------------------------------#
# Calculate heterozygosity
library(dplyr)
str(allforpopfws)
pop.list <- as.character(unique(allforpopfws$population))
pop.stats <- list()
for(i in 1:length(pop.list)){
  pop <- pop.list[i]
  pop.data <- list()
```

```
 allforpop.sub <- allforpopfws[allforpopfws$population==pop,]
 call.rate.perloci <- function(x) {
  #missing<-1-length(unique(gtdb$barcode[x]))/length(unique(gtdb$barcode))
  call.rate<-
length(unique(allforpop.sub$barcode[allforpop.sub$locus==x]))/length(unique(allforpop.sub$ba
rcode))
  return(call.rate)
 }
 numofcalls.perloci <- function(x){
  numberofsuccess<-length(unique(allforpop.sub$barcode[allforpop.sub$locus==x]))
  return(numberofsuccess)
 }
 loci.list <- as.character(unique(allforpop.sub$locus))
 call.rate <- lapply(loci.list, FUN=call.rate.perloci)
 loci.call.rate <- cbind(loci.list, call.rate)
 call.perloci <- lapply(loci.list, FUN=numofcalls.perloci)
 loci.call.rate <- cbind(loci.list, call.rate)
 call.perloci <- lapply(loci.list, FUN=numofcalls.perloci)
 numofcall.perloci<- cbind(loci.list, call.perloci)
 loci.callrate <- as.data.frame(cbind(loci.list, call.rate, call.perloci))
 loci.callrate <- as.data.frame(lapply(loci.callrate, unlist))
 loci.callrate<-loci.callrate[order(loci.callrate$call.rate),]
 loci.callrate$row.names<-NULL
 names(loci.callrate)[names(loci.callrate)=="loci.list"]<-"locus"
 pop.data$loci.callrate <- loci.callrate
 funSubsetCount <- function(x) {
  subset.int <-subset(allforpop.sub, locus==x)
  myfreq <- as.data.frame(table(subset.int$allele))
  names(myfreq) <- c("allele", "Freq")
  locusid <- rep(x,nrow(myfreq))
  output.df<-cbind(myfreq, locusid)
  #return(fun.data.frame)
  return(output.df)
 }
 ?count
 loci.allele <- lapply(loci.list, FUN=funSubsetCount)
 lociallele <-do.call(rbind, loci.allele) # Table of all allele types present in pop.
 lociallele <- as.data.frame (lociallele)
```

```
  funallelefreq <- function(x){    # Function to determine sum of all samples that were successful
at specific loci
    alleledenominator<-sum(lociallele$Freq[lociallele$locusid==x])
    return(alleledenominator)
  }
  sum.allelesperloci<-lapply(loci.list, FUN=funallelefreq)
  allelefreq.denom <-cbind(loci.list, sum.allelesperloci) # col of 27 markers, each row contains
how many samples were genotyped at each loci
  allelefreq<-merge(lociallele, allelefreq.denom, by.x="locusid", by.y="loci.list", all=FALSE)
  allelefreq$sum.allelesperloci<-as.numeric(allelefreq$sum.allelesperloci)
  allelefreq$allelefreq<-(allelefreq$Freq)/(allelefreq$sum.allelesperloci) # Calculate allele
frequency at each ith allele
  funHe <- function(x){
    He <- ((length(unique(allforpopfws$barcode)))/(((length(unique(allforpopfws$barcode)))-
1))*(1-sum((allelefreq$allelefreq[allelefreq$locusid==x]^2))))
    return(He)
  }
  He.loci.list <- lapply(loci.list, FUN=funHe)
  He.perloci<- cbind(loci.list, sum.allelesperloci, He.loci.list)
  He.perloci<- as.data.frame(He.perloci)
  allelecount <- as.data.frame(table(allelefreq$locusid))
  He.perloci<-cbind(He.perloci, allelecount)
  He.perloci$x<-NULL
  names(He.perloci)[names(He.perloci)=="He.loci.list"]<- "He"
  names(He.perloci)[names(He.perloci)=="sum.allelesperloci"]<- "TotalNo.Samples"
  names(He.perloci)[names(He.perloci)=="Freq"]<- "Freq.Uniquealleles"
  names(He.perloci)[names(He.perloci)=="loci.list"]<- "Loci"
  He.perloci$He<-as.numeric(He.perloci$He)
  He.perloci$TotalNo.Samples<-as.numeric(He.perloci$TotalNo.Samples)
  He.perloci$Loci<-as.character(He.perloci$Loci)
  pop.data$He.perloci <- He.perloci
  pop.data$allele.freq <- allelefreq
  pop.stats[[pop]] <- pop.data
}
head(pop.stats)
# summary of HE
Angola_South  = pop.stats$`Angola South`$He.perloci[, -4]
Angola_South$pop = "Angola_South"
```

```
head(Angola_South)
No_travel  = pop.stats$`No travel`$He.perloci[, -4]
No_travel$pop = "No_travel"
head(No_travel)
# combine all data
all.he <- rbind(Angola_South, No_travel)
head(all.he)
# presentation of He by travel
diag7_p2<-ggplot(data= all.he, aes(pop, He, colour=pop)) +
  geom_boxplot() + geom_jitter(alpha=0.3)+ geom_hline(yintercept = mean(all.he$He), linetype
= 2)+
  stat_compare_means(method = "t.test")+
  labs(title = "He distribution by travel") +
  xlab("population") +
  guides(col=FALSE)+
  ylab("He")
#Fws
head(allforpopfws)
funSubsetAlleleCount <- function(x) {
  subset.int <-subset(allforpopfws, barcode==x)
  countlocus <- dplyr::count(subset.int, locus)
  barcode <- rep(x,nrow(countlocus))
  output.df<-cbind(countlocus, barcode)
  #return(fun.data.frame)
  return(output.df)
}
barcodelist<-c(unique(allforpopfws$barcode))
allele.count<- lapply(barcodelist, FUN=funSubsetAlleleCount)
AlleleCount.table <-do.call(rbind, allele.count)
addrows1 <-expand.grid(id = unique(AlleleCount.table$barcode),
                       locus = unique(AlleleCount.table$locus))
names(addrows1)
allelecount.loci<-merge(addrows1, AlleleCount.table, by.x=c("id","locus"),
by.y=c("barcode","locus"), all=FALSE)
# Dataframe where row = barcodes and col = allele count per loci
allelecount<-reshape(allelecount.loci, dir="wide", idvar="id", timevar="locus")
names(allelecount)<-gsub("freq.", "", names(allelecount), fixed=TRUE) # Remove "freq." from
all locus markers
```

```
names(allelecount)[names(allelecount)=="id"]<-"barcode"
allelecount[is.na(allelecount)]<-0
names (allelecount)
## Sum of number of alleles present at each locus, disregarding if they are unique or not.
sum.numalleles.perloci<- as.data.frame(apply(allelecount[,2:27], 2, sum, na.rm=TRUE))
names(sum.numalleles.perloci)[names(sum.numalleles.perloci)=="apply(allelecount[, 2:27], 2,
sum, na.rm = TRUE)"]<-"allele.sum"
library(data.table)
setDT(sum.numalleles.perloci, keep.rownames = TRUE)[]
names(sum.numalleles.perloci)[names(sum.numalleles.perloci)=="rn"]<-"locus"
allforpopfws.sort <- allforpopfws[order(allforpopfws$locus,allforpopfws$allele), ]
allelefreq$locus<-allelefreq$locusid
allelefreq.sort<-allelefreq[order(allelefreq$locus, allelefreq$allele), ]
allforpopfws.allele.freq<-merge(allforpopfws.sort, allelefreq.sort, by=c("locus", "allele"),
all=FALSE)
allforpopfws.allele.freq$locusid<-NULL
allforpopfws.allele.freq$allelefreq2<-(allforpopfws.allele.freq$allelefreq)^2
fws <- matrix(0, nrow = length(barcodelist), ncol = length(loci.list))
rownames(fws) <- barcodelist
colnames(fws) <- loci.list
for(i in 1:(length(barcodelist))) {
  barcode <- barcodelist[i]
  for (j in 1:(length(loci.list))){
    locus <- loci.list[j]
    Hs<-1-sum(allforpopfws.allele.freq$allelefreq2[allforpopfws.allele.freq$barcode==barcode &
allforpopfws.allele.freq$locus==locus])
    Hw<-1-(length(allforpopfws.allele.freq$allele[allforpopfws.allele.freq$barcode==barcode &
allforpopfws.allele.freq$locus==locus]))*(1/length(allforpopfws.allele.freq$allele[allforpopfws.a
llele.freq$barcode==barcode & allforpopfws.allele.freq$locus==locus])^2)
    fws[i,j]<-1-Hw/Hs
  }
}
## fws[i,j] will not work if the rows or columns are not in the same order as the matrix.
## fws[barcode, locus] = will find that specific barcode row and locus column and stick that
calculation into that cell.
Fws.barcode<-as.data.frame(fws)
#Fws.barcode[is.na(Fws.barcode)]<-1 ## For all missing alleles at loci, Fws = 1
setDT(Fws.barcode, keep.rownames = TRUE)[]
```

```
names(Fws.barcode)[names(Fws.barcode)=="rn"]<-"barcode"
Fws.barcode<-as.data.frame(Fws.barcode)
Fws.barcode$mean.Fws<- rowMeans(Fws.barcode[,2:24], na.rm=TRUE)
Fws.barcode = Fws.barcode[, c(1, 28)]
head(Fws.barcode)
summary(Fws.barcode$mean.Fws)
#|-----------------------------------|-------------------------------|---------------------------------|#
# merge with popualtion and determine the mean fws, calcualte p-value
Fws.pop <- merge(Fws.barcode,sample_moi, by="barcode")
plot(Fws.pop$mean.Fws, Fws.pop$moi)
#Presentation of data
diag8_p2<-ggplot(data=Fws.pop, aes(Travel_history, mean.Fws, colour= Travel_history)) +
  geom_boxplot() + geom_jitter(alpha= 0.2) +geom_hline(yintercept =
mean(Fws.pop$mean.Fws), linetype = 2)+
  stat_compare_means(method = "t.test")+
  labs(title = "Fws distribution by travel history") +
  xlab("population") +
  guides(col=FALSE)+
  ylab("Fws")
# fws with the age data
library(gridExtra)
library(ggpubr)
Fws.pop$Age = as.numeric(as.character(Fws.pop$Age))
Fws.pop$age.grp = cut(Fws.pop$Age, c(0,5,10,15,40,80))
diag9_p2<-ggplot(data = Fws.pop, aes(Age,moi,colour= Travel_history ))+
  geom_point()+geom_smooth()+ ggtitle("MOI distribution by age and travel history")
diag10_p2<-ggplot(data = Fws.pop, aes(Age,mean.Fws, colour= Travel_history))+
    geom_point()+geom_smooth() +ggtitle("Mean Fws distribution by age and travel history")
diag11_p2<-ggplot(data = Fws.pop, aes(PrimaryResidence, Fws.pop$mean.Fws, colour=
PrimaryResidence))+ geom_boxplot() + geom_jitter(alpha = 0.3) + ggtitle("Mean Fws
distribution by primary residence")+ ylab("Fws") + theme(axis.text.x = element_text(angle=270,
hjust=.1))
grid.arrange(diag9_p2,diag10_p2, nrow = 2)
grid.arrange(diag3_p2, diag11_p2, nrow = 2)
Fws.pop %>% group_by(age.grp) %>% dplyr::summarise(mean(moi))
Fws.pop %>% group_by(age.grp) %>% dplyr::summarise(mean(mean.Fws))
### transmission dynamics by epi data
setwd("/Users/gp-admin/Dropbox/Munya.Genotyping/Script from ST/Data")
```

```
library(tidyverse)
library(dplyr)
library(gridExtra)
library(ggplot2)
library(reshape2)
age_res_data<-read.csv("GeneticEpiCombined11082018.csv",as.is=T)
age_res_data<-age_res_data[,-c(1:2)]
###
# create travel data
names(age_res_data)
age_res_data$angola.travelled = ifelse(rowSums(age_res_data[, 7:12]) > 0, 1, 0)
#age_res_data$other.country.travelled = ifelse(rowSums(age_res_data[, 13:16]) > 0, 1, 0)
age_res_data$namibia.travelled = ifelse(rowSums(age_res_data[, 17:38]) > 0, 1, 0)
age_res_data$namibia.outsidestudysite = ifelse(rowSums(age_res_data[, 17:31]) > 0, 1, 0)
age_res_data$namibia.studysite = ifelse(rowSums(age_res_data[, 32:38]) > 0, 1, 0)
table(age_res_data$namibia.outsidestudysite)
# merge with location info
loc = read.csv("Location_clinics_updated.csv")
age_res_data = merge(age_res_data, loc, by.x = "RDTHealthFacility", by.y="Facility.name",
all.x=T)
head(age_res_data)
#age distribution
library(reshape2)
names(age_res_data)
age_res_data_DF <- melt(age_res_data, id.vars = c("RDTBarcode", "RDTHealthFacility",
"PrimaryResidence","Age", "RDTDate", "Admin2"), measure.vars = c("angola.travelled",
"namibia.outsidestudysite", "namibia.studysite"))
head(age_res_data_DF)
ggplot(data =age_res_data_DF , aes(x= variable, y = as.numeric(Age), col=factor(value)))+
  geom_boxplot() + ggtitle("Travel history age distribution by destiniation") +
xlab("Destination") + ylab("Age") +
  facet_wrap(~variable, scales = "free")+
  theme(axis.text.x  = element_text(angle=60, vjust=0.95, size=9,hjust=0.95))
ggplot(data =age_res_data_DF , aes(x= variable, y = as.numeric(Age), col=factor(value)))+
  geom_boxplot() + ggtitle("Travellers age distribution by destiniation") + xlab("Destination") +
ylab("Age") +
  facet_wrap(~Admin2, scales = "free")+
  theme(axis.text.x  = element_text(angle=60, vjust=0.95, size=9,hjust=0.95))
```

```
age_res_data_DFc = age_res_data_DF[complete.cases(age_res_data_DF$Age),]
age_res_data_DFc = age_res_data_DFc %>% filter(variable=="namibia.outsidestudysite")
pairwise.t.test(as.numeric(age_res_data_DFc$Age), as.factor(age_res_data_DFc$value),
p.adjust.method = "none")
age_res_data_DFc$age.grp5 = ifelse(age_res_data_DFc$Age < 5, 1, 0)
age_res_data_DFc %>% group_by(age.grp5) %>% summarise(sum=sum(value), n = n()) %>%
mutate(prop=sum/n)
##############################################
#summarised data
df = read.csv("KavangoTS04092018.csv")
loc = read.csv("Location_clinics_updated.csv")
df = merge(df, loc, by.x = "RDTHealthFacility", by.y="Facility.name", all.x=T)
head(df)
df$angola.travelled = ifelse(is.na(df$ResidDest), 0, 1)
table(as.factor(df$angola.travelled), exclude = NULL)
df$age.grp = ifelse(df$Age < 5, "<5",
                 ifelse(df$Age >=5&df$Age <=15, "5-15",
                    ifelse(df$Age >=16, ">16",NA)))
#Alternative age grps
#df$age.grp = ifelse(df$Age < 5, "<5",
        ifelse(df$Age >=5&df$Age <=10, "5-10",
                ifelse(df$Age >=11&df$Age <=15, "11-15",
                    ifelse(df$Age >=16&df$Age <=25, "16-25",
                        ifelse(df$Age>=26&df$Age<=40, "25-40",
                            ifelse(df$Age >=40, ">40", NA)))))
table(df$age.grp)
df$age.grp <- factor(df$age.grp, levels = c("<5", "5-15", ">16"))
names(df)
df$n = 1
df %>% group_by(age.grp, angola.travelled) %>% dplyr::summarise(n = n()) %>%
mutate(prop=sum/n)
# filter travelled
df.travelled  = df %>%  filter(NewDestination != "<NA>")
hist(df.travelled$Age, breaks =25)
table(df.travelled$age.grp)
df.travelled$grp = "Angola travellers"
# filter residence
df.resid  = df %>%  filter(NewResidence != "<NA>")
```

```
hist(df.resid$Age, breaks =25)
table(df.resid$age.grp)
df.resid$grp = "Angola residents"
# filter local
df.local  = df %>%  filter(is.na(ResidDest) ==TRUE)
hist(df.local$Age, breaks =25)
table(df.local$age.grp)
df.local$grp = "Namibia local"
# combine
df.comb = rbind(df.resid, df.travelled, df.local)
df.comb$age.grp = ifelse(df.comb$Age < 5, "<5",
                  ifelse(df.comb$Age >=5&df.comb$Age <=15, "5-15",
                         ifelse(df.comb$Age > 15, ">15", NA)))
df.comb$age.grp2 = ifelse(df.comb$Age < 5, "<5",
                       ifelse(df.comb$Age >=5&df.comb$Age <=14, "5-14",
                              ifelse(df.comb$Age >=15&df.comb$Age <=29, "15-29",
                                     ifelse(df.comb$Age >= 30&df.comb$Age <=44, "30-44",
                                            ifelse(df.comb$Age >= 45&df.comb$Age <=59, "45-59",
                                                   ifelse(df.comb$Age >=60, ">=60", NA))))))
table(df.comb$age.grp2)
sumAge = df.comb %>%
  dplyr::group_by(age.grp2, grp) %>%
  dplyr::summarise(n = n()) %>%
  dplyr::group_by(grp) %>%
  dplyr::mutate(prop= n/sum(n))
sumAge = sumAge[complete.cases(sumAge$age.grp2),]
#Use observed sample proportions to test hypothesis about unobserved pop proportions
table(sumAge$age.grp2)
# for <5 age group
prop.test(x=c(36, 431), n=c(1649, 3626))
# for 5-14 age group
prop.test(x=c(66, 1562), n=c(165, 3626), correct = F)
# for 15-29 age groups
prop.test(x=c(39, 869), n=c(165,3626))
# for 30-44 age groups
prop.test(x=c(11, 340), n=c(165,3626))
# for 45-59 age groups
prop.test(x=c(6, 144), n=c(165,3626))
```

```
sumAge$age.grp2 <- factor(sumAge$age.grp2, levels = c("<5", "5-14", "15-29", "30-44", "45-
59", ">=60"))
#plot
## plot theme
my_theme <- theme_classic() +
  theme(legend.title=element_blank())+
  theme(plot.background = element_blank(), panel.grid.major = element_blank(),
panel.grid.minor = element_blank() )+
  theme(axis.line.x = element_line(color="black", size = 0.3),axis.line.y =
element_line(color="black", size = 0.3))+
  theme(text=element_text(size=14))
ggplot(sumAge %>%  filter(grp != "Angola travellers"), aes(age.grp2, prop, fill=grp)) +
  geom_bar(stat = "identity", position ="dodge", alpha=0.5) +
  labs(x = "Age group", y = "Proportion", title = "") +
  scale_fill_manual(values = c("red", "darkolivegreen3", "blue"))+
  my_theme
#histogram plot
ggplot(df.comb %>%  filter(grp != "Angola travellers"), aes(Age, fill=grp)) +
  geom_histogram(aes(Age, ..density..), binwidth = 1, alpha=0.5)+
  scale_y_continuous(expand = c(0.01,0))+
  scale_x_continuous(expand = c(0.01,0))+
  #geom_vline(xintercept=c(10, 12, 62.5), color =c("red", "blue", "grey"), lwd=0.5, lty=4)+
  labs(x = "Age", y = "Proportion", title = "")+
  scale_fill_manual(values = c("red", "darkolivegreen3", "blue"))+
  my_theme
# Age group risk by travel
df.comb.risk = df.comb %>%  filter(grp != "Angola residents")
# odds ratio
df.comb.risk$age.grp2 = as.factor(df.comb.risk$age.grp2)
df.comb.risk$Admin2 <- relevel(df.comb.risk$Admin2, ref="Andara")
df.comb.risk$age.grp2 <- factor(df.comb.risk$age.grp2, levels = c("<5", "5-14", "15-29", "30-
44", "45-59", ">=60"))
df.comb.risk$angola.travelled = ifelse(df.comb.risk$grp == "Namibia local", 0,1)
df.comb.risk$age.grp2 <- relevel(df.comb.risk$age.grp2, ref="<5")
# admin
summary(m1 <- glm(angola.travelled ~ Admin2, family=binomial, data = df.comb.risk))
est <- cbind(estimate = coef(m1), confint(m1)) %>% as.data.frame()
est
```

```
exp(est)
table(df.comb.risk$Admin2, df.comb.risk$grp)
# Age grp
names(df.comb.risk)
summary(m1 <- glm(angola.travelled ~ age.grp2, family=binomial, data = df.comb.risk))
est <- cbind(estimate = coef(m1), confint(m1)) %>% as.data.frame()
est
exp(est)
#Adjusted by district residence
names(df.comb.risk)
summary(m1 <- glm(angola.travelled ~ age.grp2 + Admin2, family=binomial, data =
df.comb.risk))
est <- cbind(estimate = coef(m1), confint(m1)) %>% as.data.frame()
est
exp(est)
table(df.comb.risk$Admin2, df.comb.risk$age.grp2)
table(df.comb.risk$age.grp2, df.comb.risk$grp)
################################################################################
#################
# add MOI
names(df.comb)
sample_moi<-read.csv("sample_moi_p2.csv")
#df.comb.moi = merge(df.comb, sample_moi, by="barcode", all.y=T) %>% filter(grp=="local")
df.comb.moi = merge(df.comb, sample_moi, by="barcode", all.y=T)
table(df.comb.moi$grp)

write.csv(Fws.barcode, "Fws.barcode_p2.csv")
ggplot(df.comb.moi %>% filter(grp != "Angola travellers"), aes(grp, moi, fill=grp)) +
  geom_boxplot(alpha=0.5) +
  labs(x = "Residence", y = "MOI", title = "") +
  scale_fill_manual(values = c("red", "darkolivegreen3", "blue"))+
  my_theme
df.comb.moi<-merge(df.comb.moi, Fws.barcode, by = "barcode")
ggplot(df.comb.moi %>%  filter(grp != "Angola travellers"), aes(grp, mean.Fws, fill=grp)) +
  geom_boxplot(alpha=0.5) +
  labs(x = "Residence", y = "Fws", title = "") +
  scale_fill_manual(values = c("red", "darkolivegreen3", "blue"))+
  my_theme
```

```
# use median for namibian residents
df.comb.moi$age.grp2 <- factor(df.comb.moi$age.grp2, levels = c("<5", "5-14", "15-29", "30-
44", "45-59", ">=60"))
df.comb.moi %>%
  filter(grp == "Namibia local")%>%
  group_by(age.grp2) %>%
  dplyr::summarise(median = median((moi), na.rm = TRUE),
                   sd = sd((moi), na.rm = TRUE),
                   `50%`=quantile(moi, probs=0.5),
                   `25%`=quantile(moi, probs=0.25),
                   `75%`=quantile(moi, probs=0.75),n=n()) %>%
  dplyr:: mutate(se = sd/sqrt(n),
                 lower.ci = median - qt(1 - (0.05 / 2), n - 1) * se,
                 upper.ci = median + qt(1 - (0.05 / 2), n - 1) * se)
n_namres<-filter(df.comb.moi, grp == "Namibia local")
table(n_namres$age.grp2)
# use median for Angolan residents
df.comb.moi$age.grp2 <- factor(df.comb.moi$age.grp2, levels = c("<5", "5-14", "15-29", "30-
44", "45-59", ">=60"))
df.comb.moi %>%
  filter(grp == "Angola residents") %>%
  group_by(age.grp2) %>%
  dplyr::summarise(median = median((moi), na.rm = TRUE),
                   sd = sd((moi), na.rm = TRUE),
                   `50%`=quantile(moi, probs=0.5),
                   `25%`=quantile(moi, probs=0.25),
                   `75%`=quantile(moi, probs=0.75),n=n()) %>%
  dplyr:: mutate(se = sd/sqrt(n),
                 lower.ci = median - qt(1 - (0.05 / 2), n - 1) * se,
                 upper.ci = median + qt(1 - (0.05 / 2), n - 1) * se)
n_namres<-filter(df.comb.moi, grp == "Angola residents")
table(n_namres$age.grp2)
################################################################################
#################
################################################
# by health facility local
local.df = df.comb %>% filter(grp =="Namibia local")
local.df = local.df[local.df$RDTHealthFacility != "Takawasa C",]
```

```
table(local.df$age.grp )
table(local.df$RDTHealthFacility)
# MOI for 3 age groups <5, 5-15 and >15years
local.df$age.grp = ifelse(df.comb$Age < 5, "<5",
                    ifelse(df.comb$Age >=5&df.comb$Age <=15, "5-15",
                      ifelse(df.comb$Age > 15, ">15", NA)))
local.df = local.df[complete.cases(local.df$age.grp),]
sumAge2 = local.df %>%
  group_by(RDTHealthFacility, Age) %>%
  dplyr::summarise(n = n()) %>% mutate(prop= n/sum(n))
local.df$clinic <- reorder(local.df$RDTHealthFacility, local.df$Long)
median.Age = local.df %>%  group_by(RDTHealthFacility) %>% summarise(med.age =
median(Age, na.rm = T))

#MOI for 3 age grps <5, 5-15 and>15years
sumAge2 = local.df %>%
  group_by(RDTHealthFacility, age.grp) %>%
  dplyr::summarise(n = n()) %>% mutate(prop= n/sum(n))
local.df$clinic <- reorder(local.df$RDTHealthFacility, local.df$Long)
ggplot(sumAge2, aes(age.grp, prop, colour = RDTHealthFacility)) +
  geom_smooth(se=F) +
  theme_classic() + facet_wrap(~RDTHealthFacility) + labs(x = "Age", y = "Proportion", title =
"Malaria case distribution by health facility")
sumAge3 = local.df %>% group_by(clinic, Age) %>% summarise(n = n()) %>% mutate(prop=
n/sum(n))
ggplot(local.df, aes(Age, col=clinic)) +  geom_density(position = "dodge")+
theme_classic() +facet_wrap(~Admin2, scales = "free_y") + labs(x = "age", y = "Density", title
= "Malaria case distribution by age and district")
##############################################
  # add MOI
names(df.comb)
sample_moi<-read.csv("sample_moi_p2.csv")
#df.comb.moi = merge(df.comb, sample_moi, by="barcode", all.y=T) %>% filter(grp=="local")
df.comb.moi = merge(df.comb, sample_moi, by="barcode", all.y=T)
median.Age = df.comb.moi %>%  group_by(RDTHealthFacility) %>% summarise(med.age =
median(Age.x, na.rm = T), moi=median(moi))
df.comb.moi$age.grp <- factor(df.comb.moi$age.grp, levels = c("<5", "5-15", ">15"))
df.comb.moi = df.comb.moi[df.comb.moi$RDTHealthFacility != "Takawasa C",]
```

```
df.comb.moi$clinic <- reorder(df.comb.moi$RDTHealthFacility, df.comb.moi$Long)
df.comb.moi = df.comb.moi[complete.cases(df.comb.moi$age.grp),]
#ggplot(data=df.comb.moi, aes(age.grp, moi, colour = clinic))+facet_wrap(~Admin2)+
#  geom_smooth(method = "lm", se=F) + labs (x = "Age", y = "MOI", title = "MOI distribution
by age and health facility")
my_comparisons <- list( c("Rundu", "Nyangana"), c("Rundu", "Andara"), c("Nyangana",
"Andara"))
p2_1<-ggplot(data=df.comb.moi, aes(age.grp, moi, col = Admin2))+ geom_boxplot() +
geom_jitter(alpha = 0.1) + geom_hline(yintercept = mean(df.comb.moi$moi), linetype = 2) +
stat_compare_means(comparisons = my_comparisons) +  stat_compare_means(method =
"anova", label.y = 10.5) + labs(title = "Malaria risk by age group measure by MOI", x = "Age
group", y = "MOI")
p2_1
  #geom_point()+
  #facet_wrap(~Admin2)+
  #geom_smooth(method = "lm", se=F)+
  #labs (x = "Age", y = "MOI", title = "MOI distribution by age per district in local residence")
df.comb.moi<-merge(df.comb.moi, Fws.pop[,c(1,2)], by = "barcode")
p2_2<-ggplot(data=df.comb.moi, aes(age.grp, mean.Fws, col = Admin2))+ geom_boxplot() +
geom_jitter(alpha = 0.1) + labs (x = "Age group", y = "Fws", title = "Malaria risk by age group
measured by Fws")
 p2_2
library(gridExtra)
grid.arrange(p2_1, p2_2, nrow = 2)
cor.test()
##############################################
ggplot(df.comb_mean_age, aes(x = grp, y = prop, colour = Admin2))+ geom_boxplot() +
ggtitle("Proportion of local residence, residence in Angola and Travellers by district") +
xlab("variable") + ylab("Proportion")
# median Age by health facility
names(age_res_data)
x = df.comb %>% group_by(grp,Admin2) %>% count(grp)
df.comb%>% group_by(Admin2) %>% summarise(total = n()) %>% left_join(x) %>%
  mutate(prop = 100*n/total)
df.comb_mean_age<-as.data.frame(df.comb%>% group_by(Admin2) %>% summarise(total =
n()) %>% left_join(x) %>% mutate(prop = 100*n/total))
d1p2_1<-aggregate(df.comb$Age, na.rm = T, by=list(df.comb$Admin2), FUN=mean)
d1p2_2<-aggregate(df.comb$Age, na.rm = T, by=list(df.comb$Admin2), FUN=median)
```

```
d1p2<-merge(d1p2_1, d1p2_2, by = "Group.1")
colnames(d1p2)<- c("District","Mean_age","Median_age")
d1p2_3<-aggregate(df.comb$Age, na.rm = T, by=list(df.comb$grp), FUN=mean)
d1p2_4<-aggregate(df.comb$Age, na.rm = T, by=list(df.comb$grp), FUN=median)
d2p2<-merge(d1p2_3, d1p2_4, by = "Group.1")
colnames(d2p2)<- c("Residence","Mean_age","Median_age")
d1p2_5<-aggregate(as.numeric(age_res_data_DF$Age), na.rm = T,
by=list(age_res_data_DF$variable), FUN=mean)
d1p2_6<-aggregate(age_res_data_DF$Age, na.rm = T, by=list(age_res_data_DF$variable),
FUN=median)
d3p2<-merge(d1p2_5, d1p2_6, by = "Group.1")
colnames(d3p2)<- c("Destination","Mean_age","Median_age")
write.csv(d1p2, 'summary_by_district.csv')
write.csv(d2p2, 'summary_by_residence.csv')
write.csv(d3p2, 'summary_by_destination.csv')
write.csv(d4p2, 'summary_by_health_facility.csv')
d1p2_7<-aggregate(df.comb$Age, na.rm = T, by=list(df.comb$RDTHealthFacility),
FUN=mean)
d1p2_8<-aggregate(df.comb$Age, na.rm = T, by=list(df.comb$RDTHealthFacility),
FUN=median)
d4p2<-merge(d1p2_7, d1p2_8, by = "Group.1")
colnames(d4p2)<- c("Health_facility","Mean_age","Median_age")
#median age by residence
names(df.comb)
x = df.comb %>% group_by(grp,PrimaryResidence) %>% count(grp)
x = df.comb%>% group_by(PrimaryResidence) %>% summarise(total = n()) %>% left_join(x)
%>%
  mutate(prop = 100*n/total)
ggplot(x, aes(x = PrimaryResidence, y = prop, colour = grp))+ geom_point() +
ggtitle("Proportion of local residence, residence in Angola and Travellers by Primary residence")
+ xlab("variable") + ylab("Proportion") + theme(axis.text.x  = element_text(angle=60,
vjust=0.95, size=9,hjust=0.95))
# proportion travelled
age_res_data$travelled  = ifelse(age_res_data$NewDestination =="NA", 0, 1)
table(age_res_data$NewDestination, exclude = F)
x = age_res_data %>%
  group_by(RDTHealthFacility, Admin2) %>%
  summarise(sum = sum(travelled), n = n(), age= median(Age, na.rm = T)) %>%
```

```
  mutate(prop = 100*sum/n)
ggplot(data =x, aes(age, prop),fill=Admin2)+
  geom_point()
# slope
clinic = as.character(unique(df.comb.moi$clinic))
names(df.comb.moi)
summary(lm(moi~Age.x, data = df.comb.moi[df.comb.moi$clinic %in% var,]))
# create data frame to store results
results.slope <- data.frame()
# loop through the scales and each variable
for(var in as.character(unique(df.comb.moi$clinic))){
  # dynamically generate formula
  fmla <- as.formula(paste0("moi ~ ", "Age.x"))
  # fit glm model
  fit <- lm(fmla, data = df.comb.moi[df.comb.moi$clinic %in% var,])
  ## capture summary stats
  intercept <- coef(summary(fit))[1]
  slope <- coef(summary(fit))[2]
  p.value <- coef(summary(fit))[8]
  AIC <- AIC(fit)
  Deviance <- deviance(fit)
  # get coefficents of fit
  cfit <- coef(summary(fit))
  #get ci
  est <- cbind(Estimate = coef(fit), confint(fit))
  # create temporary data frame
  df <- data.frame(clinic = var,
                   intercept = cfit[1],
                   std.err = cfit[4],
                   slope = (est[2]),
                   slope2.5 = (est[4]),
                   slope97.5 = (est[6]),
                   p.value = cfit[8],
                   AIC = AIC(fit), Deviance = deviance(fit), stringsAsFactors = F)
  # bind rows of temporary data frame to the results data frame
  results.slope <- rbind(results.slope, df)
}
results.slope.loc = merge(results.slope, loc, by.x= "clinic", by.y="Facility.name", all.x=T)
```

```
results.slope.loc = results.slope.loc %>% arrange(Long)
pd <- position_dodge(0.15)
results.slope.loc$clinic <- reorder(results.slope.loc$clinic, results.slope.loc$Long)
names(df.comb.moi)
m.age = df.comb.moi %>%  group_by(clinic) %>% dplyr::summarise(age= mean(Age.x, na.rm
= T))
results.slope.loc =  m.age %>% right_join(results.slope.loc)
clinic = as.character(results.slope.loc$clinic)
ggplot(results.slope.loc, aes(x=as.factor(Long), y=(slope))) +
  geom_errorbar(aes(ymin=slope+std.err, ymax= slope-std.err ),
width=.1,size=0.7,position=pd)+
  geom_point(size=2.5,position=pd)+
  #geom_line(size=1,position=pd)+
  geom_hline(yintercept = 0, linetype=4)+
  labs(title = "") +
  theme_classic()+
  xlab("") + ylab("Slope")+
  guides(fill=FALSE)+
  theme(axis.text.x  = element_text(angle=60, vjust=0.95, size=12,hjust=0.95))+
  scale_x_discrete(labels= clinic)
#All clinics slope
loc1<- read_csv("Location_clinics_updated.csv")
results.slope.loc1 = merge(results.slope, loc1, by.x= "clinic", by.y="Facility.name", all.x=T)
results.slope.loc = results.slope.loc1 %>% arrange(Long)
pd <- position_dodge(0.15)
results.slope.loc1$clinic <- reorder(results.slope.loc1$clinic, results.slope.loc1$Long)
names(df.comb.moi)
m.age = df.comb.moi %>%  group_by(clinic) %>% dplyr::summarise(age= mean(Age.x, na.rm
= T))
results.slope.loc1 =  m.age %>% right_join(results.slope.loc1)
clinic = as.character(results.slope.loc1$clinic)
ggplot(results.slope.loc1, aes(x=as.factor(Long), y=(slope))) +
  geom_errorbar(aes(ymin=slope+std.err, ymax= slope-std.err ),
width=.1,size=0.7,position=pd)+
  geom_point(size=2.5,position=pd)+
  #geom_line(size=1,position=pd)+
  geom_hline(yintercept = 0, linetype=4)+
  labs(title = "") +
```

```
theme_classic()+
xlab("") + ylab("Slope")+
guides(fill=FALSE)+
theme(axis.text.x  = element_text(angle=60, vjust=0.95, size=12,hjust=0.95))+
scale_x_discrete(labels= clinic)
```

9.3 Appendix C: Data analysis Chapter 5

```
library(tidyverse)
library(ggplot2)
library(dplyr)
library(ggpubr)
#Set working directory
setwd("~/Dropbox/Munya.Genotyping/Script from ST/Data/Paper 3 data")
#Read incidence data
df_p3<-read.csv("~/Dropbox/Munya.Genotyping/Script from ST/Data/Paper 3 data/CleanP3data copy.csv")
#df_p3<-read.csv("~/Dropbox/Munya.Genotyping/Script from ST/Data/Paper 3 data/Allelic richness.csv")
#Read popperclinic
popclinic<-read.csv("PopPerClinic.csv")
#Rename columns to join by "Facility"
colnames(df_p3)[colnames(df_p3) == "facility"] <- "Facility"
#count number of cases per village
df_p3<-as.data.frame(table(df_p3$Facility))
#Change column names
colnames(df_p3) <- c("Facility", "ncases")
#Join with population data at health facility level
df_p3<-merge(df_p3, popclinic, by = "Facility")
#Annual population growth by year to 2017 (2%) source:http://worldpopulationreview.com/countries/namibia-population/
df_p3<- transform(df_p3, POP2016 = (POP2015*0.02)+POP2015)
df_p3<- transform(df_p3, POP2017 = (POP2016*0.02)+POP2016)
#Calculate incidence at healthfacility level
df_p3<- transform(df_p3, incidence = (ncases/POP2017)*1000)
#Save the incidence file
#write.csv(df_p3, "incidence_HF.csv")
incid_HF<-read.csv("incidence_HF.csv")
#incid_HF$Facility<- factor(incid_HF$Facility, levels = c("Choi_Clinic", "Katima_District_Hospital", "Sachona_Clinic", "Sangwali_HC", "Sibbinda_HC", "Batubaja_Clinic", "Linyanti_Clinic", "Chinchimani_Clinic", "Kanono_Clinic", "Kasheshe_Clinic", "Masokotwane_Clinic", "Mafuta_Clinic", "Sesheke_Clinic"))
#Incidence per health facility orderd from west to east representation
#ggplot(data= incid_HF, aes(Facility, incidence2014, fill = Facility)) + guides(fill=FALSE) +
```

```
 # geom_bar(stat = "identity") + labs(title = "Malaria incidence across health facilities in the Zambezi region") +
 #   xlab("Health facility") + ylab("Incidence") + theme(axis.text.x = element_text(angle=-45, hjust=.1))
#Calculate MOI
## MOI
library(tidyverse)
library(ggplot2)
library(dplyr)
all_alleles <- read.csv('MERFAT-all-alleles-27102017.csv') # The column name for sample ID should be namesd as "Sample"
#all_alleles<-merge(df_p3[,2],all_alleles, by.x=T, by.y = "barcode" )
head(all_alleles)
#colnames(all_alleles)[colnames(all_alleles) == "x"] <- "barcode"
samples <- dplyr::filter(all_alleles, !grepl(pattern = 'NTC|POS', x = barcode))
negatives <- dplyr::filter(all_alleles, grepl(pattern = 'NTC', x = barcode))
positives <- dplyr::filter(all_alleles, grepl(pattern = 'POS', x = barcode))
loci <- colnames(samples)[-c(1)]
sample_alleles = data.frame(barcode=c(), Allele=c(), Locus=c())
for(i in 1:length(loci)) {
  locus = loci[i]
  res <- cbind(tidyr::separate_rows_(samples, `locus`)[, c('barcode', `locus`)], c(`locus`))
  colnames(res) <- c('barcode', 'Allele', 'Locus')
  res <- dplyr::filter(res, Allele != "")
  sample_alleles <- rbind(sample_alleles, res)
}
by_sample <- dplyr::group_by(sample_alleles, barcode)
by_locus <- dplyr::group_by(sample_alleles, Locus)
head(by_sample)
moi_offset = 2 # takes the second highest
sample_moi <- dplyr::summarise(by_sample[complete.cases(by_sample$Allele),],
                               moi = sort(table(Locus), decreasing = T)[moi_offset])
#Save MOI results by sample
#write.csv(sample_moi, "Sample_moi_p3.csv")
#Merge data with facility data
HF_data<-read.csv("MERFAT-genotyping-key.csv")
sample_moi<-merge(sample_moi, HF_data, by = "barcode") %>% filter(barcode!=1148500508)
sample_moi_avg = sample_moi %>% group_by(Facility= healthfacility) %>% dplyr::summarise(moi=mean(moi))
```

```
sample_moi_avg.inc<-merge(sample_moi_avg, incid_HF, by="Facility", all.x=T)
sample_moi_inc<-merge(sample_moi, incid_HF, by.x="healthfacility", by.y="Facility", all.x=T)
#Save sample_moi_HF data
#write.csv(sample_moi, "Sample_moi_HF_p3.csv")
#Order Healthfacilities from west to East
sample_moi$healthfacility<- factor(sample_moi$healthfacility,
                                   levels = c("Choi C", "Sibbinda HC", "Chinchimani C", "Kanono C", "Kasheshe C",
"Sesheke C"))
ggplot(data= sample_moi, aes(moi))+geom_histogram()+facet_wrap(~healthfacility.x, scales = "free")
#|-----------------------------------|-------------------------------|-----------------------------------|#
#|-----------------------------------|-------------------------------|-----------------------------------|#
#Figure 2A
library(ggrepel)
moi = ggplot(data= sample_moi_avg.inc, aes(ncases, moi)) +
  geom_smooth(method = lm, se=F)+
  geom_point() + labs(title = "") +
  scale_y_continuous(limits = c(1.85,2.2))+
  geom_text_repel(aes(label = Facility))+
   xlab("Number of reported cases") + ylab("Multiplicity of Infection (MOI)") +
  theme_bw()
#correlation
cor.test(sample_moi_avg.inc$ncases, sample_moi_avg.inc$moi)
#Figure 2B
#Presentation of data
allelecountDf = allelecount
allelecount$pmulti <- (rowSums(allelecount[,2:27] >= 2,na.rm = T))/26
dfComb = merge(Fws.pop, allelecount[, c(1, 28)], by="barcode")
plot(dfComb$mean.Fws, dfComb$pmulti)
Fws_avg = dfComb %>% filter(barcode!=1148500508) %>%
  group_by(Facility=healthfacility.x) %>%
  summarise(fws = mean(pmulti))
Fws_avg.inc<-merge(Fws_avg, incid_HF, by="Facility", all.x=T)
fwsp = ggplot(data= Fws_avg.inc, aes(ncases, fws)) +
  geom_smooth(data= Fws_avg.inc %>% filter(Facility != "Kanono C"),
              aes(ncases, fws), method = "lm", se=F)+
  geom_point(data= Fws_avg.inc, aes(ncases, fws)) + labs(title = " ") +
  geom_text_repel(aes(label = Facility))+
  xlab("Number of reported cases") + ylab("Within host diversity (1-Fws)") +
```

```
  theme_bw()
library(cowplot)
figure2 <- plot_grid(moi, fwsp,
                    nrow = 1,
                    labels = c("A","B"),
                    align = "v")
figure2
pdf("~/Dropbox/Munya.Genotyping/Script from ST/Data/Paper 3 data/ figure2.pdf", width = 8, height = 4)
figure2
dev.off()
#|------------------------------------|-------------------------------|----------------------------------|#
#|------------------------------------|-------------------------------|----------------------------------|#
# Heterozygosity
all_alleles<-merge(HF_data,all_alleles, by = 'barcode')
head(all_alleles)
cleaned = all_alleles[,c(1,3, 19:44)] # id, pop, and alleles
names (cleaned)
cleaned = dplyr::rename(cleaned, pop = healthfacility) # rename healthfacility to pop
loci <- colnames(cleaned)
loci <- loci[loci != 'barcode' & loci != 'pop']
loci # lsit of loci
long_form_results <- data.frame("barcode"=integer(0), "locus"=character(0), "allele"=integer(0), "population"=character(0));
for(i in 1:(dim(cleaned)[1])) {
  entry = cleaned[i,]
  sample_id = entry["barcode"]
  population = as.character(entry["pop"]$pop)
  print(sample_id[1,]);
  print(i)
  for(j in 1:(length(loci))) {
    locus = loci[j];
    alleles_list = entry[locus];
    #print(alleles_list);
    alleles <- unlist(strsplit(gsub(" ", "", unlist(alleles_list)), ";"));
    for(k in 1:(length(alleles))) {
      allele <- as.integer(alleles[k]);
      if(length(allele) > 0) {
```

```
        long_form_results <- rbind(long_form_results, data.frame("barcode" = sample_id, "locus" = locus,
"allele" = allele, "population" = population));
      }
    }
  }
}
dim(long_form_results)#13927
head(long_form_results)
is.na (long_form_results)
long_form_results<- na.omit(long_form_results)# removes NA
dim(long_form_results) # 13259
str(long_form_results)#104534 including NA
#convert allele to numeric
hist (long_form_results$allele)##Check if there are zeros
#rename to allforpopfws
allforpopfws  <-  long_form_results[complete.cases(long_form_results),]
#Omit if samples have less than 10 number of successful loci ----#
# Number of successful genotyping per unique barcode - will exclude if >10 missing
SuccessCountPerSample <- function(x){
  SuccessCount<-length(unique(allforpopfws$locus[allforpopfws$barcode==x]))
  return(SuccessCount)
}
barcodelist<-c(unique(allforpopfws$barcode)) # List of unique barcodes
gtsuccesscount.barcode<-lapply(barcodelist, FUN=SuccessCountPerSample)
gtsuccesscount.barcode<-cbind(barcodelist, gtsuccesscount.barcode)
gtsuccesscount.barcode<-as.data.frame(gtsuccesscount.barcode)  #  row  =  230  barcodes,  col  =  barcode,
successful genotype count
gtsuccesscount.barcode$num.locipresent <- gtsuccesscount.barcode$gtsuccesscount.barcode
gtsuccesscount.barcode$gtsuccesscount.barcode<- NULL
gtsuccesscount.barcode <- as.data.frame(lapply(gtsuccesscount.barcode, unlist))
gtsuccesscount.barcode$num.locimissing <- 26 - gtsuccesscount.barcode$num.locipresent
head(gtsuccesscount.barcode)
names(gtsuccesscount.barcode)[names(gtsuccesscount.barcode)=="barcodelist"]<-"barcode"
hist(gtsuccesscount.barcode$num.locimissing) # Histogram shows that only a small number of
abline(v=11, col="red") # not substed as I already remove based on the home MS
allforpopfws.locimissing<-(merge(gtsuccesscount.barcode, allforpopfws, by="barcode"))
allforpopfws<-as.data.frame(allforpopfws.locimissing)
#allforpopfws <-subset(allforpopfws, num.locimissing<=11)
```

```
head(allforpopfws)
#---------------------------------------------------------------------------#
# Calculate heterozygosity
library(dplyr)
str(allforpopfws)
pop.list <- as.character(unique(allforpopfws$population))
pop.stats <- list()
for(i in 1:length(pop.list)){
  pop <- pop.list[i]
  pop.data <- list()
  allforpop.sub <- allforpopfws[allforpopfws$population==pop,]
  call.rate.perloci <- function(x) {
    #missing<-1-length(unique(gtdb$barcode[x]))/length(unique(gtdb$barcode))
    call.rate<-
length(unique(allforpop.sub$barcode[allforpop.sub$locus==x]))/length(unique(allforpop.sub$barcode))
    return(call.rate)
  }
  numofcalls.perloci <- function(x){
    numberofsuccess<-length(unique(allforpop.sub$barcode[allforpop.sub$locus==x]))
    return(numberofsuccess)
  }
  loci.list <- as.character(unique(allforpop.sub$locus))
  call.rate <- lapply(loci.list, FUN=call.rate.perloci)
  loci.call.rate <- cbind(loci.list, call.rate)
  call.perloci <- lapply(loci.list, FUN=numofcalls.perloci)
  loci.call.rate <- cbind(loci.list, call.rate)
  call.perloci <- lapply(loci.list, FUN=numofcalls.perloci)
  numofcall.perloci<- cbind(loci.list, call.perloci)
  loci.callrate <- as.data.frame(cbind(loci.list, call.rate, call.perloci))
  loci.callrate <- as.data.frame(lapply(loci.callrate, unlist))
  loci.callrate<-loci.callrate[order(loci.callrate$call.rate),]
  loci.callrate$row.names<-NULL
  names(loci.callrate)[names(loci.callrate)=="loci.list"]<-"locus"
  pop.data$loci.callrate <- loci.callrate
  funSubsetCount <- function(x) {
    subset.int <-subset(allforpop.sub, locus==x)
    myfreq <- as.data.frame(table(subset.int$allele))
    names(myfreq) <- c("allele", "Freq")
```

```
   locusid <- rep(x,nrow(myfreq))
   output.df<-cbind(myfreq, locusid)
   #return(fun.data.frame)
   return(output.df)
  }
  ?count
  loci.allele <- lapply(loci.list, FUN=funSubsetCount)
  lociallele <-do.call(rbind, loci.allele) # Table of all allele types present in pop.
  lociallele <- as.data.frame (lociallele)
  funallelefreq <- function(x){    # Function to determine sum of all samples that were successful at specific loci
   alleledenominator<-sum(lociallele$Freq[lociallele$locusid==x])
   return(alleledenominator)
  }
  sum.allelesperloci<-lapply(loci.list, FUN=funallelefreq)
  allelefreq.denom <-cbind(loci.list, sum.allelesperloci) # col of 27 markers, each row contains how many samples were genotyped at each loci
  allelefreq<-merge(lociallele, allelefreq.denom, by.x="locusid", by.y="loci.list", all=FALSE)
  allelefreq$sum.allelesperloci<-as.numeric(allelefreq$sum.allelesperloci)
  allelefreq$allelefreq<-(allelefreq$Freq)/(allelefreq$sum.allelesperloci) # Calculate allele frequency at each ith allele
  funHe <- function(x){
   He      <-      ((length(unique(allforpopfws$barcode)))/(((length(unique(allforpopfws$barcode)))-1))*(1-sum((allelefreq$allelefreq[allelefreq$locusid==x]^2))))
   return(He)
  }
  He.loci.list <- lapply(loci.list, FUN=funHe)
  He.perloci<- cbind(loci.list, sum.allelesperloci, He.loci.list)
  He.perloci<- as.data.frame(He.perloci)
  allelecount <- as.data.frame(table(allelefreq$locusid))
  He.perloci<-cbind(He.perloci, allelecount)
  He.perloci$x<-NULL
  names(He.perloci)[names(He.perloci)=="He.loci.list"]<- "He"
  names(He.perloci)[names(He.perloci)=="sum.allelesperloci"]<- "TotalNo.Samples"
  names(He.perloci)[names(He.perloci)=="Freq"]<- "Freq.Uniquealleles"
  names(He.perloci)[names(He.perloci)=="loci.list"]<- "Loci"
  He.perloci$He<-as.numeric(He.perloci$He)
  He.perloci$TotalNo.Samples<-as.numeric(He.perloci$TotalNo.Samples)
```

```
  He.perloci$Loci<-as.character(He.perloci$Loci)
  pop.data$He.perloci <- He.perloci
  pop.data$allele.freq <- allelefreq
  pop.stats[[pop]] <- pop.data
}
head(pop.stats)
# summary of HE
Choi_C  = pop.stats$`Choi C`$He.perloci[, -4]
Choi_C$pop = "Choi C"
head(Choi_C)
Sibbinda_HC  = pop.stats$`Sibbinda HC`$He.perloci[, -4]
Sibbinda_HC$pop = "Sibbinda HC"
Chinchimani_C  = pop.stats$`Chinchimani C`$He.perloci[, -4]
Chinchimani_C$pop = "Chinchimani C"
Kanono_C  = pop.stats$`Kanono C`$He.perloci[, -4]
Kanono_C$pop = "Kanono C"
Kasheshe_C  = pop.stats$`Kasheshe C`$He.perloci[, -4]
Kasheshe_C$pop = "Kasheshe C"
Sesheke_C  = pop.stats$`Sesheke C`$He.perloci[, -4]
Sesheke_C$pop = "Sesheke C"
# combine all data
all.he <- rbind(Choi_C, Sibbinda_HC, Chinchimani_C, Kanono_C,Kasheshe_C,Sesheke_C)
head(all.he)
#Save unique alleles data
#write.csv(allelefreq, "Unique_alleles_p3.csv")
#mean and se by popualtion
het <- all.he %>% dplyr::group_by(pop)
dd.he = het %>% dplyr::summarise_each(funs(mean,sd,se=sd(.)/sqrt(n())), He)
he_avg = all.he %>% group_by(Facility=pop) %>% summarise(he = mean(He))
he_avg.inc<-merge(he_avg, incid_HF, by="Facility", all.x=T)
# #|-----------------------------------|-------------------------------|----------------------------------|#
# #|-----------------------------------|-------------------------------|----------------------------------|#
#Figure3A
he.plot = ggplot(data= he_avg.inc, aes(ncases, he)) +
  geom_smooth(method = "gam",formula = y ~ s(x, k=3), se=F)+
  geom_point() + labs(title = " ") +
  geom_text_repel(aes(label = Facility))+
  xlab("Number of reported cases") + ylab("Heterozygosity") +
```

```
  theme_bw()
# figure 3B
names(het)
ar= het %>%  group_by(Facility= pop) %>%  summarise(mean.allele = mean(Freq.Uniquealleles))
ar <- merge(ar,incid_HF, by="Facility")
#Figure3B
mean.all.plot = ggplot(data= ar, aes(ncases, mean.allele)) +
  geom_smooth(method = "gam",formula = y ~ s(x, k=3), se=F)+
  geom_point() + labs(title = " ") +
  geom_text_repel(aes(label = Facility))+
  xlab("Number of reported cases") + ylab("Mean # of alleles") +
  theme_bw()
library(cowplot)
figure3 <- plot_grid(he.plot, mean.all.plot,
                    nrow = 1,
                    labels = c("A","B"),
                    align = "v")
figure3
pdf("~/Dropbox/Munya.Genotyping/Script from ST/Data/Paper 3 data/ figure3.pdf", width = 8, height = 4)
figure3
dev.off()
# #|------------------------------------|--------------------------------|-----------------------------------|#
# #|------------------------------------|--------------------------------|-----------------------------------|#
## Fws
head(allforpopfws)
funSubsetAlleleCount <- function(x) {
  subset.int <-subset(allforpopfws, barcode==x)
  countlocus <- dplyr::count(subset.int, locus)
  barcode <- rep(x,nrow(countlocus))
  output.df<-cbind(countlocus, barcode)
  #return(fun.data.frame)
  return(output.df)
}
barcodelist<-c(unique(allforpopfws$barcode))
allele.count<- lapply(barcodelist, FUN=funSubsetAlleleCount)
AlleleCount.table <-do.call(rbind, allele.count)
addrows1 <-expand.grid(id = unique(AlleleCount.table$barcode),
                      locus = unique(AlleleCount.table$locus))
```

```
names(addrows1)
allelecount.loci<-merge(addrows1, AlleleCount.table, by.x=c("id","locus"), by.y=c("barcode","locus"),
all=FALSE)
# Dataframe where row = barcodes and col = allele count per loci
allelecount<-reshape(allelecount.loci, dir="wide", idvar="id", timevar="locus")
names(allelecount)<-gsub("freq.", "", names(allelecount), fixed=TRUE) # Remove "freq." from all locus
markers
names(allelecount)[names(allelecount)=="id"]<-"barcode"
#allelecount[is.na(allelecount)]<-0
names (allelecount)
## Sum of number of alleles present at each locus, disregarding if they are unique or not.
sum.numalleles.perloci<- as.data.frame(apply(allelecount[,2:27], 2, sum, na.rm=TRUE))
names(sum.numalleles.perloci)[names(sum.numalleles.perloci)=="apply(allelecount[, 2:27], 2, sum, na.rm
= TRUE)"]<-"allele.sum"
library(data.table)
setDT(sum.numalleles.perloci, keep.rownames = TRUE)[]
names(sum.numalleles.perloci)[names(sum.numalleles.perloci)=="rn"]<-"locus"
allforpopfws.sort <- allforpopfws[order(allforpopfws$locus,allforpopfws$allele), ]
allelefreq$locus<-allelefreq$locusid
allelefreq.sort<-allelefreq[order(allelefreq$locus, allelefreq$allele), ]
allforpopfws.allele.freq<-merge(allforpopfws.sort, allelefreq.sort, by=c("locus", "allele"), all=FALSE)
allforpopfws.allele.freq$locusid<-NULL
allforpopfws.allele.freq$allelefreq2<-(allforpopfws.allele.freq$allelefreq)^2
fws <- matrix(0, nrow = length(barcodelist), ncol = length(loci.list))
rownames(fws) <- barcodelist
colnames(fws) <- loci.list
for(i in 1:(length(barcodelist))) {
  barcode <- barcodelist[i]
  for (j in 1:(length(loci.list))){
    locus <- loci.list[j]
    Hs<-1-sum(allforpopfws.allele.freq$allelefreq2[allforpopfws.allele.freq$barcode==barcode &
allforpopfws.allele.freq$locus==locus])
    Hw<-1-(length(allforpopfws.allele.freq$allele[allforpopfws.allele.freq$barcode==barcode &
allforpopfws.allele.freq$locus==locus]))*(1/length(allforpopfws.allele.freq$allele[allforpopfws.allele.freq
$barcode==barcode & allforpopfws.allele.freq$locus==locus])^2)
    fws[i,j]<-1-Hw/Hs
  }
}
```

```
## fws[i,j] will not work if the rows or columns are not in the same order as the matrix.
## fws[barcode, locus] = will find that specific barcode row and locus column and stick that calculation into that cell.
Fws.barcode<-as.data.frame(fws)
#Fws.barcode[is.na(Fws.barcode)]<-1 ## For all missing alleles at loci, Fws = 1
setDT(Fws.barcode, keep.rownames = TRUE)[]
names(Fws.barcode)[names(Fws.barcode)=="rn"]<-"barcode"
Fws.barcode <-as.data.frame(Fws.barcode)
names(Fws.barcode)
Fws.barcode$mean.Fws<- rowMeans(Fws.barcode[,2:27], na.rm=TRUE)
head(Fws.barcode)
summary(Fws.barcode$mean.Fws)
#|-----------------------------------|------------------------------|----------------------------------|#
# merge with popualtion and determine the mean fws, calcualte p-value
Fws.pop <- merge(Fws.barcode,sample_moi, by="barcode")
plot(Fws.pop$moi, Fws.pop$mean.Fws)
#Order HF
Fws.pop$healthfacility.x<- factor(Fws.pop$healthfacility.x, levels = c("Choi C", "Sibbinda HC", "Chinchimani C", "Kanono C", "Kasheshe C", "Sesheke C"))
#Presentation of data
Fws_avg = Fws.pop %>% filter(barcode!=1148500508) %>% group_by(Facility=healthfacility.x) %>% summarise(fws = mean(1-mean.Fws))
Fws_avg.inc<-merge(Fws_avg, incid_HF, by="Facility", all.x=T)
#FFigure2B
ggplot(data= Fws_avg.inc %>% filter(Facility != "Kanono C"), aes(ncases, fws)) +
  geom_smooth(method = "gam",formula = y ~ s(x, k=5), se=T)+
  geom_point() + labs(title = " ") +
  geom_text_repel(aes(label = Facility))+
  xlab("Number of reported cases per health facility") + ylab("1-Fws") +
  theme_bw()
#ggplot(data= Fws_avg.inc %>% filter(Facility != "Kanono C"), aes(incidence2014, fws)) +
 # geom_smooth(method = "gam",formula = y ~ s(x, k=5), se=T)+
 # geom_point() + labs(title = " ") +
 # geom_text_repel(aes(label = Facility))+
  #xlab("Incidence") + ylab("HE") +
  #theme_bw()
# ggplot(data=Fws.pop, aes(healthfacility, mean.Fws, colour=healthfacility)) +
#   geom_boxplot() + geom_jitter(alpha = 0.3) +
```

```
#   labs(title = "Fws distribution per healthfacility") +
#   xlab("Health facility") +
#   ylab("Fws")
#Create data for LD
ld_df<-read.csv("MERFAT_all_27102017_Dominant_Peaks.csv")
ld_df<-merge(ld_df, HF_data[,c(1,3)], by = "barcode")
write.csv(ld_df, "LD_p3_main.csv")
#################_#################_#################_#################_#############
####
#Allelic richness
# ar<-merge(sample_alleles, HF_data, by = "barcode")
#
# ar <-ar%>%
#   group_by(healthfacility) %>%
#   summarise(count = n_distinct(Allele))
# colnames(ar) <- c("Facility", "count")
# ar <- merge(ar,Fws_avg.inc, by="Facility")
names(het)
ar= het %>%  group_by(Facility= pop) %>%  summarise(mean.allele = mean(Freq.Uniquealleles))
ar <- merge(ar,incid_HF, by="Facility")
#Figure3B
ggplot(data= ar, aes(ncases, mean.allele)) +
  geom_smooth(method = "gam",formula = y ~ s(x, k=3), se=F)+
  geom_point() + labs(title = " ") +
  geom_text_repel(aes(label = Facility))+
  xlab("Number of reported cases") + ylab("Mean # of alleles") +
  theme_bw()
# #|--------------------------------------|--------------------------------|-----------------------------------|##
# #|--------------------------------------|--------------------------------|-----------------------------------|#
#Travel
trav<-read.csv("Travel.csv")
colnames(trav) <- c("Facility", "travellers", "total", "prop.travel", "ncases")
#Figure4A
trav.plot = ggplot(data= trav, aes(ncases, prop.travel)) +
  geom_smooth(method = "lm", se=F)+
  geom_point() + labs(title = " ") +
  geom_text_repel(aes(label = Facility))+
  xlab("Number of reported cases") + ylab("% reproted international travel") +
```

```
  theme_bw()
#realtedness
zamRelated = read.csv("ZambeziRelatedness.csv")
names(zamRelated)
district.rltd <- count_(zamRelated %>% filter(relat.ibs>=0.6), c("clinic.x", "clinic.y"))
district.all <- count_(zamRelated,  c("clinic.x", "clinic.y"))
district.all = dplyr::rename(district.all, nn = n)
x.district.rltd = right_join(district.rltd, district.all)
x.district.rltd[,3][is.na(x.district.rltd[,3])] <-0 #replace NA Zero
x.district.rltd$ratio.hrelated=(x.district.rltd$n/x.district.rltd$nn)
x.district.rltd
realtednessWithinclinic =  x.district.rltd %>% filter(clinic.x==clinic.y) %>%
                          dplyr::select(Facility=clinic.x, ratio.hrelated)
df.related = left_join(realtednessWithinclinic, incid_HF)
#Figure4B
prop = ggplot() +
  geom_smooth(data= df.related %>% filter(Facility != "Kanono C"), aes(ncases, ratio.hrelated), method =
lm, se=F)+
  geom_point(data= df.related, aes(ncases, ratio.hrelated)) +
  labs(title = " ") +
  geom_text_repel(data= df.related,aes(ncases, ratio.hrelated,label = Facility))+
  xlab("Number of reported cases") + ylab("Prop. highly related") +
  theme_bw()
library(cowplot)
figure4 <- plot_grid(trav.plot, prop,
                nrow = 1,
                labels = c("A","B"),
                align = "v")
figure4
pdf("~/Dropbox/Munya.Genotyping/Script from ST/Data/Paper 3 data/ figure4.pdf", width = 8, height = 4)
figure4
dev.off()
```

9.4 Appendix D: Figure 8, page 75, map code

```
library('rgdal')      # Reading and projecting shapefiles
 library('plotrix')    # Creating color scales
 library('classInt')   # Assigning colors to data
 library(rasterVis)
 library(RColorBrewer)
 library(tidyverse)
 library(viridis)
 library(ggraph)
 #------------------------------------------#------------------------------------------#
 # Reading map (source:http://www.diva-gis.org/gdata)

 nam <- readOGR("~/Dropbox/Munya.Genotyping/Script from
 ST/MapScript/Kav_zambezi_merged.shp")
 plot(nam)
 namZambezi = nam[nam$NAME_1=="Zambezi",] #only Zambezi
 nam_df = fortify(namZambezi)


 rundu1 = fortify(readOGR("~/Dropbox/Munya.Genotyping/Script from
 ST/MapScript/Shapefiles_Split/Rundu/NAM_adm2_ID_2__22.shp"))
 rundu2= fortify(readOGR("~/Dropbox/Munya.Genotyping/Script from
 ST/MapScript/Shapefiles_Split/Rundu/NAM_adm2_ID_2__26.shp"))
 rundu3 = fortify(readOGR("~/Dropbox/Munya.Genotyping/Script from
 ST/MapScript/Shapefiles_Split/Rundu/NAM_adm2_ID_2__27.shp"))
 rundu4 = fortify(readOGR("~/Dropbox/Munya.Genotyping/Script from
 ST/MapScript/Shapefiles_Split/Rundu/NAM_adm2_ID_2__28.shp"))
 nyanganna = fortify(readOGR("~/Dropbox/Munya.Genotyping/Script from
 ST/MapScript/Shapefiles_Split/Nyanganna/NAM_adm2_ID_2__25.shp"))
 andara = fortify(readOGR("~/Dropbox/Munya.Genotyping/Script from
 ST/MapScript/Shapefiles_Split/Andara//NAM_adm2_ID_2__24.shp"))

 z1 = fortify(readOGR("~/Dropbox/Munya.Genotyping/Script from
 ST/MapScript/Shapefiles_Split/Zambezi//NAM_adm2_ID_2__102.shp"))
 z2 = fortify(readOGR("~/Dropbox/Munya.Genotyping/Script from
 ST/MapScript/Shapefiles_Split/Zambezi//NAM_adm2_ID_2__103.shp"))
 z3 = fortify(readOGR("~/Dropbox/Munya.Genotyping/Script from
 ST/MapScript/Shapefiles_Split/Zambezi//NAM_adm2_ID_2__104.shp"))
 z4 = fortify(readOGR("~/Dropbox/Munya.Genotyping/Script from
 ST/MapScript/Shapefiles_Split/Zambezi//NAM_adm2_ID_2__105.shp"))
```

```
z5 = fortify(readOGR("~/Dropbox/Munya.Genotyping/Script from
ST/MapScript/Shapefiles_Split/Zambezi//NAM_adm2_ID_2__106.shp"))
z6 = fortify(readOGR("~/Dropbox/Munya.Genotyping/Script from
ST/MapScript/Shapefiles_Split/Zambezi//NAM_adm2_ID_2__107.shp"))


#number of samples
loc = read.csv("~/Dropbox/Munya.Genotyping/Script from
ST/MapScript//Location_clinics_updated_22Feb2018.csv")
names(loc)
loc = loc %>%  dplyr::filter(Admin1=="Caprivi")
#-----------------------------------------#-----------------------------------------#
#plot
g = ggplot() +
  geom_polygon(data=nam_df, aes(x=long, y=lat, group=group), color="black", fill
= NA, size=0.25) +
  geom_polygon(data=z1, aes(x=long, y=lat, group=group), fill=NA, color="black",
size=0.25) +
  geom_polygon(data=z2, aes(x=long, y=lat, group=group),  fill=NA, color="black",
size=0.25, alpha=0.5) +
  geom_polygon(data=z3, aes(x=long, y=lat, group=group), fill=NA, color="black",
size=0.25) +
  geom_polygon(data=z4, aes(x=long, y=lat, group=group), fill=NA, color="black",
size=0.25) +
  geom_polygon(data=z5, aes(x=long, y=lat, group=group), fill=NA,  color="black",
size=0.25) +
  geom_polygon(data=z6, aes(x=long, y=lat, group=group), fill=NA,  color="black",
size=0.25) +
  coord_map(projection="mercator")+
  coord_equal() +
  theme_graph()+
  #scale_fill_gradientn(colors = colorRampPalette(rev(brewer.pal(6, "Blues")))(8),
guide = FALSE)+
  #scale_fill_gradient(low ="#B4D3E9"  , high ='#2573B3',name = "# of
samples",guide = guide_legend(keyheight = unit(5, units = "mm"),title.position =
'top'))+
  geom_point(data=loc, aes(x= Long, y=Lat, fill= n.total, size=n.total), col="black"
,pch=21)+
  theme(legend.position="bottom")

g
#-----------------------------------------#-----------------------------------------#
```

```
library(Cairo)
cairo_pdf(filename = "~/Dropbox/Munya.Genotyping/Script from
ST/MapScript/Map_fig1Paper2.pdf", width = 12 ,height = 6)
plot(g)
dev.o
```

9.5 Appendix E: Raw data

	barcode	clinic	residence	date	Lat	Long	Admin1	Admin2	AS2	AS3	AS32	AS7	AS8	AS11	AS12	AS14	AS15	AS19	AS21	AS34	AS25	B7M19	TA109	AS31	Ara2	PfPK2	TA1	TA87	TA81	TA60	PolyA	PFG377	TA40
1	1071878953	AndaraDH	NotRecorded	4/21/16	-18.0599	21.44215	Kavango	Andara	195		227		198		165	212	119;122				123;120;104;101		152;177			191	181	113	131	209		103	268;271
2	1106663868	AndaraDH	NotRecorded	4/19/16	-18.0599	21.44215	Kavango	Andara	192			174	198		165	212	131	179	157		98;101		165		149	200	190	106	121	218	152	107	
3	1106664099	AndaraDH	Mukwe	4/25/16	-18.0599	21.44215	Kavango	Andara	198;195		227		204;201;198		165	212;215	128;122		157		110;101		165;168;189		136;139	191;170	174;177;171		121;128;125			103;100;110	244;238;241;256;259
4	110666	Nyanga	NotRecor	4/27/	-18.00	20.676	Kavan	Nyanga	192		242		198		165	212	122	179	157	184	113				136		187	113	118	221		103;107	268;247

	5920	naDH	ded	16	337	55	go	na																									
5	1117532688	NyanganaDH	Ndonga Linena	4/28/16	-18.00337	20.67655	Kavango	Nyangana	198;189;192	170;173;167;158	227;242;248;233	171;177	201;198	162;156	165	212;206;221;215	116;128;122	179;182	157;160	187;184	110;91;120	172;155	152;165;168;155	197;200;212	149;146	209;179;176;188;200;191;197;170	168;171	110;100;106;113;103;97	134;128	215;221;218	136;185;146;173;149;161;158;155	103;100	268;271;274;277;250;253
6	1130856274	NyanganaDH	Not Recorded	4/10/16	-18.00337	20.67655	Kavango	Nyangana	198		227		198	162	162	218	128	179	160	184	88		152;149	224;221;227	139		165;162	110;113;116	125	205		100;97	271
7	1138700276	AndaraDH	Not Recorded	4/25/16	-18.05599	21.44215	Kavango	Andara	189		227	171	198		165	224	122	167	157		107;98;101		165		143	191	171		121	209	155	100	
8	1138700664	NyanganaDH	Not Recorded	4/11/16	-18.00337	20.67655	Kavango	Nyangana	192		273		201;198	162;165	162;165	212;206	131;122	179;182	157;160	184	120;101		177;174	197;212	139;143		181;171	110;106;103	128;137	209		103;107	268;283;265
9	113	Nya	NotR	4/1	-18	20.	Kav	Nya			227;242		201;	162;156	171;	212;206	134;140;	179;182	157;160;	187;184	110;120;101		152;180;177;	200;206	139;146		174;171	110;136;106;	140;131;125	215;212;209		113;103;	280

	8700689	ngana DH	ecorded	1/16	.003337	67655	ango	ngana					198		162;165		128;122		154				165;168;155;149					113;103;97				100	
10	1138701082	Nyangana DH	Not Recorded	4/9/16	-18.003337	20.67655	Kavango	Nyangana	198;192		242;273		201	162;165	162;165	212;206	131;122	179;182	157;160	184	110;113;120;104;101		180;177;193;155;189	197;212	136;139;143		187;171	100;106;103;97;91	118;128;137;125	215;221;209		113;107	250
11	1138702225	Andara DH	Mukwe	4/14/16	-18.0599	21.44215	Kavango	Andara	189;195		258	171	201;198		162	206;209	134;122	182	157		110;101		165;168		152;146	179;170	174;168;181;150		128	205;218	146	103;107	235;277;253;259
12	1138702299	Andara DH	Namibia Other	4/14/16	-18.0599	21.44215	Kavango	Andara	198;195		227	174	198		165	206;215	140		160		107;110		152		136;139	179	205	100	134;128	212	152	103;100	
13	1138702333	Nyangana DH	Not Recorded	3/29/16	-18.003337	20.67655	Kavango	Nyangana	192		227;258		204	165;159	165	206	122	179	157	187;184	110		168;155	203	139		171	100	118;131	221		103;107	217;253

14	1138702892	NyanganaDH	Not Recorded	3/26/16	-18.00337	20.67655	Kavango	Nyangana	198;195		242		219;198			212;206	131;125	179;182	157	187;184	132;129;104;101		152;165		136		174	113		209;218		103;100	280;262;247;265;259
15	1147452810	NyanganaDH	Not Recorded	4/7/16	-18.00337	20.67655	Kavango	Nyangana	198		248			159			134		160	187	101		165;168	197	133		171	110;85;113;88;116	125	205		107;100	
16	1147452856	NyanganaDH	Not Recorded	3/31/16	-18.00337	20.67655	Kavango	Nyangana	198;195		242		219;198		165	212;206	131;125		157	187;184	76;129;104;101		152;165		136		174	113	121;137	209;218		103;100	274;280;277;262;247;265;259
17	1147452860	AndaraDH	Namibia Other	4/14/16	-18.05999	21.44215	Kavango	Andara	192		242		201		165	209	122	179	160;154	187	98		155		158		174;187;177	110;106;113;116	112;125	209		103;100	274
18	11474528	Nyangana	Not Recorded	4/10/16	-18.003	20.67655	Kavango	Nyangana	192		227		201;198	162;156	165	206		182	157;160	184	91		152;165;155	194;212	149;143;146		168;171	94;97	128;112;131	221		107;100	

	85	DH			37																												
19	1147453230	SibbindaHC	Sachinga	2/19/15	-17.787745	23.83366	Caprivi	Katima	195	173	227;242	171	201	162;165	162;165	206	125		157	187;184	113;104	146;155	171;165	221;209	139	173;191	143;184	110;106	128	218	170	113;103	238;241;253
20	1147453238	SibbindaHC	Namushasha	2/13/15	-17.787745	23.83366	Caprivi	Katima	198	170		171	198	165	162	212	131	185	157	187	107;110	155	155	200;203	152	182	184	103	137	209	149	100	268
21	1147453243	SibbindaHC	Sibbindavillage	4/8/15	-17.787745	23.83366	Caprivi	Katima	192	167	242	171	198	165	165	206	128	179	157	184	123	164	165	200	139	173;170	177	113	128	218	161	100	286
22	1147454145	NyanganaDH	NdongaLinena	4/12/16	-18.00337	20.67655	Kavango	Nyangana	192	173;164	258;242	165;174	204;201;198	168;156		212	125;122	182	163	187;184	110;120	164;155	177;168	200;206	139;146	173;197	174	136;106	131;125	215	167;161;164	113;103	271;253;265
23	11484	Sibbi	Kansoko	3/2/	-17.7	23.83	Capri	Katima			273	165;1		165		221	125	179	157		110		168	194	130	188;191		110;103;97	121	212		103	268

	98561	ndaHC		16	8745	366	vi					71																					
24	1148499601	SibbindaHC	Kaswabenga village,kasheshe area	2/26/16	-17.787445	23.833366	Caprivi	Katima	192	161	242		198	159	165	212	128	182	157	187	110;113	164	155	209	139	194	168	106	128	209	173	113	247
25	1148499615	SibbindaHC	Building together	4/11/16	-17.787445	23.833366	Caprivi	Katima	202;189;195	167;176	258	174	201;198	162	165		137;131;128;122	179;182	157		94;98;101	155	168	197;203;206	139;149;146	173;194;182;203	184;171;140	82	134	221;212;209;205	173;167;176;161;158	103;100	238;280;262
26	1148499618	SibbindaHC	Muzimbwa village	4/14/16	-17.787445	23.833366	Caprivi	Katima	192	164	242	171	201	156		206	128	182	157	187	120;116	155	165	215;212	146	185	174	103;97	118	212	158	103	268
27	11484999	Sibbind	Nampengu	4/13/16	-17.787	23.833	Caprivi	Katima			242	171;174	201	162		212	125	179	157		94;110;113;104		152;168	194;197	146		168;171	119	125	215		107	268;250;247;253

	645	aHC			45	66																											
28	1148499652	SibbindaHC	Masida	4/13/16	-17.78745	23.83366	Caprivi	Katima			251	171		165		206	143		157				165	194	130	167;146		110	121	212			262
29	1148499655	SibbindaHC	Matanka,sibbinda	3/2/16	-17.78745	23.83366	Caprivi	Katima			245	171;174	201	162;165			131;122	182	157		110;120		168;155	200;206	136	155;212;146		103;116	134;121	209;205		103	271
30	1148499668	SibbindaHC	Kaenda	3/16/16	-17.78745	23.83366	Caprivi	Katima			242	171	201	156	165	212	122	179	157;160		110;113		158	203	139;143	149;146	137	94	125	218		103;107	268;283;271;280
31	1148499672	SibbindaHC	Ngonga	3/11/16	-17.78745	23.83366	Caprivi	Katima	192	167	242	174	198	165	168	221	125	182	157	184	94	155	177	203	136	176	168	94;110	121	205	158	103	268
32	1148	Sibb	Mabanga	3/9/	-17.	23.8	Capri	Kati			258;242	171	201;1	162;165		206;215	137;122	185;179	157		110;101		165;155	200;206	136;139	191		113;97	121	215;209		103;100	250

	499673	indaHC		16	78745	3366	vi	ma					98																				
33	1148499674	SibbindaHC	Nakakwa,kaedaarea	2/26/16	-17.78745	23.83366	Caprivi	Katima	202	173	227	171	201	162	168	221	125	179	160	187			177	209	146	182	181	88;116	121	205	161	103	250
34	1148499677	SibbindaHC	Kenyani,kasheshearea	2/19/16	-17.78745	23.83366	Caprivi	Katima	192	167	242	174	198	165	168	221	125	182	157	184	94	155	177	200;203	136	176	168;184	110;88	121	205	158	103	268
35	1148499679	SibbindaHC	Izwii	1/7/16	-17.78745	23.83366	Caprivi	Katima	195	167	242	171;174	201;198	162	162	206	140;137;128;125	179;182	160	187;184	107;110	164;155	168	197;212	149	191;170	184	103	128;131	199	143;146;152;149;155	103;107	244;280;262;259
36	1148499683	SibbindaHC	Sibbinda	1/27/16	-17.78745	23.83366	Caprivi	Katima	192	185	258	171;177	201	162	165	206	122	182	157	184	110	155	165	194	143	176	171	97	112	221	136;134	103	253

37	1148499721	SibbindaHC	Sachinga	4/27/15	-17.78745	23.83366	Caprivi	Katima	198;195	164	258	174	219;198	165	162;165	206;215	122	179;167		184	101	164	152;155	197;206	139;143	179;170	187	122	128		167;101;161;155	103	253
38	1148499739	SibbindaHC	Namushasha	2/12/15	-17.78745	23.83366	Caprivi	Katima	192	173;191	227	171;174	198	165	168;162	212;206	119	182;167	157;160	187;184	104;101;116	164;155	152;165;168;155	197;200	136;146	212;191;170	184;171;208;196	110;113;119;116	121;125	215;221;218	143;146;152;149		268;271;274
39	1148499751	SibbindaHC	Malundu	2/12/15	-17.78745	23.83366	Caprivi	Katima	195	170;179	227	165	201	162	165	212	125;122	179	157	187;184	126;94;82;98;129;91	164;172	165;168	200;191	139	188;185	174;177	110;91	134;128	209	146;161;155	103	250
40	1148499773	SibbindaHC	Sibbinda	2/17/15	-17.78745	23.83366	Caprivi	Katima	195	173	227;242		201	162;165	162;165	206	125	179	157;160	187;184	94;113;104	146;155	171;165	221;209	139	173;152;191;170	184	110;106	128	218	170	113;103	238
41	11484997	Sibbinda	Sachinga	3/29/16	-17.787	23.83366	Caprivi	Katima	195	173;176	227	171;174	201	162	162	212;206	128;125	179	157	187	110;101	155	168	197	152;136	173;191	193;177	113	118;131	215;205	191;200;167;176;158	103	271

	81	HC			45																												
42	1148499787	SibbindaHC	Sachinga	3/10/16	-17.78745	23.83366	Caprivi	Katima	195	176	258	171	201	162	165	221	122	179	157	184	120	164	155	197	133	170	184	97	121		173	103	268
43	1148499799	SibbindaHC	Intobo village, Kaenda area	3/16/16	-17.78745	23.83366	Caprivi	Katima	189	176	258		201;198	162	162	224;212	128	167		184	120;101	164;155	152;165	197;200	146	173	165	94;110;91	121;112	209	161;158	103;100	229;274
44	1148499800	SibbindaHC	Makusi	2/22/16	-17.78745	23.83366	Caprivi	Katima	198;195	167;158	258;242	171	198	162;165	165	206;221	122	179;182	160	187	132;110	155	177	197		176;188;194;170	137;181	103;116	128;131;125	215;199	170;164	103;107	268;262
45	1148499824	SibbindaHC	Masida	4/8/16	-17.78745	23.83366	Caprivi	Katima	184;189	173	242	171;174	219;201;198	162	165	212;221;215	125;122	179	157	187	110;101	164;155	152	197	136;139	176;188;185	153	113;97	121;131	205	161;158	103;97	262
46	1148	Sibb	Sibbinda	1/27	-17.	23.8	Capri	Kati	192	185	258	177	201	162	165	206	122	182	157	184	110	155	165	194	143	176	171	97	112	221	136;134	103;100	250;253

	499835	indaHC		/16	78745	3366	vi	ma																								
47	1148499854	SibbindaHC	Makanga	4/22/16	-17.78745	23.83366	Caprivi	Katima	189;195	170;161;179;164	227;242;273	171	201;198	168	162;165		140;122	179;182	157	187;184	110	164;155	152;165;155	194;200;203;191;206	139;143;146	173;182	174;140;162		140;128;112;125	230	161;164	103	286;268;289;271
48	1148499857	SibbindaHC	Makusi Village	3/9/16	-17.78745	23.83366	Caprivi	Katima	202	164	242	171	201	165	168	197	125	179	160	187	82	172	152	197	136	209	174;187	110;106	121	218	197;161	100	268
49	1148499866	SibbindaHC	Ngonga	3/11/16	-17.78745	23.83366	Caprivi	Katima	192	167	233		201	162	162	212		185	157	184	91	155	152;177	206	143	182	174	110	125	218	146;149	103;100	
50	1148499884	SibbindaHC	Munwewoke village, Sach	4/13/16	-17.78745	23.83366	Caprivi	Katima	189	170;167	242		201	153;165	165	206;209	128;125	182	157;160	187;184	123;110;113;120	164	152;165;155	194;200	136	173;188	174;187	110;113;103;116	128;131	215;212	167;164	103;107	271

			ingaarea																														
51	1148498885	SibbindaHC	Kasheshe	3/2/16	-17.787445	23.833366	Caprivi	Katima	198	170	258		198	165	165	218	122	167	157	187	113	155	165	197	139	191	187	106	125	205	164	103;100	
52	1148499903	SibbindaHC	Masida	4/12/16	-17.787445	23.833366	Caprivi	Katima	184;189;195	170;173	258;242	171;174	219;201;198		165	212;206;215;209	131;125;122	179;182	157;160	187;184	110;101	164;155	152;155	197	136;139;155	206;176;185;197;182	153	110;113	121;128;131;125	205	170;179;182;161;158	103;97	262;265
53	1148499904	SibbindaHC	Lubuta	3/23/16	-17.787445	23.833366	Caprivi	Katima	202;195	173;158	227;242	171	198	162	165	206	128;122	179	160	187	107;110;113;101	164;155	168	200	139;146	191;197	165;181;171	103;116	134;125	212;209	161	94;97	268;271
54	1148499907	SibbindaHC	Makanga	4/7/16	-17.787445	23.833366	Caprivi	Katima			242	168;174	198	165	165	197	122	179	160	184	101		180	209	133		168	110	128	212		110	259
55	114	Sib	Mas	4/1	-17	23.	Cap	Kati			242		198	162	165	206;209	128;125	179;182	157;160		101		152;168	194;197	143	149	171	113;122	121;131	209		107;110	274;262

	8499908	bindaHC	ida	3/16	.787745	833366	rivi	ma																									
56	1148499914	SibbindaHC	Kachilamupepo	2/26/16	-17.787745	23.833366	Caprivi	Katima	198	167	273	171	198	165	165	221	125	179	157	184	110	155	168	194	130	203	190	110	121	212	158		268
57	1148499919	SibbindaHC	Masida	4/8/16	-17.787745	23.833366	Caprivi	Katima			242	171	198	159	162	215	125	179	157		120		168	203	136	149		113;97	134	215		100	268
58	1148499925	SibbindaHC	Nampengu	4/13/16	-17.787745	23.833366	Caprivi	Katima			242	171;174	201;198	162		212	125	179	157		94;110;113;104		168	197	143;146	149		113;119	125	215		107	250;247;253
59	1148499933	SibbindaHC	Makanga	4/25/16	-17.787745	23.833366	Caprivi	Katima			261;227	165;171	201	156	168		128;125;122	179;182	157		126;120;116		180;168	215;200	136;143			110;119	118;131;125	212;209;205		103	268;271

60	1148499937	SibbindaHC	Mabanga	3/23/16	-17.78745	23.83366	Caprivi	Katima	189;192	170;173	251	171	201	165;156	162;165	212;206	143	179	157	184	132;129;113;104;101	172;155	165	194	130;143	179	177	110;88;103	121;115	212	136;191;188;146;149;182;155	103;100	268;271;262;259
61	1148499941	SibbindaHC	Kaliyangile	4/13/16	-17.78745	23.83366	Caprivi	Katima			242	165;171	198	162	165	206	137	179	157	187	91		152	218	139		177;199	100	125	205		100	271
62	1148499961	SibbindaHC	Masida	4/12/16	-17.78745	23.83366	Caprivi	Katima			227;258;242		219;201;198	162			131;125;122	179;182	157;160	187	110;120;101		152;165;155	197;200	136;139;158;155	149;146		110;113	121;128;131;125	215;221;205		103;97	268;271;262
63	1148499970	SibbindaHC	Mpancha,sibbinda	4/20/16	-17.78745	23.83366	Caprivi	Katima	198;195	170;176;164	258;242	171	204	162	162;165	206;218;215;209	134;128;122	179	157	187	110;113;120	172;155	171;152	197;209	136;139	176;191;197;170	168;181	128;106;113	121;128;131	212;209;205	167;176;158	97	268;262
64	1148500000	Nyangana	NdongaLinena	4/8/16	-18.003	20.67655	Kavango	Nyangana	208;198;195	170;161	242;248	171	201	159	165	212;215	128;122		157	187;184	107;110	164	168	206;209	139;146	173	165;177	100;103;97	125	212;205	188	103;107	268;262;265

	54	DH			37																												
65	1148500071	SibbindaHC	Lubuta	4/21/16	-17.787445	23.83366	Caprivi	Katima	195	176;164	258	171	204;201;198	162	162	206;215	134;122	179	157	187	120	172;155	152	197;209	136;139;143	176	168	128;125;106	121;128	212;209;205	176;158	103;100	268;262
66	1148500074	SibbindaHC	Kaliyangile	4/19/16	-17.787445	23.83366	Caprivi	Katima			242	171	198	162	165	206	137		157	187	91		152	218	139	161	177	100	125	205		100	271
67	1148500076	SibbindaHC	Makanga	4/28/16	-17.787445	23.83366	Caprivi	Katima	192	170	258	174	201	156	162	206	134	179	160	184	98;101	155	155	197	143	176	177	110	125	218	179	103;100	268
68	1148500077	SibbindaHC	Makanga	4/26/16	-17.787445	23.83366	Caprivi	Katima	195		242	174	198	162	165	212	122		160	187	110		155	197	136	191	187	106	121	205		103;100	271
69	11485	Sibbi	Makanga	4/19/	-17.7	23.83	Capri	Katima	192		258	174	201	156	162	206	134		160	184	101		155	197	143		177	110	125	218		103;100	268

	00083	ndaHC		16	8745	366	vi																												
70	1148500086	SibbindaHC	Makanga	4/22/16	-17.78745	23.83366	Caprivi	Katima	189	173	251	171	201	165	162	206	143;140	179	157	184	132;129;113	172	165	194	130	179;176	177	110	121	212	188	103	262
71	1148500088	SibbindaHC	Naikakhela,kasheshe	4/19/16	-17.78745	23.83366	Caprivi	Katima	198;192	164	258;245	171	198	162	165	206	128;122	179;167	160		110;120;101	155	155	203	152;146	179;176	174	106	125		170;167;164	107;100	274;277
72	1148500096	SibbindaHC	Masida	4/19/16	-17.78745	23.83366	Caprivi	Katima	198;195;192	170;176;164	258;242	171	204;201;198	162	162;165	206;215	134;125;122	179	157	187;184	120	172;155	171;152	197;203;209	136;139	176;191;182	168;181	128;106;113	121;128	212;205	176;158	103	268;262
73	1148500099	SibbindaHC	Nukwa Village	4/22/16	-17.78745	23.83366	Caprivi	Katima	195	167	242	171	198	162	165	206	131	179	157	187	120	164	152	200	146	197	171	106	128	215	179		271

74	1148500100	SibbindaHC	Makanga	4/19/16	-17.78745	23.83366	Caprivi	Katima	192	170	258	174	201	156	162	206	134	179	160	184	98;101	155	155	197	143	176	177	110	125		179	103	268
75	1148500102	SibbindaHC	Katongo village, sibbinda	4/19/16	-17.78745	23.83366	Caprivi	Katima	189;195;192	173;176			201;198	162	165	215	137;131;122	179;182	157		94;82;98;91;101	155	177;165;168	197;203;206	149;146	173;194;182	171;140	103	134		170;173;167;161	103	238;262
76	1148500103	SibbindaHC	Masida	4/28/16	-17.78745	23.83366	Caprivi	Katima	204	173	227	171	198	168	165	212	125;122	173;179	157;160	187	120	155	177	206	139	179;191	137	91		215	167;149	103	283
77	1148500107	SibbindaHC	Kasese	4/28/16	-17.78745	23.83366	Caprivi	Katima	195;192	167;185	258;242	171;177	201	162	162;165	212;206	122	179;182	157;160	187;184	110;101	155	165;168	194;200;212	149;143	176;191	177;171	103;97	115;112	199;221		103;100	253
78	1148500	Sibbind	Makanga	4/19/16	-17.787	23.833	Caprivi	Katima	198	161	242	171	201	168	165	206	125	188	157		113	164	165	209	152	197	171	100	125	221	146;170	103;100	271

	114	aHC			45	66																											
79	11485001116	SibbindaHC	Shaile	4/18/16	-17.78745	23.83366	Caprivi	Katima	192	164	242	171	201	156	165	206	128	182	157	187	120	155	165	215	146	185	174	103	118	212	158	103	268
80	11485001119	SibbindaHC	Goldenhalfway	4/14/16	-17.78745	23.83366	Caprivi	Katima			242		201	153;165	165		128;125		157;160	187;184	123;110;113		152;165;155	194;200	136	152;149	174;187	103;116	128;131	215;212		103;107	271
81	11485001121	SibbindaHC	Nampengu	4/18/16	-17.78745	23.83366	Caprivi	Katima			242	171	219;201;198	162;165;156	162;165	212;203	140;128;125;122		157	187	110;91;120;116		165;202;189	197;200;206	136;143;158		174;190;177	106;91	134;131	218;224			244;268;271;262;250;247;253;265
82	11485001123	SibbindaHC	Ngonga	4/27/16	-17.78745	23.83366	Caprivi	Katima	187;192	167	258;242	171;174	201;198	165	165	221	134		157		123;98;101	155	152;177	197;203;206	136	176;191	137;147;168;165;181;184	103	134;121		112;161;158	103	268;277
83	1148	Sibb	Nabulon	4/19	-17.	23.8	Capri	Kati	198	164	245	179;1	198	162	165	206	128	179	160	187	82;110;120;101;116	155	155	203	152	179;176	174	106	125	215	167;164	107	277

	500133	indaHC	gwevillage, kasheshearea	/16	78745	3366	vi	ma				71																					
84	1148500138	SibbindaHC	Masida	4/29/16	-17.78745	23.83366	Caprivi	Katima	198	167	242	171	198	162;159	162	212	134;131	179	160	187	110	155	180;177	209	139	179	147	110	121	215	176	103	271
85	1148500145	SibbindaHC	Sinjwara,makanga	4/27/16	-17.78745	23.83366	Caprivi	Katima	198;192	167	242	174	219;198	165	168;162	221	134;125	179;182	157;160	184	94;98;113;91;116	155	152;177	203	136;133	173;152;176;197	168;184	103	134;121	205;218	191;152;158	100	268
86	1148500146	SibbindaHC	Mpacha Village	4/21/16	-17.78745	23.83366	Caprivi	Katima	189;192	170;167	242		204;201	153;165	165	206;209	128;125	182	160	187;184	123;94;110;113;120;101	164	165;155	194;200	136	173;188	174;137;165;187	103;116	134;128;131	215;212	167	103;107	271
87	114850	Sibbin	Makanga	4/15/	-17.78	23.833	Caprivi	Katima	202;189;195;192	173;167;176	258;273	171;174	198	162		215	137;131;122	179;182	157;160	187	94;98;101	155	165;168;155	194;197;203	139;149;146	173;194;182	171;140	103	134	221;212;209;205	191;170;173;167;176	103	235;238;280;262;256

	0147	daHC		16	745	66																											
88	1148500149	SibbindaHC	Kaenda	4/22/16	-17.787745	23.833366	Caprivi	Katima	192		258	171	198	165	165		128				123		180	194;203		215	174	110	131			103	253
89	1148500152	SibbindaHC	Muguza	4/19/16	-17.787745	23.833366	Caprivi	Katima	195	173;167	248	174	201;198	165;156	165	218	116	182	157	184	94;98;101	155	152	197;203	152;136	176;185;182	184	110;113	121;137		149	103	256
90	1148500153	SibbindaHC	Sibbinda	4/21/16	-17.787745	23.833366	Caprivi	Katima			258;273	174	201;198	162	165	215	131;122		157;160	187	101		165;168	197;203	139		184;171;140	103	134	205		103;100	277;253
91	1148500157	SibbindaHC	Masidia	4/18/16	-17.787745	23.833366	Caprivi	Katima	192	170	242		198	162	165	221	122	179		184	107;110;101	155	155	200	143	179	193;171	110	121;131	205	167	103	268
92	114	Sib	Chinc	3/1	-17	23.	Cap	Kati	189;192	170;173	251		201	165;156	162;	212;206	143	179	157	184	132;129;110;101	172;155	165	194	130;143	179;176	177	110;103	121;115	212	136;188;146;	103	262

	8500168	bindaHC	himani	1/16	.787745	833366	rivi	ma							165																149;182;155		
93	1148500169	SibbindaHC	Sibbinda	3/16/16	-17.787745	23.833366	Caprivi	Katima	192	170;173	242	171	201;198	162;165	165	206;215	137;122	185;179	157	184	110;101	164;155	165;168;155	200;206	136;139	179;188	165	113	121	215;209	173;152;161;155	103;100	250
94	1148500172	SibbindaHC	Kapani,masida area	3/11/16	-17.787745	23.833366	Caprivi	Katima			242		198	162		212				187	110		152	197	146		196	94	125			103	262
95	1148500174	SibbindaHC	Sibbinda	4/12/16	-17.787745	23.833366	Caprivi	Katima			248			162		206	125				139		155	197	136		181	94	121	205		103	268
96	1148500193	SibbindaHC	Njanganjanga	4/4/16	-17.787745	23.833366	Caprivi	Katima			242		198	165		209	125				101		152	209	146		171	110	131	205		110	268

97	1148500202	SibbindaHC	Ngonga	3/11/16	-17.787445	23.833366	Caprivi	Katima	192	167	242	174		165	168	221	125	182	157	184	94;91	155	177	203	136	176	168	110	121		158	103	268
98	1148500207	SibbindaHC	Chinchimane	3/2/16	-17.787445	23.833366	Caprivi	Katima	195	170	242	171	201	165	165	209	128	179	154	187	110	155	152	203	136	185	174	106	131	218	112;170	103	253
99	1148500217	SibbindaHC	Nampengu	2/26/16	-17.787445	23.833366	Caprivi	Katima			258;248		201	162;165		221	125			187	120		168	209;212	139;146		181;177	106	121	209;205		103;119	250
100	1148500224	SibbindaHC	Lubuta	3/11/16	-17.787445	23.833366	Caprivi	Katima	189	173	251			165	162	206	143	179	157	184	94;132;129;113	172	165	194	130	179;176	168;177	110	121		188;158		262
101	11485002	Sibbinda	Lubunjetwo	2/8/16	-17.787	23.833366	Caprivi	Katima	195	173	242	171	198	168	162	206	131	182	160	184	110;113	164	168	206	143	188;191	171	103	134	209	161	103	274

	25	HC			45																												
102	1148500249	SibbindaHC	Sachinga	3/17/16	-17.78745	23.83366	Caprivi	Katima	195;192	173;176	258		198	162	162	206;197;209	128	179	160	187	98	155	171	197	139;149	173;170	205;181	116	128;125	224	191	103;100	277
103	1148500272	SibbindaHC	Sibbinda	5/18/15	-17.78745	23.83366	Caprivi	Katima	195;192	161	258;242		204;201;198	165	165	206	137;125;122	179	160	184	120	155	152;168;155	206	139;149;146	173;176	174;181;177	110	118;131		109;170	103;100	268
104	1148500282	SibbindaHC	Kaliyangile	4/26/16	-17.78745	23.83366	Caprivi	Katima			288		198	162	162	206	119	185	157	187	101		152	200	146		171;199	103	134	221		103	
105	1148500292	SibbindaHC	Kanono	2/20/15	-17.78745	23.83366	Caprivi	Katima	192	191	227	174	198	165	162	212	131	182	157	187	116	155	152	197	136	191	196	119;91	121	218	143;146	103	271;274
106	11485	Sibbi	Masida	4/15/	-17.7	23.83	Capri	Katima			242	171	198	162	162	212	131		157	184	110;113;120		152	197	152;143		174;137	100;106	121;131	215;227		103;97	244;262;247

	00297	ndaHC		16	8745	366	vi																										
107	1148500300	SibbindaHC	Nampengu	4/28/16	-17.78745	23.83366	Caprivi	Katima	195;192		242;236	171;162	201;198	162	165		119;128			184	101		152	197;200;191		191	171	103;97	118;134;128		146	103	268
108	1148500303	SibbindaHC	Sikubi	4/19/16	-17.78745	23.83366	Caprivi	Katima			258	171	201	165	165	221	134		157	184	101		152	206	136	158	181	103	134	218		103	277
109	1148500308	SibbindaHC	Masida	4/11/16	-17.78745	23.83366	Caprivi	Katima			242	171	198		162	206	131		157	184	113;120		152		152;143		174;137	100;106	131	227		103;100	262;247
110	1148500313	SibbindaHC	Makanga	4/19/16	-17.78745	23.83366	Caprivi	Katima	198;192	170;173	227	171	201	162	165	212	119	185	157		104;101	172;155	152	224	152;146	197	168;177	113	121;128		179	103	271;265

111	1148500314	SibbindaHC	Kahunikwa	4/21/16	-17.78745	23.83366	Caprivi	Katima	198	173	242		198	162	165	212	131;128	179	157	184	88;91	155	152	200	136	173;170	168	119	137	230	109;164		265
112	1148500316	SibbindaHC	Nampengu	4/20/16	-17.78745	23.83366	Caprivi	Katima			242		201	165	165	209	128		160	184	110		155	200	136		174	116	131	212		107	271
113	1148500321	SibbindaHC	Shaile	4/29/16	-17.78745	23.83366	Caprivi	Katima	198	167	242	179;171	198	162	162	233	140	179	157	184	88;82;91;85	164	165	209	152	179;176	174	97	155	215	158	103;100	247
114	1148500323	SibbindaHC	Makanga	4/26/16	-17.78745	23.83366	Caprivi	Katima			258	174	198	162	165	215	131;122		157	187	101		165;168	197;203	139;146	191	171	103	134	205		103	280;262
115	11485003	Sibbinda	Kahunikwa villag	4/18/16	-17.787	23.83366	Caprivi	Katima	195	167;161	258;242		201	162	165	197;194	128	179;182	157;160		120	164	165	162;159;165	136;146	200	187	103	134		176;161	100	268

	24	HC	e, Kasheshe area		45																												
116	1148500326	Sibbinda HC	Kaliyangile	4/19/16	-17.787445	23.83366	Caprivi	Katima			258	174	201;198	162			131;122	182	157		88;94;82;91;101		177;165	197;203;206	139;149	176;146		103	134;128	209;205		103;100	238;280;262;256
117	1148500327	Sibbinda HC	Kahunikwa village, Kasheshe Area.	4/19/16	-17.787445	23.83366	Caprivi	Katima	198	173	242	171	198	162	165	206	137	179	157	187	91	155	152	215;221;218	139	182	177	100	125	205	161;158;164	100	271
118	1148500330	Sibbinda HC	Makanga	4/28/16	-17.787445	23.83366	Caprivi	Katima			258	174	198	162	165	215	131;122		157;160	187	101		165;168	197;203	139;146		171	103	134	205		103	280
119	11485	Sibbi	Nampe	4/28/	-17.7	23.83	Capri	Katima			242	171	198	162	165	206;215	119			187;184	101		152;149	197;200			171	97	118	221		103	

	00335	ndaHC	ngu	16	8745	366	vi																										
120	11485003340	SibbindaHC	Makanga	4/20/16	-17.787445	23.833366	Caprivi	Katima	192		258	174	201	156			134		160	184	101		155		143	191	177	110		218		103	268
121	11485003341	SibbindaHC	Makusi	4/18/16	-17.787445	23.833366	Caprivi	Katima	192		221	171	198	165	165	206	122		157	187	110;120;116		168	212	146	191;146	171	110	121	215			292
122	11485003343	SibbindaHC	Makanga	4/15/16	-17.787445	23.833366	Caprivi	Katima	195	173	242	171		168	162	206	131	182	160	184	110;113	164	168	206	143	188;191	171	103	134	209	161	103	274
123	11485003345	SibbindaHC	Masida	4/27/16	-17.787445	23.833366	Caprivi	Katima			273	171	201	162	162	206	128		157	187	110		152	200	139	167	181	119	125	221		100	271

124	11485003446	SibbindaHC	Kaliyangile	4/26/16	-17.78745	23.83366	Caprivi	Katima			273		191;201	162	165	206	122		157	187	101		165;168	203	139	158;146	184	103	134	205		103	280
125	11485005510	SibbindaHC	Masesese village ,sibbinda constituency	3/1/16	-17.78745	23.83366	Caprivi	Katima			227	171	198	165		206	131	167	157		120		152	206	155			103	121	215		103	268
126	11485005518	SibbindaHC	Njanganjanga	4/4/16	-17.78745	23.83366	Caprivi	Katima			258	177	201		165	206	122	182	157	184	110		165	194	143		171	97	112	221		103	253
127	11485005519	SibbindaHC	Kaliyangile	3/22/16	-17.78745	23.83366	Caprivi	Katima			258	171	198	165	165	218	122	167	157	187	113		165	197	139	191		106	125	205		103	271

128	1149099753	Nyangana DH	Mashare	4/7/16	-18.00337	20.67655	Kavango	Nyangana			227;242	171		162;156	165		125	182	160	187	126;129			203;206	133	173	174		128	218		103;100	
129	1149099754	Nyangana DH	Not Recorded	4/7/16	-18.00337	20.67655	Kavango	Nyangana			227			162	162		128	179	160	184	88		152	224;197	139	173	165		125	205		103;100;97	
130	1149101684	Nyangana DH	Ndonga Linena	4/17/16	-18.00337	20.67655	Kavango	Nyangana	195;192		227;258;242	171		162		212;206	128;125	179	157;160	187;184	110;101		171;165	197;200;203	136;143;146	176;182	165;177	125;116;97		199		103;107;100	
131	1149101685	Nyangana DH	Ndonga Linena	4/26/16	-18.00337	20.67655	Kavango	Nyangana	195		227	171	198	162	155	212	128	179	154	184	120		168	197	136	176	165	100	118	199;209		103;107;100	
132	11491016	Nyangana	Ndonga Linena	5/15/16	-18.003	20.67655	Kavango	Nyangana	202;195		227;258	171;174	201;198	168;162	155;165	212;206	128;125	179;182	157;154	187;184	107;110;113;120;101;116		168	197;200	136;143	176;191	193;165	100;113	118;134	215;212;209;218		103;107;100	

	86	DH			37																												
133	11491016888	NyangananaDH	Ndonga Linena	5/10/16	-18.00337	20.67655	Kavango	Nyangana	198		242	174	201	156	165	212	122	179	157	187	82;139;110;91;120		168	194	139	173	177	85;113;88	128	215		100	274
134	11491016889	NyangananaDH	Ndonga Linena	4/20/16	-18.00337	20.67655	Kavango	Nyangana	198;195		258	171;174	201	162;165	162	212;221	128	179;182	157;160	187	123;98;101		152;177	224;191	139;146	176;182	165	110	121;131	218		107	250
135	11491016890	NyangananaDH	Not Recorded	4/7/16	-18.00337	20.67655	Kavango	Nyangana	198;189;192		227;242	171;177	201;198	162;156	162;165	212;206;215	134;116;128;122	179;182	157;160	187;184	110;91;120		152;165;155	197;200;212	149;143;146	179;176;188;170	168;171	110;100;106;113;103;97	134;128	221;218		103;100;97	
136	11491016891	NyangananaDH	Ndonga Linena	4/25/16	-18.00337	20.67655	Kavango	Nyangana	189		242	174	201	162	165	206	122	182	157	184	120		168	197	143	173	177	119	121			103;100	247
137	11491	Nyang	Ndonga Li	4/6/	-18.0	20.67	Kavan	Nyang	187		242	171	201;1	162;165	165	206	122	179	157;160		101		168	197	143	173;158		100;103	128	209		103;100	268

	01692	anaDH	nena	16	0337	655	go	ana					98																				
138	1149101694	NyanganaDH	NdongaLinena	5/19/16	-18.00337	20.676555	Kavango	Nyangana	181		227	171		162	162	212	140;125	179	157	187	101		177	206	139	191	190	103	128	221;218;224		103;100	
139	1149101695	NyanganaDH	NdongaLinena	6/8/16	-18.00337	20.676555	Kavango	Nyangana	198		242	171	204	162	162	215	122	179	157	184	120;101		152	194;197	136	200	177	110	121	205	152	103;100	
140	1149101697	NyanganaDH	NotRecorded	4/6/16	-18.00337	20.676555	Kavango	Nyangana	195		258;242	171	198	162;156	165	206;215	119;125	179	157	187;184	110;101		177;165;168	200;203	139;143	173;188;200	174;165;177	110;85;125;106;88;91	134;125	205		103;107;100	
141	1149101698	NyanganaDH	NdongaLinena	4/18/16	-18.00337	20.676555	Kavango	Nyangana	192		258	171	198	162	165	206	125	179	157	187			171	200	146	182	177	116	137	199		100;97	271

142	1149101699	NyangananaDH	Ndonga Linena	4/27/16	-18.00337	20.67655	Kavango	Nyangana	198;189		242	174	201	156	165	212	122	179	157	187	139;110		165;168	194;197	152;139	173	174;177	113;116	121;128	215;212;209		100	
143	1149101700	NyangananaDH	Not Recorded	4/20/16	-18.00337	20.67655	Kavango	Nyangana	192		273	171	198	162	162	221	125	179	157	184	139		168	194	136	173	190	110	121	218		103;100	274
144	1149101701	NyangananaDH	Not Recorded	4/7/16	-18.00337	20.67655	Kavango	Nyangana	192		242	174	198	162	165	212	122	179	157	184	110;113		155	197	136	170	165;187	113	118	221			247
145	1149101702	NyangananaDH	Not Recorded	4/1/16	-18.00337	20.67655	Kavango	Nyangana	195;192		242	171	198	156;159	165	206;218	134;122	179	157;160	184	110;113;104		152;165;168;155	197;200;191	136;139	170	174	110	118;115	215;199;212;218		113	271
146	11491017	Nyangana	Ndonga Linena	4/27/16	-18.003	20.67655	Kavango	Nyangana	192		258;242	171	198	162;159	155;165	212;206	125;122	179	157;154		101		171;168	197;200;203	136;143	176;182	165;177;171	113	118;137;125			107;100	262

	09	DH			37																												
147	1149101710	NyanganaDH	NdongaLinena	4/27/16	-18.00337	20.67655	Kavango	Nyangana	198		248		198	162	162	215	128				120		152	197		197	181	110	121			103	
148	1149101711	NyanganaDH	NdongaLinena	4/19/16	-18.00337	20.67655	Kavango	Nyangana	195		242	174	201;198	156	162	212	134;140	179	157	187	120		168	200;209	143	194	181;177	110	128	215;218		100;97	271
149	1149101712	NyanganaDH	NdongaLinena	4/6/16	-18.00337	20.67655	Kavango	Nyangana	198		248	174	201	159	165	206	134	182	160	187	101		168	159;197	133	185	171	113	125	205			253
150	1149101713	NyanganaDH	RunduRuralW	4/23/16	-18.00337	20.67655	Kavango	Nyangana	198		227	171	198	162	162	218	128	179	160	184	88		152	224;221;227	139	173	165	113	125			100;97	271
151	11491	Nyang	NotRecor	4/6/	-18.0	20.67	Kavan	Nyang	208;195		242	171	201	156	165	212;209	122	179	157	187	101		152	200	139	197;203	202;156	106;113	121;137	205		107;100	268;271;250

	017114	anaDH	ded	16	0337	655	go	ana																									
152	1149101715	NyangananaDH	NdongaLinena	4/26/16	-18.00337	20.67655	Kavango	Nyangana	195		242	171	201;198	168	162	215	128	182	157;160	184	98		155	197	152;149	173;182	177	116	134	218		100	
153	1149101716	NyangananaDH	NdongaLinena	6/9/16	-18.00337	20.67655	Kavango	Nyangana	198;189		227;242	171	201;198	162;165	162;165	212	140;125;122	179	157;160	187	110;101		165;168	194;197;206	152;139;146	173;176;191;182	174;177	100;106;116	121;128	212;209		103;100;97	286;268;274
154	1149101717	NyangananaDH	NdongaLinena	4/22/16	-18.00337	20.67655	Kavango	Nyangana	192		227			162	162		128	182	157	187	101		165;168	200;212	136;139	176;167	187;177	110;106;113;116	125	215;205		107;110	
155	1149101718	NyangananaDH	Ndiyona	4/7/16	-18.00337	20.67655	Kavango	Nyangana	189		242	171		162	165	212	134;137;125;122	179	157	187	110		165	197	152	176;182	174	116	121	212			

156	1149101719	NyanganaDH	Ndonga Linena	4/5/16	-18.003337	20.676555	Kavango	Nyangana	189;192		227;258;242;248;233	177	201;198	162;156	162;165	212;206;215	116;122	179;182	157;160	187;184	110;91;120		152;165;168;155	197;212	149;143;146	179;176;188	168;171	110;100;106;113;103;97	134;128	218		100	
157	1149101720	NyanganaDH	Not Recorded	4/16/16	-18.003337	20.676555	Kavango	Nyangana	195		227	174	198	162	162	206	122	179;167	157;160	184	110;113;104;101		152;168	197;200	139;143	173	174;177	110;100	121;125			103	271
158	1149101721	NyanganaDH	Ndonga Linena	4/27/16	-18.003337	20.676555	Kavango	Nyangana	198		258	174	198	162	165	206	122	179	160	187	107;110		155	200;203	152	176	177	103	131	205			
159	1149101722	NyanganaDH	Ndonga Linena	4/23/16	-18.003337	20.676555	Kavango	Nyangana	192		258;242	171	201;198	162	162	197	119;125	179;167	157	187	101		152;180;177;155	203;209	143	173;149;161	181;177	110;100;97	115	209;205		103;107;100	235;253
160	11491017	Nyangana	Not Recorded	4/7/16	-18.003	20.676555	Kavango	Nyangana	192		258;242	171		168		212	125;122				101			194	143;146	173;176	184;177	110	118;121				271

	23	DH			37																												
161	1149101724	NyanganaDH	NotRecorded	4/6/16	-18.00337	20.67655	Kavango	Nyangana	198		258	171	198	162;165	162	212;206	140;122	179	157;154	187			152;189	212	149	179;200;182;203	174	100;91	121;125	212		113;103;100	271;253
162	1149101725	NyanganaDH	NotRecorded	4/7/16	-18.00337	20.67655	Kavango	Nyangana			227			162	162	218	128	179	160	184	88		152	224	139	173	165	113	125	205		100	271
163	1149101733	NyanganaDH	NotRecorded	4/4/16	-18.00337	20.67655	Kavango	Nyangana	192		258;242	171	198	162	162;165	212;209	134	182	160	187	101		152;177;155	197;212	152;155	173;182	190	106;91	128;131	218		107;110	286
164	1149101734	NyanganaDH	NotRecorded	4/18/16	-18.00337	20.67655	Kavango	Nyangana	195		258	171	198	156	165	212	143	179	163	184	120		168	194	149	179	190	113	131	212		94;97	
165	11491	Nyang	NotRecor	4/5/	-18.0	20.67	Kavan	Nyang	195		258	171	198	165	165	206	134	182	160	184	91		165;162	200	139	170	177	106	125	215		103;100	268

	01735	anaDH	ded	16	0337	655	go	ana																									
166	1149101736	NyanganaDH	Ndonga Linena	5/28/16	-18.00337	20.67655	Kavango	Nyangana	192		258	171	198	162;156;159	165	206	128	167	160	184	101		165	162;197;200	139	185	143	110	125	205		100	271
167	1149101739	NyanganaDH	Ndiyona	4/4/16	-18.00337	20.67655	Kavango	Nyangana	192		242	171	201	156	165	212	122	179	160	187	101		168	197	139	182	184	116	115	209		100;97	280
168	1149101741	NyanganaDH	Not Recorded	4/19/16	-18.00337	20.67655	Kavango	Nyangana	187;192		242	171;174		162;159	165	206	134;122	179;182	157;160	184	110;113;104		168	200	139;149	185;170	174;177	94;110;103	121;128	215		103;100	253
169	1149101742	NyanganaDH	Ndonga Linena	4/28/16	-18.00337	20.67655	Kavango	Nyangana	187;198;192		242	171		162	162;165	206;197;218;209	119	179;182	157	184	104;101		168	200	139;149	185;170	174;184;177	110;100;103	128;131	215;205		103;107	271;253

170	1149101743	NyanganaDH	Ndonga Linena	4/26/16	-18.00337	20.67655	Kavango	Nyangana	187;198;195;192		258;242	171	201	162;156;159	162;165	212;206;215	134;131;119	179	157	187;184	107;98;110;101		165;168	221;197;200;191	130;139;149	176;185;170;182	174;184;177	110;100;113;103;116	134;121;128;115	215;212;209;218		113;103;107;100	256
171	1149101744	NyanganaDH	Ndonga Linena	4/20/16	-18.00337	20.67655	Kavango	Nyangana	192		242	171		165	162	206	122	179	160	187	107;113;116		165	197	143	179	168;165	122	121			103;107;100	265
172	1149101745	NyanganaDH	Not Recorded	4/16/16	-18.00337	20.67655	Kavango	Nyangana	189		242	171	198	153;165	165	215	125	182	157	187	98		180	191	143	173	174	113	121	215		103;97	
173	1149101746	NyanganaDH	Not Recorded	4/20/16	-18.00337	20.67655	Kavango	Nyangana	192		242	174	201	162;159	162	221	131	179	157	187	129		177	162;191	136	188	184	113	128	215		94;97	
174	11491017	Nyangana	Ndonga Linena	4/5/16	-18.003	20.67655	Kavango	Nyangana	198;189;192		227;242;248	171;177	201	162;156	165	212;206;215	116;122	179;182	157	187;184	110;91;120		152;165;168;155	197;200;212	149;143;146	179;176;188	168;171	110;100;106;103;97	134;128				

	47	DH			37																												
175	1149101748	NyanganaDH	Not Recorded	4/1/16	-18.00337	20.67655	Kavango	Nyangana	189;192		227;242;248	177	201;198	162;156	165	212;206;215;209	134;116;128;122	179;182	157	187	110;91;120		152;165;168	197;200;203;212	149;143;146	179;188	168;171	110;100;106;113;97	128;131			100;97	
176	1149101749	NyanganaDH	Ndonga Linena	4/28/16	-18.00337	20.67655	Kavango	Nyangana	198		227	174	198	162;159	168	197	122	182	160	184	101		155	162;197;200	152	191	177	103	118	209		103;107	256
177	1149101750	NyanganaDH	Not Recorded	4/2/16	-18.00337	20.67655	Kavango	Nyangana	192		242	171	201		165	206	122	179	160	187	110		171	203	136	194	184	113	131	212		103	262
178	1149101757	NyanganaDH	Ndonga Linena	4/7/16	-18.00337	20.67655	Kavango	Nyangana	198;189;192		227	171	201	162;165;159	162;165	206	134;128	179;182	157	187	110;91;120		155	162;197;209;212	152;149;146	179;176	171	106;97	128	218		94;100;97	238;271
179	11491	Nyang	Ndonga Li	4/22/	-18.0	20.67	Kavan	Nyang	195;192		258	171	201	162	162	221	125	179	157	184	101		168	194;197	136;158	176	165;187	110	121	215		103;100	271

	01758	anaDH	nena	16	0337	655	go	ana																									
180	1149101759	NyangananaDH	NotRecorded	4/5/16	-18.00337	20.67655	Kavango	Nyangana	195		242	171	198	162;165	165	221	116	182	160	187	82;129		214;217	203;206	136	191	165	91	125	218		103;100	271
181	1149101760	NyangananaDH	NdongaLinena	4/4/16	-18.00337	20.67655	Kavango	Nyangana			227;258;242	171;174	201;198	168;162;165	155;165		125;122	179	157;160;154	187;184	110;91;120;101		152;155	194;197;200	152;139;146	173;179;194;182	168;184	106;103;116	134;121;128;131	209;205;218		103;107	
182	1149101761	NyangananaDH	NdongaLinena	4/26/16	-18.00337	20.67655	Kavango	Nyangana	189		242	174	201	162	162	206	125	179	157	187	82;129;120		168	212	146	173	174	110;106	134	218		103;100	
183	1149101762	NyangananaDH	NotRecorded	4/1/16	-18.00337	20.67655	Kavango	Nyangana			242	171	201	165	162		122	179	160	187	107;113;116		165	197	143	179	168;165	122	121	205		100	265

184	1149101764	NyanganaDH	NotRecorded	4/21/16	-18.00337	20.67655	Kavango	Nyangana	198;195;192		242;248	171;174	201;198	162	155;165		128;122	179;182	157;160	184	123;132;113;120		152;177	197;191	136;143;146	173;194;182	165;187	110;119	128;125	221;209;218		103;100	247;265
185	1149101765	NyanganaDH	NotRecorded	4/16/16	-18.00337	20.67655	Kavango	Nyangana			242	174		162	165	212;206;197;215	122	179;182	157;160	184	123;132;107;113		152	197	143	194	187	119	128;125	221;218;224		100	
186	1149101767	NyanganaDH	NotRecorded	4/3/16	-18.00337	20.67655	Kavango	Nyangana	192		242	174	198	162	165	212;206;197;203	128;122	179	157;160	184	123;132;107;129;110;113		152	194	143	194	187	119	128;125	221;218;224		100	286;283
187	1149101768	NyanganaDH	NotRecorded	4/15/16	-18.00337	20.67655	Kavango	Nyangana	195;192		258	171	198	168;165	162;165	206	125			184	91		165	194;200		188;170	177	82;110;85;106	118;125			107;100;110	
188	11491017	Nyangana	NdongaLinena	4/14/16	-18.003	20.67655	Kavango	Nyangana	198;189		242;245	171	198	162;165	162	206	119	179	157	187;184	91;101		155	194;200	130;136	173;182	165	106;103	121;125	205		103;97	256

	69	DH			37																												
189	11491017770	NyanganaDH	Ndonga Linena	4/23/16	-18.00337	20.67655	Kavango	Nyangana	198;195		242	174	201;198	165		206	131	179;182	157	187	98;129		177;168	200	143;161	194	168;165;184	106	128;131	215	146	113;100;110;116	268;274
190	11491017772	NyanganaDH	Not Recorded	4/23/16	-18.00337	20.67655	Kavango	Nyangana	198;192		227;242	177;174	201	162	165	212;206	125;122	179	157	184	110;120;101		171;177;168;155;174	221;197;200;203	136;139;133	173;176;188;194;170;182	187;184	100;113;119	118;134;131;137	212;209;205		103;107;100	271;247
191	11491017773	NyanganaDH	Not Recorded	4/4/16	-18.00337	20.67655	Kavango	Nyangana	195;192		242	171;174	201	162	165	206	125	179	157	187	107;98;101		165	200	146	194	174	110	115	215	152	103	235;268;238
192	11491017774	NyanganaDH	Ndonga Linena	4/25/16	-18.00337	20.67655	Kavango	Nyangana	198		242	171	198		162	206	119	179	160	187	126;129		168		139	173	177	113	128	221		107;110	274
193	11491	Nyang	Ndonga Li	3/31/	-18.0	20.67	Kavan	Nyang	192	167	242	174	198	162		212	122	179	157	184	113	155	155	197	136	170;167	187	113	118	221	161	103;107	247

	02550	anaDH	nena	16	0337	655	go	ana																									
194	1149102560	NyanganaDH	NdongaLinena	6/13/16	-18.00337	20.67655	Kavango	Nyangana	195		227	171		162		215	125	179	157	187	120		152	200	149	191		103		227			
195	1149102564	RunduDH	RunduRuralE	5/30/16	-17.91639	19.78028	Kavango	Rundu	187;198;189;195;192	170;173;167;176;161;179;164	227;258;242	171;174	201;198	162;165;156	168;162;165	212;206;209	131;119;128;125;122	179;182	157;160	187;184	110;113;91;120	172;155	152;165;168;155	194;197;200;203;209	136;139;133;143;146	155;173;179;185;170;182;149;161	181;177;171	110;100;106;113;103;116	134;121;128;115;131;125	215;221;212;209;205;218		113;103;107;100;110	268;271;274;262;250;253;256
196	1149102576	NyanganaDH	NdongaLinena	4/26/16	-18.00337	20.67655	Kavango	Nyangana		173	242	171			165						129;120	164	152;165	203	136		205;165		118;125	218	158	103;107	
197	1149102586	NyanganaDH	NdongaLinena	5/29/16	-18.00337	20.67655	Kavango	Nyangana			242			156	165	212	122		160	187	101			197	139	182	184		115	209		100	

198	1149102597	RunduDH	Rundu Urban	4/16/16	-17.916639	19.78028	Kavango	Rundu	202	176	242	171	201	171	162	209	134	179	157	187	110	155	165	209	136	200	174	94	112;146	218	185;182	103	271
199	1149102601	NyanganaDH	Ndonga Linena	5/13/16	-18.003337	20.676555	Kavango	Nyangana			242	171		165	165		122	179	160	187	98			197	143	173	177		128	209	146	103	271
200	1149102608	NyanganaDH	Ndonga Linena	5/10/16	-18.003337	20.676555	Kavango	Nyangana	192		258;242	171	198	162;165	162;165	206	134;122	179	157;160	187	110;113		171;158;168;155	197;203	139;143	173;194;182;203	165;184	100	121	212;209;218		103;100	271;277
201	1149102610	NyanganaDH	Ndiyona	6/6/16	-18.003337	20.676555	Kavango	Nyangana	202;189		245	171;174	201	165;159	165		134;125	179;167	157	184			152	200;206	139		181;177						
202	11491026	AndaraDH	Mukwe	5/6/16	-18.05999	21.44215	Kavango	Andara	192	164	227	171	198	162	165	206	149;131	179	169	184	110;120	137;146	155	206	155	176;197	187	94	128	218	170;167	103	

	11																																
203	1149103122	NyanganaDH	Ndiyona	4/4/16	-18.00337	20.67655	Kavango	Nyangana	187		227	171	201	165	165	206	122	179	160	187	120		168	197	143	200;224;146	177	88;91	128	209		100	
204	1149103123	NyanganaDH	Ndonga Linena	4/18/16	-18.00337	20.67655	Kavango	Nyangana	189		242	174	219	162	162	206	140	179	154	187	129		168	197	139	179	184	125	131	224		103	271
205	1149103124	NyanganaDH	Mashare	5/4/16	-18.00337	20.67655	Kavango	Nyangana	195		258	171	201	162;156	165	206	122	179;182	157	187	120;101		165;155	203	139;146	179;212;200;191;170	168	106;91	128;125	224	143	103;107	268
206	1149103125	NyanganaDH	Ndonga Linena	4/28/16	-18.00337	20.67655	Kavango	Nyangana	198		242;248	171	204;201;198	162		215	128;122	179			120		152	197	136	200;197		110	121	215;205	152	103	271
207	11491	Nyang	NotRecor	4/16/	-18.0	20.67	Kavan	Nyang	198		242	174	201	165	152	215	125	182	157	184	129		168	212	152	206;149	174	113	131	218		103	283

	03126	anaDH	ded	16	0337	655	go	ana																									
208	1149103127	NyanganaDH	Mukwe	5/12/16	-18.00337	20.67655	Kavango	Nyangana	198		248	171	201	162	165	215	122	182	160	184	113		177	191	143	182	165	110	125	218		103;100	247
209	1149103128	NyanganaDH	NamibiaOther	4/2/16	-18.00337	20.67655	Kavango	Nyangana	198;192		242	171	204;201	162;156	162;165	212	134;125	179	160	187;184	98;101		168;155	197;200	152;143	170	181;177	113;103;97	121	215;205		103	271;262;250
210	1149103129	NyanganaDH	NdongaLinena	5/7/16	-18.00337	20.67655	Kavango	Nyangana	198		242	174	201	156	162	212	134	179	160	184			155	200	152	170	205	97	125	215		103;100	262
211	1149103130	NyanganaDH	NdongaLinena	4/6/16	-18.00337	20.67655	Kavango	Nyangana	198;192		258;242	171;174	201	168;165	162;165	212;206	125;122	179	160	187;184	101		165;168	194;197	143;146	173;188	184	110;100	118;121	209		103;107	268;271

212	1149103131	NyangananaDH	Ndonga Linena	5/10/16	-18.00337	20.67655	Kavango	Nyangana	189;195		258;242;245	171	198	162	162	212	131	182	157	187	101		152	200	139	179;194	171	110	118	215		103;100	268
213	1149103132	NyangananaDH	Ndiyona	5/4/16	-18.00337	20.67655	Kavango	Nyangana	198		242	171	204	162	162	215	128;122	179	157	184	120		152	197	136	200;197	181;177	110	121	215;205			
214	1149103134	NyangananaDH	Ndonga Linena	5/7/16	-18.00337	20.67655	Kavango	Nyangana	192		242	171	198	162	165	209	128	179	157	187	101		168	197	143	173	165	116	131	215		107	229
215	1149103135	NyangananaDH	Not Recorded	4/22/16	-18.00337	20.67655	Kavango	Nyangana	192		258	171	201	162	162	221	125	179	157	184	101		168	194	158	176	187	110;106	121	215		103;100	271
216	11491031	Nyangana	Ndonga Linena	4/5/16	-18.003	20.67655	Kavango	Nyangana	198;189;192		227;242	171;177	201;198	162;156	165	212;206;215	134;116;128;122	179;182	157	187;184	110;91;120		152;165;168;155	197;200;203;212	149;143;146	179;176;188	168;171	100;106;113;103;97	134;128	221;218		103;100;97	

	36	DH			37																												
217	1149103137	NyanganaDH	NdongaLinena	4/22/16	-18.00337	20.67655	Kavango	Nyangana	192			174	201	162	162	221	131	179	157	187	82;129;120		177	191	136	188	184	113	128	215		97	268
218	1149103138	NyanganaDH	NdongaLinena	5/3/16	-18.00337	20.67655	Kavango	Nyangana	198		242	171	201	162	165	206	131	179	157	187	139		155	194	143	191;182	177	113	140	215	155	103	268
219	1149103139	NyanganaDH	NdongaLinena	4/27/16	-18.00337	20.67655	Kavango	Nyangana	184		242	171	201	162	165	212	125	179	160	184			165	206	152	179	190	110;106	115	205		103	253
220	1149103140	NyanganaDH	NdongaLinena	6/18/16	-18.00337	20.67655	Kavango	Nyangana			242	171	201	165	165	212	122	179	160	187	101		168	197	146	173	177	100;97	121	209		103	
221	11491	Nyang	NdongaLi	4/16/	-18.0	20.67	Kavan	Nyang	198		242		201	165	152	215	125		157	184	129		168		152	206	174	113	131	218		103	

	03141	anaDH	nena	16	0337	655	go	ana																									
222	1149103142	NyanganaDH	NotRecorded	4/19/16	-18.00337	20.67655	Kavango	Nyangana	195		258	174	219	162	165	209	128	179	157	187	82;129		177	218	136	173	184	100	131	218		103	
223	1149103143	NyanganaDH	NotRecorded	4/7/16	-18.00337	20.67655	Kavango	Nyangana	198		242	171	198	162	165	206	128	182	160	187	101		189	206	146	182	181	116	121	215		97	265
224	1149103146	NyanganaDH	NotRecorded	4/2/16	-18.00337	20.67655	Kavango	Nyangana	192		242	171	198	162	162	212	122	179	157	184	104		171	203	158	179	171	110	128	205	146	103	265
225	1149103147	NyanganaDH	NotRecorded	4/7/16	-18.00337	20.67655	Kavango	Nyangana	192		242	174	198	162	165	212	122	179	157	187;184	113		155	197	136	170	187	113	118	221	149	107	247

226	1149103148	NyanganaDH	Ndonga Linena	4/18/16	-18.00337	20.67655	Kavango	Nyangana	192		242	171	201	156	165	212	122	179	160	187	101		168	197	139	182	184	116	115	209		100	280
227	1149103149	NyanganaDH	Ndonga Linena	4/26/16	-18.00337	20.67655	Kavango	Nyangana	192		242	171;177	201	153;162	162;165	206	128;125;122	182	157	184	110;101		177;165	194;212	136;149;143	176;191	171	94;106;103;97	121;112	215		103;107	268;271;250;253
228	1149103150	NyanganaDH	Not Recorded	4/18/16	-18.00337	20.67655	Kavango	Nyangana	198;189		242	171;174	201;198	162;165	162	206	134;128;125	179;182	157	187;184	110;101		165;193	203	139	173;152;179		110;113	131;125	212;218		103;100	235;238
229	1149103151	NyanganaDH	Mashare	5/11/16	-18.00337	20.67655	Kavango	Nyangana			258	174	226	162	165	206	122	179	157	187	113		155	206	139	200		91	128	224		103	274
230	11491031	Nyangana	Not Recorded	4/16/16	-18.003	20.67655	Kavango	Nyangana	187		227	171	201	165	165	206	122	179	160	187	120		168	197	143	200		88;91	128	209		100;97	280

	53	DH			37																												
231	1149103154	NyanganaDH	Ndiyona	4/21/16	-18.00337	20.67655	Kavango	Nyangana	198			171	201	162	165	215	122	182	160	184	113		177	191	143	182	165	110	125	218		103;100	247
232	1149103155	NyanganaDH	NdongaLinena	4/27/16	-18.00337	20.67655	Kavango	Nyangana	187		227	171	198	165	165	206	122	179	160	190;187	98		152	200	143	200	181	91	128	209		103;100	271
233	1149103156	NyanganaDH	NdongaLinena	5/2/16	-18.00337	20.67655	Kavango	Nyangana	187;195		242	165;174	198	162;159	162;165	206;215	122	179;182	157	184	82;132;139;129;110;113		152;165;168;155	197;206	139;149;143	173;179	174;171	94;119;103;116	140;128	209;205	146	103;100	250;253
234	1149103158	NyanganaDH	NdongaLinena	5/18/16	-18.00337	20.67655	Kavango	Nyangana	187		248	171	201;198	162	162	212	143;125;122	185	157	187	110;113		168;155	200	149;143	179;185	177	110;106	131	205;218		103;100	256
235	11491	Nyang	NotRecor	4/26/	-18.0	20.67	Kavan	Nyang	198		227;242	171	201;1	162	168;1	197;209	119;122	182	157;160	184	101		168	200	152;139	206;191;185;170	184;177	100;103	118;131	209;205		103;107	256

	03159	anaDH	ded	16	0337	655	go	ana					98		62																		
236	1149103160	NyanganaDH	Ndonga Linena	5/3/16	-18.00337	20.67655	Kavango	Nyangana	181		227	171	198	162;165		212	125	179	157	187;184	110;91;101		180;177;165	206;212	139	173;176;191	137;190	106;103		218		103;107	274
237	1149103161	NyanganaDH	Ndonga Linena	4/28/16	-18.00337	20.67655	Kavango	Nyangana	189;195			171		162	165	206;215		179	160	184	110;120		171;158;168	209;212	143;146	194;203	177	110;106	134;128	205		103	268;271;253
238	1149103163	NyanganaDH	Ndonga Linena	5/3/16	-18.00337	20.67655	Kavango	Nyangana	192		227	171	198	165	162	212	134	185	157	184	110;120		152	212	136	182	181	100	137	212	152	107	250;247;253
239	1149103164	NyanganaDH	Ndonga Linena	5/4/16	-18.00337	20.67655	Kavango	Nyangana	192		258	174	198	162	165	206	125	179	160	187	107;104		152	197	158	200		106	125	212		100	247

240	1149103165	Nyangana DH	Ndonga Linena	4/18/16	-18.00337	20.67655	Kavango	Nyangana	192		242	171	201	156	165	212	122	179	160	187	101		168	197	139	209;182	184	116	115	209	164	100	280
241	1149103166	Nyangana DH	Ndonga Linena	4/18/16	-18.00337	20.67655	Kavango	Nyangana	198;189		242	171;174	201;198	162;165	162	212;206	134;128	179;182	157	187;184	110		152;165;193	203;206;212	139	173;152;179	174;177	110;113	131;125	212;218		103;100	238
242	1149103167	Nyangana DH	Ndonga Linena	4/20/16	-18.00337	20.67655	Kavango	Nyangana	198;195		258	171;174	201	162;165	162;165	212;221	128	179;182	157	187	123;98;110;101		152;180;177	224;191	146	176;182	165	110;113;103	121;131	218		103;107;110	262;250;253
243	1149103168	Nyangana DH	Not Recorded	4/7/16	-18.00337	20.67655	Kavango	Nyangana	202;198;192		242	171;177	201;198	153;162	162;165	206;209	128;125;122	179;182;167	157	184	110;101		177;165	194;212	152;136;149;143	176;191;182	174;171	113;103;97	121;112;131	215;209		103;107	271;253
244	11491031	Nyangana	Ndonga Linena	5/2/16	-18.003	20.67655	Kavango	Nyangana	195		242	171	201;198	165	165	212;206;221;215	134	179	157	187	110;113		165;168	194;197	136;146	191	168	103	140;131	212	130	103	283;274

	69	DH			37																												
245	1149103170	NyanganaDH	Ndonga Linena	4/6/16	-18.00337	20.67655	Kavango	Nyangana	198			174	198	162	168	197	122	182	160		101		155	200	152	191;146		103	118	209			256
246	1149103171	NyanganaDH	Ndonga Linena	4/7/16	-18.00337	20.67655	Kavango	Nyangana	192		242	174	198	162	165	212	119;122	173;179	157;160	187;184	110;113		152;155	197	136;146	173;152;170	187;171;196	94;113	118;125	221		103;107	262;247
247	1149103172	NyanganaDH	Not Recorded	4/5/16	-18.00337	20.67655	Kavango	Nyangana	198		227	174	198	162	168	197	122	182	160	184	101		155	200	152	191	177	103	118	209		103	256
248	1149103173	NyanganaDH	Ndonga Linena	5/13/16	-18.00337	20.67655	Kavango	Nyangana	192		258;242	171;174	204;201;198	162	162;165	212;206	119;125	179	160;154	184	94;107;98;110;91		177;168	221;212	139	191;170;203	171	110;100	118;128	205		103;107;100	238;220;217;253
249	11491	Nyang	Ndonga Li	5/3/	-18.0	20.67	Kavan	Nyang	195		242	174	198	162	165	206	128	179	160	187	120		168	197	149	188	187	106	137	227		107	271

	03174	ana DH	nena	16	0337	655	go	ana																									
250	1149103175	Nyangana DH	Not Recorded	4/2/16	-18.00337	20.67655	Kavango	Nyangana	198;189;192;204		227;258;242	171;174	201;198	162;159	165	215	140;131;128;125;122	179;182	157;160	187;184;181	88;91;101		158;152;165;168;155;162	200;203	136;139;133;143	179;176;188;185;170;182	174;168;165;184;177;171	113;103	118;121;128;131;125	215;199;212;209;205	146	113;103;107;100	268;271;250;253
251	1149103177	Nyangana DH	Ndonga Linena	6/23/16	-18.00337	20.67655	Kavango	Nyangana	195		227;242	174	198	162	168	206;197	128;122	179;182	160	187;184	120;101		155	197;200	152;149	209;188;191;170	187;177	106;103	137	227;209		107	271;256
252	1149103178	Nyangana DH	Not Recorded	4/15/16	-18.00337	20.67655	Kavango	Nyangana	198			171	201	162	165	212	122	182	160	187	110		168	200	143	170	184	106	131	221		100	241
253	1149103179	Nyangana DH	Ndonga Linena	5/11/16	-18.00337	20.67655	Kavango	Nyangana	187		227	171;174	198	165	168;165	206;197	122	179;182	160	187;184	120;101		168;155	197;200	152;143	200;191	177	88;103;91	118;128	209		100	280;262;256

254	1149103180	NyanganaDH	Ndonga Linena	4/17/16	-18.00337	20.67655	Kavango	Nyangana	192		242	174	198	162	165	212	122	179	157	184			155	197	136	170	187	113	118	221	149	107	247
255	1149103183	NyanganaDH	Ndonga Linena	5/3/16	-18.00337	20.67655	Kavango	Nyangana	198		242;273	171	201	162;159	165	212	140	179	160	184	107;113;116		168	206	139	179;188	174	94;113	134;128	205		100	271
256	1149103184	NyanganaDH	Ndonga Linena	5/4/16	-18.00337	20.67655	Kavango	Nyangana	198		242	171	201	162	165	206	131	179	157	187	139		155	194	143	182	177	113	140	215		103	268
257	1149103185	NyanganaDH	Not Recorded	4/14/16	-18.00337	20.67655	Kavango	Nyangana	189			171	201	162	162	221	125	179	157	187	82;129		155	218	143	191	165	110	125	218		110	247
258	11491031	Nyangana	Ndonga Linena	4/7/16	-18.003	20.67655	Kavango	Nyangana	192		242	171	201	162	165	206	122	179	160	187	110		171	203	136	194	184	113	131	212		100	262

	86	DH			37																											
259	11491031187	NyanganaDH	Ndiyona	4/27/16	-18.00337	20.67655	Kavango	Nyangana	195		227	171	198	162	155	212	128	179	154	184	120		168	197	136	176	165	100	118	209		107	268
260	11491031188	NyanganaDH	NdongaLinena	5/4/16	-18.00337	20.67655	Kavango	Nyangana			242	171	201	159	162	221	137;149	179		184	120		155	200	139	200	184	116	131	218	158	103;97	265
261	11491031189	NyanganaDH	NotRecorded	4/18/16	-18.00337	20.67655	Kavango	Nyangana	192		242	174	201	168	162	212	125	179	157	184	120		168	206	139	173	177	106	131	218		103	265
262	11491031190	NyanganaDH	RunduRuralW	4/28/16	-18.00337	20.67655	Kavango	Nyangana	195		227	171;174	201;198	162;159	162	206;215	125	167	157	187;184	91		155	197	146	173;170	177	110;116	134;125	209			271;241
263	11491	Nyang	NdongaLi	4/20/	-18.0	20.67	Kavan	Nyang	198;195		258	171;1	201	162;165	162;1	212;221	128	179;182	157;160	187	123;98;110;101		152;177	191	139;146	176;182	165;177	110;113	121;131	218		107	262;250;253

	031191	anaDH	nena	16	0337	655	go	ana				74			65																		
264	11491031192	NyanganaDH	Ndonga Linena	4/20/16	-18.00337	20.67655	Kavango	Nyangana	204		211	174	201	162	162	206	122	179	157	187	110		168	206	139	191	171	94;110	125	221	146	107	256
265	11491031193	NyanganaDH	Not Recorded	4/14/16	-18.00337	20.67655	Kavango	Nyangana	184		242	171	201	162	165	212	125	179	160	184	101		165	206	152	179	190	110;106	115	205		103;100	253
266	11491031194	NyanganaDH	Ndonga Linena	4/20/16	-18.00337	20.67655	Kavango	Nyangana	184		242	174	198	162	165	212	125	173	160	187	67;110		152	197	146	173	196	94;110	125		155	103	262
267	11491031195	NyanganaDH	Not Recorded	4/14/16	-18.00337	20.67655	Kavango	Nyangana	189		258;242	171	201;198	162;165	162;165	221;230	125;122	179	157;160	187	98;101		152;165;168;155	218;191	133;143	179;176;188;191	174;165	110;113;122;119	121;131;125	209		113;107;110	

268	1149103196	NyanganaDH	Ndonga Linena	4/20/16	-18.00337	20.67655	Kavango	Nyangana	181		242	171	201;198	162;165	162;165	212;221;215	134;140;137;131;128;125;122	179	157;160	187;184	107;110;120;101		180;165;155	218;197;191;212	136;133;146	176	165;184;177	103	115;131	215;221;209;205;218		113;103;107;110	247
269	1149103197	NyanganaDH	Ndonga Linena	5/20/16	-18.00337	20.67655	Kavango	Nyangana	198		242	171	204	162	162	215	128;122	179	157	184	120		152	197	136	200	177	110	121	215		103	271
270	1149103198	NyanganaDH	Not Recorded	4/21/16	-18.00337	20.67655	Kavango	Nyangana	192		242	171	201	156	165	206	131	179	157	184	110		189	197	152	179	202	103	128	215		107	256
271	1149103199	NyanganaDH	Ndonga Linena	4/15/16	-18.00337	20.67655	Kavango	Nyangana	195			171	204	162			128;122	179	157;160		120			209	136	173	171	110	121;128			103	271
272	11491032	Nyangana	Ndonga Linena	5/3/16	-18.003	20.67655	Kavango	Nyangana	192		242	171	201	156	162	212	128	182	160	187	110		155	203	146	173	174	110	121	209			262

	00	DH			37																												
273	11491032001	NyangananaDH	NotRecorded	4/4/16	-18.003337	20.67655	Kavango	Nyangana	198		258;242	174	204;201	168;162	162	224;197	116	179;182	160	187;184	110		165	209	136	173;170	174;168;165;171	110;106;97	128	215		103;100	268;256;259
274	11491032002	NyangananaDH	Ndiyona	5/13/16	-18.003337	20.67655	Kavango	Nyangana	189			171;174	198	162	165	212	116;128;122	179	175	187	101		171	200	139	173;176;170	177	91	128;115	218		103	256;259
275	11491032003	NyangananaDH	RunduRuralW	4/22/16	-18.003337	20.67655	Kavango	Nyangana	195		258	174	204	165	165	212	125	182	160	187	110		155	197	143	149;203	187	97	128	215	149	100;97	274
276	11491032005	NyangananaDH	Mashare	6/1/16	-18.003337	20.67655	Kavango	Nyangana	198		258	171	201	168;165	165	206;221	134;140	179	160	187	110;113;101		152;165;168;155	197;200	152	176;191	174;184	113;103	118	212;209	170	103	286;283;289;280;265
277	11491	Nyang	NotRecor	4/17/	-18.0	20.67	Kavan	Nyang	202;198;204		227;242	171;1	201	162;159	162;1	206;209	134;125	179;167	157	187	110;101		152;165	197;206	149;143	173;197	168	100;113	118;121	199		103	265;259

	03207	anaDH	ded	16	0337	655	go	ana				74			65																		
278	1149103208	NyanganaDH	NotRecorded	5/5/16	-18.00337	20.67655	Kavango	Nyangana	202;198;189		227;242	171	201	162;159		212;206	134;137	167	157		94;110;101		152;165	194;197;206	149;143	173;197		100;113	118;121;128	199		103	265;259
279	1149103209	NyanganaDH	Mashare	5/14/16	-18.00337	20.67655	Kavango	Nyangana	189			174	198	162	165	206	128	179	157	181	101		158	200	136	182		82;94;85;100;106;103;91	131	209		103	268
280	1149103210	NyanganaDH	NotRecorded	4/2/16	-18.00337	20.67655	Kavango	Nyangana	189;195		227	171;174	201;198	174;159	165	212	137;128	179	157	187;184	94;98;91;101		168	194;197	139;143	191;185	171	125;113	121;128	215;205		103	268;271
281	1149103212	NyanganaDH	NdongaLinena	5/18/16	-18.00337	20.67655	Kavango	Nyangana	202		258	174	201	168	165	206	125	182	157		110		168	200	143	191	193	113	134	218		103	271

282	1149103213	NyanganaDH	NdongaLinena	4/22/16	-18.00337	20.67655	Kavango	Nyangana	192		242	174	198	162	165	206	140	182	160	184	113		152	200	139	185	177	103		212			271
283	1149103214	NyanganaDH	NotRecorded	4/6/16	-18.00337	20.67655	Kavango	Nyangana	198		258			162;165	162	212;206	140;128	179		187	120		152;189	212	149		174	100;91	121;125	212		113;103	271;253
284	1149103215	NyanganaDH	NdongaLinena	4/26/16	-18.00337	20.67655	Kavango	Nyangana	204		227	171	198	162	165	215	125	179	157	187	91		155;162	200	139	176;188	177	110	121	215		103	268
285	1149103216	NyanganaDH	NotRecorded	4/26/16	-18.00337	20.67655	Kavango	Nyangana	192			171	198	162		221	125	185	157		101		168	194	161	179		110	121	215		103	268
286	11491035	Nyangana	NdongaLinena	5/21/16	-18.003	20.67655	Kavango	Nyangana	195;192		258	171		162;165	162;165		125;122	179	157;160	187			155	197;203;191	136;146		181;177	103	121				

	06	DH			37																											
287	1149103507	Nyangana DH	Ndonga Linena	4/3/16	-18.00337	20.67655	Kavango	Nyangana	192		258;242	171	201;198	162;165	162;165	206	128;125;122	179;182	157;160	187;184			152;177;155	197;203;191	136;143;146		190;181;177		121;140			
288	1149103508	Nyangana DH	Ndonga Linena	6/17/16	-18.00337	20.67655	Kavango	Nyangana	192		242	174		165	165		122	182	160	184			155	197	136		187		131			
289	1149103511	Nyangana DH	Ndonga Linena	5/7/16	-18.00337	20.67655	Kavango	Nyangana	195		242	174	219	162	165	212	128	182	157	184			177	218	143		184	103	131			
290	1149103523	Nyangana DH	Not Recorded	4/17/16	-18.00337	20.67655	Kavango	Nyangana	202;198		227;242	171;174	201	162;159	162;165	206;209	134;125	179;167	151;157	187;184			152;165	197;206	149;143		168;181	100	118;121			
291	11491	Nyang	Ndonga Li	5/29/	-18.0	20.67	Kavan	Nyang	198		242	171	204	162	162		122	179	157	184			152	197	136		177		121			

	03524	anaDH	nena	16	0337	655	go	ana																									
292	1149103526	NyanganaDH	Ndonga Linena	6/1/16	-18.00337	20.67655	Kavango	Nyangana	195		273	171		162	165	197	128		160	178			168	218	143		174	100					
293	1149103528	NyanganaDH	Ndonga Linena	6/15/16	-18.00337	20.67655	Kavango	Nyangana	198		242	171	204	162	162	215	122	179	157	184			152	197	136		177	110	121				
294	1149103530	NyanganaDH	Ndonga Linena	5/3/16	-18.00337	20.67655	Kavango	Nyangana	181		242	171	201	162	162	212	137	179	157	187			180	212	146		184	103	115				
295	1149103531	NyanganaDH	Ndonga Linena	5/17/16	-18.00337	20.67655	Kavango	Nyangana	198		242	171		165	165	206	122		160	187			168	197	146		177		118				

296	11491035334	NyangananaDH	Ndiyona	5/18/16	-18.00337	20.67655	Kavango	Nyangana	202		242	171	201	162	162	212	116	179	157	187			152	200	146		177;171	125					
297	11491035339	NyangananaDH	NdongaLinena	5/3/16	-18.00337	20.67655	Kavango	Nyangana	198		227	171;174	198	162	168		122	182	157;160	184			155	200	152;139		177	103					
298	11491035549	NyangananaDH	NdongaLinena	4/18/16	-18.00337	20.67655	Kavango	Nyangana	198;189;192		242	171	198	162;165	162	212	125		157	184			165;193	203	139		177						
299	11491035551	NyangananaDH	NdongaLinena	4/2/16	-18.00337	20.67655	Kavango	Nyangana	198;195		258	171	201	162	165		122	179	154	184			165	200	139		181	100					
300	11491035	Nyangana	NdongaLinena	7/23/16	-18.003	20.67655	Kavango	Nyangana	195		258	174	219	162	165		128;125;122	179	157	187			177	218;197	136		184						

	53	DH			37																											
301	11491035555	NyanganaDH	Ndonga Linena	4/27/16	-18.00337	20.67655	Kavango	Nyangana	192		258	171	201	162	162	221	125	179	157	184			168	194	158	149	187	110	121			
302	11491035556	NyanganaDH	Mashare	5/1/16	-18.00337	20.67655	Kavango	Nyangana	192		242	171	201	156	165	215	134	182	157	184			152	200	143		174	113	140			
303	11491035559	NyanganaDH	Ndonga Linena	5/2/16	-18.00337	20.67655	Kavango	Nyangana	195		227	171		159	162	206	125	167	157	184			155	197	146		177	110				
304	11491035570	NyanganaDH	Ndonga Linena	4/30/16	-18.00337	20.67655	Kavango	Nyangana	198		227	174	198	162	168	197	122	182	160	184			155	200	152		177	103	118			
305	11491	Nyang	Rundu Rur	4/30/	-18.0	20.67	Kavan	Nyang	192		258	171	198	162	162	209	134	182	160	187			177	212	152		190	106	131			

	03571	anaDH	alW	16	0337	655	go	ana																									
306	1149103572	NyanganaDH	Ndiyona	4/29/16	-18.00337	20.67655	Kavango	Nyangana	198;195		258	171	201	156	165	206	131;128	179;182	157	184				197	139;149		202		134;125				
307	1149103573	NyanganaDH	RunduUrban	6/3/16	-18.00337	20.67655	Kavango	Nyangana	195		242	174	198	162		212	125;122	179	160	187			152	197	133		171	106					
308	1149103576	NyanganaDH	NdongaLinena	4/29/16	-18.00337	20.67655	Kavango	Nyangana	198		242	171	198	156	165	209	122	182	157	184			168	191	143		137	110					
309	1149103579	NyanganaDH	NdongaLinena	5/2/16	-18.00337	20.67655	Kavango	Nyangana	192		258	177	201	162;159	165		122	182	157	184			165	194	143		171		121;112				

310	1149103581	NyanganaDH	NdongaLinena	6/3/16	-18.00337	20.676555	Kavango	Nyangana	198;192		248	171	198	162	162		122	179	157	184			152	197	136		177						
311	1149103583	NyanganaDH	NdongaLinena	5/4/16	-18.00337	20.676555	Kavango	Nyangana	195		258	171	201		165	215	122	179	157				155	197	133		184	97	128				
312	1149103594	NyanganaDH	NdongaLinena	5/21/16	-18.00337	20.676555	Kavango	Nyangana	195		242		219	162	165	212		182	157	184			177	218	143		184	103					
313	1149103597	NyanganaDH	NdongaLinena	6/5/16	-18.00337	20.676555	Kavango	Nyangana	195		227	171		162	165		125	179	157	187			152	200	149		193	103					
314	11491036	Nyangana	NdongaLinena	5/2/16	-18.003	20.676555	Kavango	Nyangana	189		242	171	198	165	165		128	179	160	184			165	218	152		171	122	128				

	00	DH			37																												
315	11491036003	NyanganaDH	RunduRuralW	5/2/16	-18.00337	20.67655	Kavango	Nyangana			227			162	168	197	122		160	184				200	152	191	177		118	209		103	256
316	11491036004	NyanganaDH	Ndiyona	4/4/16	-18.00337	20.67655	Kavango	Nyangana			242			159	162	221	149		157		120			200	139	200	184		131	218	158	103	
317	11491036005	NyanganaDH	NotRecorded	6/8/16	-18.00337	20.67655	Kavango	Nyangana			242			162	162	215	122		157	184	120			197	136	200	177		121	205	152	103	
318	11491036007	NyanganaDH	Ndiyona	5/19/16	-18.00337	20.67655	Kavango	Nyangana			258			162	162	221	125		157	187	120			194	158	176	190		121	215	146	113	
319	11491	Nyang	NdongaLi	6/13/	-18.0	20.67	Kavan	Nyang			227;258;242			162	165	206;215	125;122		157;154	184	107;110;120;101;116			200	136;139	173;200	187;181		121	209;205	158	107	268

	036008	anaDH	nena	16	0337	655	go	ana																									
320	1149103609	NyanganaDH	NdongaLinena	5/11/16	-18.00337	20.67655	Kavango	Nyangana			227;242			162	162;165	206	137		154	184	113;116			203;212	139;149	179;191	193;177		125	199;205	146;170	103	
321	1149103610	NyanganaDH	NdongaLinena	5/5/16	-18.00337	20.67655	Kavango	Nyangana			242	171		165	165	215	128		157		101			212	143	182	174		134	215	149		268
322	1149103611	NyanganaDH	NdongaLinena	5/18/16	-18.00337	20.67655	Kavango	Nyangana	181		227			162	162	212	125		157	184	101			206	139	191	190		128	218	161	107	
323	1149103612	NyanganaDH	NdongaLinena	5/23/16	-18.00337	20.67655	Kavango	Nyangana			242			162		206	131;128		160	187	113			206	143	179	168			215	152	100	250

324	1149103613	NyanganaDH	Ndonga Linena	5/21/16	-18.00337	20.67655	Kavango	Nyangana	181		227			162	162	212	140		157	187	101			206	139	191	190		128	224	112;161	103	
325	1149103614	NyanganaDH	Ndonga Linena	5/3/16	-18.00337	20.67655	Kavango	Nyangana			236			162	165	212	125		160	184	120			200	143	182	184		128	209	140	107	253
326	1149103615	NyanganaDH	Ndonga Linena	6/16/16	-18.00337	20.67655	Kavango	Nyangana			242			165	165	212	122		160	187	101			197	146	173	177		121	209		103	268
327	1149103616	NyanganaDH	Ndonga Linena	5/3/16	-18.00337	20.67655	Kavango	Nyangana			227			162	168	197	122		160	184	101			200	152	191	177		118	209	179	103	256
328	11491036	Nyangana	Ndiyona	5/24/16	-18.003	20.67655	Kavango	Nyangana			258;242			162;165;156			143		157;160	184	98;129			194;200	133;149;143;161	179;176;194;182	165;171		131	215;212	136;146		268

	17	DH			37																												
329	1149103618	NyanganaDH	RunduRuralW	4/15/16	-18.00337	20.67655	Kavango	Nyangana			258;248			162;156	152;165	212;221	143		157;163		129;120			194;200	133;149	179;182	171		121;131	212	136	103;97	253
330	1149103619	NyanganaDH	NdongaLinena	5/25/16	-18.00337	20.67655	Kavango	Nyangana			242			168;165	165		122		160	184	110			197	136	170	187		131	221	140		241
331	1149103620	NyanganaDH	NdongaLinena	6/11/16	-18.00337	20.67655	Kavango	Nyangana			227			162	165	215	125		157	187				200	149	191	193		115	227	155	103	
332	1149103621	NyanganaDH	NdongaLinena	5/8/16	-18.00337	20.67655	Kavango	Nyangana			242			162	165	212	128		157	184	110			218	143	176	184		131	218	143	103	256
333	11491	Nyang	NdongaLi	5/2/	-18.0	20.67	Kavan	Nyang			227;242			162	165	206	122		160		101			200;206	139	188;191	184		118;128	221;209	146	97	

	03624	anaDH	nena	16	0337	655	go	ana																									
334	1149103627	NyanganaDH	NdongaLinena	5/11/16	-18.00337	20.67655	Kavango	Nyangana			227			162	165	215	125;122		157	187	110;120			200	149;143	191	193;168		121;115	227;212;209	155	103	268
335	1149103631	NyanganaDH	NdongaLinena	5/31/16	-18.00337	20.67655	Kavango	Nyangana			258			162	165	215	122		154	184	116			200	139	200	181		121	209	158	103	
336	1149103634	NyanganaDH	NdongaLinena	6/15/16	-18.00337	20.67655	Kavango	Nyangana			258			162	165	209	128		157	187	129			218	136	173	184		131	218	140		
337	1149103635	NyanganaDH	NdongaLinena	5/4/16	-18.00337	20.67655	Kavango	Nyangana			258;248			162;156	152;165		143		157;163		129;120			194;200	139;133;149	179;182	190;171		121;131	212	136;149		253

338	11491036337	NyanganaDH	Ndiyona	5/24/16	-18.00337	20.67655	Kavango	Nyangana			242			162	165	206	131		160	181				203	136	179	168		121	209	146	103	265
339	11491036340	NyanganaDH	Mashare	5/7/16	-18.00337	20.67655	Kavango	Nyangana			245			165		212	134		157;160		113;120			203	143	188	181		121	227	167;149	103;100	
340	11491036341	NyanganaDH	Ndonga Linena	5/21/16	-18.00337	20.67655	Kavango	Nyangana			258			162;165	162		125		157;160	187	139			197;203;191	136	173	177		121	212;205	164	103;100	
341	11491036342	NyanganaDH	NotRecorded	4/16/16	-18.00337	20.67655	Kavango	Nyangana			242			165	165	206	128;125		157	187	120			212	139	173	181		128	218	146	103	265
342	11491036	Nyangana	Ndonga Linena	5/20/16	-18.003	20.67655	Kavango	Nyangana			258			162	165		128;125		157	187	129			218	136	173	184		131	218	140	103	

	43	DH			37																												
343	1149103644	Nyangana DH	Ndonga Linena	5/13/16	-18.00337	20.67655	Kavango	Nyangana			227;242			165	165	206	143;128		157;160		94;101			194;197;200	136;149;146	179;200	137;187;140		121;131	215;205	146		238
344	1149103645	Nyangana DH	Ndonga Linena	5/2/16	-18.00337	20.67655	Kavango	Nyangana			248			162	165	215	122		160		113			191	143	182	165		125	218	179	103	247
345	1149103646	Nyangana DH	Rundu Rural W	4/29/16	-18.00337	20.67655	Kavango	Nyangana			242			162	165	212	122	179	157	184	113			212	152	182	196		118	209	149	107	247
346	1149103650	Nyangana DH	Ndonga Linena	5/13/16	-18.00337	20.67655	Kavango	Nyangana			242			165	165	206	122		160	184	98			197	143	173	177		128	209	146		
347	11491	Nyang	Ndonga Li	5/1/	-18.0	20.67	Kavan	Nyang			242			165	165	212	122		160		110			197	133;149	170	187		131	221	140		

	03651	anaDH	nena	16	0337	655	go	ana																								
348	1149103653	NyanganaDH	Not Recorded	4/14/16	-18.00337	20.67655	Kavango	Nyangana			242			162	165	206	122		157		120			194	152;139	176	168		128	218		103	277
349	1149103654	NyanganaDH	Ndonga Linena	6/6/16	-18.00337	20.67655	Kavango	Nyangana			258			168	165	206	125		157	187	110			200	143	191	193		134	218	146	103	
350	1149103655	NyanganaDH	Ndonga Linena	4/30/16	-18.00337	20.67655	Kavango	Nyangana			261;258			168;165	168;162	212;206	134;125		157;160	187;184	129;101			215;194	136;143	176;188	184;171		118;125	209;218	143;152	103;107	
351	1149103656	NyanganaDH	Ndonga Linena	5/2/16	-18.00337	20.67655	Kavango	Nyangana			261			165	168	212	134		160	187	129			215	136	176	171		125	218	152	103	268

352	11491036557	NyangananaDH	Ndonga Linena	5/26/16	-18.00337	20.67655	Kavango	Nyangana			242			165	165	209	122		160	187	101			203	139	209	174		146	205	136;109	103	
353	11491036558	NyangananaDH	Ndonga Linena	4/28/16	-18.00337	20.67655	Kavango	Nyangana			258;273			162	165	206	122		160	187;184				197;209	130;143	179;203	181;177		128	205	149	103	
354	11491036661	NyangananaDH	Ndonga Linena	5/3/16	-18.00337	20.67655	Kavango	Nyangana			242			156	165	206	122		157	184	120			200	143	173	181		128	209	143	107	
355	11491036663	NyangananaDH	Rundu Rural W	4/30/16	-18.00337	20.67655	Kavango	Nyangana	181		258			165	165	212	125		157	184	110			209	146	176	165		115	221;218	146		247
356	11491036	Nyangana	Ndonga Linena	5/2/16	-18.003	20.67655	Kavango	Nyangana			258			156	165	212	137;125		157	187	101			197	146	176	181		115	221	146		

	64	DH			37																												
357	11491036669	NyanganaDH	Not Recorded	4/4/16	-18.00337	20.67655	Kavango	Nyangana			227			162	165	212	122		157	187	120			212	139	182	196		128	215	152	103	
358	11491036671	NyanganaDH	Ndonga Linena	5/11/16	-18.00337	20.67655	Kavango	Nyangana			242			162	162	215	122		157	187	110			206	133	194	184		128	215	152	97	271
359	11491036672	NyanganaDH	Ndiyona	5/3/16	-18.00337	20.67655	Kavango	Nyangana			242			156	165		134		157	184	107			200	143	188	174		140	218	143	107	271
360	11491036676	NyanganaDH	Ndiyona	4/29/16	-18.00337	20.67655	Kavango	Nyangana			242			162	165	206	125		160		129			200	139	182	184		112	212	164		
361	11491	Nyang	Mashare	5/11/	-18.0	20.67	Kavan	Nyang			242			162	165	209	128; 125		157		101			197	143	173	165		131	215	167		229

	03677	anaDH		16	0337	655	go	ana																									
362	1149103678	NyanganaDH	Ndiyona	4/5/16	-18.00337	20.67655	Kavango	Nyangana	181		242			174	165	212	125		160	187	110			200	136	200	184		115	205	167	100	247
363	1149103680	NyanganaDH	Ndonga Linena	4/4/16	-18.00337	20.67655	Kavango	Nyangana			242;248			162	165	215	134;125		157	187	110;101			200	143	173;197	168		121;140	212;205	161	103;107	
364	1149103682	NyanganaDH	Ndiyona	5/13/16	-18.00337	20.67655	Kavango	Nyangana			245			156	165	197	128		157	187	120			191	143	194	177		121	221	158		
365	1149103683	NyanganaDH	Ndiyona	5/14/16	-18.00337	20.67655	Kavango	Nyangana			258			168	165	206	125		157	187	110			200	143	191			134	218	146	103	

366	1149103687	NyanganaDH	Not Recorded	4/14/16	-18.00337	20.67655	Kavango	Nyangana			242			165	165	206;221	125;122		157	187	129			218;200	139	191	165;171		125	218		110	
367	1149103688	NyanganaDH	Ndonga Linena	5/12/16	-18.00337	20.67655	Kavango	Nyangana			242			162	165	212	122		157	184	113			197	136	170	187		118	221	149	107	
368	1149103690	NyanganaDH	Ndonga Linena	5/2/16	-18.00337	20.67655	Kavango	Nyangana			227;258			162	165	215	125		157	187	91;101			197;200	139	176;188	177		121	215	146	103	
369	1149103692	NyanganaDH	Ndonga Linena	5/12/16	-18.00337	20.67655	Kavango	Nyangana			258			165	165	206	125		157		101			194;200	143	176	177		118;121	215;209	143		
370	11491036	Nyangana	Ndonga Linena	5/7/16	-18.003	20.67655	Kavango	Nyangana			242			156	162	212	134		160	184	104;101			200	152	170	205;171		125	215	188	103	262

	93	DH			37																												
371	1149103694	NyangananaDH	Ndonga Linena	5/1/16	-18.00337	20.67655	Kavango	Nyangana	181		242			162	162	212			157	187	110			212	146	176	184		115	221	152	110	247
372	1149103695	NyangananaDH	Ndonga Linena	5/5/16	-18.00337	20.67655	Kavango	Nyangana			227			162	162	218	128		160		88			224	139	173	165		125	205	146	100	
373	1149103696	NyangananaDH	Not Recorded	4/14/16	-18.00337	20.67655	Kavango	Nyangana			242			165	165	212	122		160	187	120			197	143	173	177		121	209	146	103	
374	1149103794	AndaraDH	Mukwe	5/8/16	-18.05099	21.44215	Kavango	Andara	192	167	242	171	201	162		206	122	182	157	184	101	164	168	218	143	200;197	168	103	121	224	185	100;97	253
375	11491	Andar	Mukwe	4/23/	-18.0	21.44	Kavan	Anda		170;161		179;1	198	176;174		191;197;194	122	182	157		110	155		159	152	179;176;170	187				158		277

	03795	aDH		16	599	215	go	ra				71																						
376	1149103796	AndaraDH	Mukwe	5/18/16	-18.0599	21.44215	Kavango	Andara	192	161	242	171	201	162	165	209	134	182	160	187	101	155	152	215	139	209	190;187	110	131	212	161	103	256
377	1149103797	AndaraDH	NamibiaOther	4/17/16	-18.0599	21.44215	Kavango	Andara	198	161	258	171	198	162	155;162;159	206	137	179	160	184	82;139	164		197	139	164;161	181;184;177		121	215	173;176	97	277
378	1149103798	AndaraDH	Angolaother	5/16/16	-18.0599	21.44215	Kavango	Andara	192	173	258	179;171	198	156	165	212;215	119;128;125	160;182	160	187	126;82;129;91;120;101	164;155	162	200	143;146	179;176;182	171	100;103;116;97	128;125	218	161	103;100	244;283;229;247
379	1149103800	AndaraDH	Mukwe	5/7/16	-18.0599	21.44215	Kavango	Andara	189	161	227	174	201	162	165	206	122	182	190;184	184	101	143;140	155	203	155	188;191	190	113	131	205	167	103;100	244;247

380	1149103801	AndararaDH	Mukwe	5/9/16	-18.05999	21.442215	Kavango	Andara	195;192	176;161	227	171	201		165	212;215	131;122	179	157;160	184	91;101	155	158;152;177;183		136;139	191;182	174;187;184	110;106;103	134;125	221;209	170;161;158	103;100	271;250;247
381	1149103802	AndararaDH	Mukwe	5/12/16	-18.05999	21.442215	Kavango	Andara	195	158	261	174	201	162;165;159	162;165	197	140;125	179	160	187	101	155	168	162;194;197	130;146	176	171	106;113;103	134;128	212;205	176;161	107;100	244;247;265
382	1149103803	AndararaDH	Mukwe	3/4/16	-18.05999	21.442215	Kavango	Andara	192	170;167	242	171	198	153;150	165	221;197;215	131	182	157	187	120	155	152	197	139	173;170	140	110	121;128	209	176	103	265
383	1149103808	AndararaDH	Mukwe	5/23/16	-18.05999	21.442215	Kavango	Andara	192	161;164	242	171	201;198	162;165	165	206	134;131	179	154	187	126;129;110;120	146	165	197;165	139	173;176;170	174	119	140;137	218			268
384	11491038	AndararaDH	Angolaother	5/6/16	-18.05999	21.442215	Kavango	Andara		167	242	174	201	162			140	182	157	187	113	155	168	197	146	173;176;170	196	106	128	212	182	100	274

	09																																
385	1149103810	AndaraDH	Mukwe	5/5/16	-18.0599	21.44215	Kavango	Andara	192	167	242	174	201	162	165	197	140	182	157	187	113	155	168	197	146	173	196	106	128	212	182	100;97	274
386	1149103811	AndaraDH	Mukwe	5/11/16	-18.0599	21.44215	Kavango	Andara	198;189;195;192	167;176;185	227;242	171;174	204;201;198	162;159	155;162;165	212;206;197	125;122	179;182;167	157;160;154	187;184	88;107;98;110;113;91;104;101	155	152;177;165;155	224;162;197;200;203	136;133;143	173;191;194	174;190;165;187;171	100;113;103	121;128;131	215;221;205;218		113;107;100;97	244;232;235;238;274;250;247;256
387	1149103812	AndaraDH	Mukwe	5/6/16	-18.0599	21.44215	Kavango	Andara	208;192		258	171;174	198	165	162;165	221	122	179;167	160	184	139;101	164	168;155		130;139	173;152;179;200	187	100;122	121;125	221;205	109;152;149;161;164	100;97	268;262;253
388	1149103813	AndaraDH	Mukwe	5/16/16	-18.0599	21.44215	Kavango	Andara		176	242	171		162	165	206	125	182	160	184	91	164	152	206	143	182	171	110	118	224	176;164	103;100	277
389	11491	Andar	Mukwe	4/30/	-18.0	21.44	Kavan	Anda	192	161	242	171;1	198	159	162;1	212;206;209	137;128	179;182	157;160	187;184	139;110;113	164	152	197;206;209	139	164;191;194	168;187	106;113;103	121;128	209	170;173;176	113;100	247

	038814	aDH		16	599	215	go	ra				74			65																		
390	1149103815	AndaraDH	Mukwe	4/20/16	-18.0599	21.44215	Kavango	Andara	192	179	258	174	198	165	162	206	134		157	184	120	164	189	197	136	176	193	106	125	212	167	103	253
391	1149103816	AndaraDH	Mukwe	5/16/16	-18.0599	21.44215	Kavango	Andara	195	170;167;161	276;242;245	171	201	162;165;159	165	206;209	134;125;122	182	157	187	107;110	155	152;168	218;197;200	152;133;146	179;176;170;182	174;165;181;177;171;150;196	116	128	212;209;205	161;158;155	107;100	277;247
392	1149103817	AndaraDH	Mukwe	5/17/16	-18.0599	21.44215	Kavango	Andara		173;164	242	171	201	165	162	206	140	179	157	187	110	164	152	197	139	179;176;182	168	116	121	212	164	94	247
393	1149103818	AndaraDH	Mukwe	5/9/16	-18.0599	21.44215	Kavango	Andara	189;195;192	161;158	258;242	171	201;198	174;162;165	165	212;206;221;197;203	125;122	179	166;163;169	187	107;98;110;120;101	143;134;140;149;158;146	152;177;168	197;200;191	155	170;182	165;177;171	110;106;122	134;121;131;125	221;212;209;205;218;224	136;191;194;173;167;179;149;176	100	271;274;250;247;253;256

394	1149103819	AndaraDH	Mukwe	5/13/16	-18.0599	21.44215	Kavango	Andara	195	173	242	191;171	201	162;159	165	206	143;140;137;122	157;179	157	187	110;120;101;116	172	155	162;200	136;139	179;188;191	177	116	134;137	205		107;110	
395	1149103820	AndaraDH	Mukwe	4/14/16	-18.0599	21.44215	Kavango	Andara	195	167	242	174	198	162	165	212	125	179	160	187	107	155	152	197	133	182	171	106	125	212	176	100	247
396	1149103821	AndaraDH	Mukwe	4/15/16	-18.0599	21.44215	Kavango	Andara		167	242	174		168;162	168;165	206	128	173	160	187	113	164	143	197	149	179;176	187	100	134;125	215	161	107	271
397	1149103822	AndaraDH	Mukwe	5/11/16	-18.0599	21.44215	Kavango	Andara	195		258		198	165	165	212	122	182	160	184	101	164	168;155	218;197	139	173	174;184	113;122	121	221;218	188;161	94	253
398	11491038	AndaraDH	Mukwe	5/7/16	-18.0599	21.44215	Kavango	Andara	202;189;195;192	161;179	227;242	171	201;198	162;165;156	162;165	212;206	134;128;125;122	179;182;167	157;160	184	110;91;120;101	164;172;155	152;177;165;168	197;200;203;206	136;139;149;143;146	179;200;191;170	190;168;165;187;184;171	94;110;85;100;88;103;97;91	121;128;125	215;221;209;205	200;170;179;176;182;161;158	107;100	286;262;265

	23																																	
399	1149103824	AndaraDH	Mukwe	5/8/16	-18.0599	21.44215	Kavango	Andara	192	176	227	171	198	162;159	162	206	122	157;167	157	187	110	155	165	162;197;200	143	191;194	171	103	128;131	221	170;167;164	107	235;238;241
400	1149103825	AndaraDH	Mukwe	5/11/16	-18.0599	21.44215	Kavango	Andara	189	167	227	171	201	165	165	215	128	182	157	187	113	164	155	197	143	200	181	113	134	215	161	100	271
401	1149103826	AndaraDH	Mukwe	5/11/16	-18.0599	21.44215	Kavango	Andara		167	242	174	201		165		140	182	157	187	113	155	168	197	146	173	143;196	106	128	212	179;182	100;97	274
402	1149103827	AndaraDH	Mukwe	5/4/16	-18.0599	21.44215	Kavango	Andara		188	227	171	198	165	165		122	179	160	187	116		180	212	136	191	165;162	106	128	215	158	103	268
403	11491	Andar	Mukwe	5/8/	-18.0	21.44	Kavan	Anda	192	173	242	171	201	156	162	212	128	182	160	187	110	164	155	203	146	173	174	110	121	209	158;155	103	262

	03832	aDH		16	599	215	go	ra																									
404	11491038833	AndaraDH	Mukwe	5/22/16	-18.0599	21.44215	Kavango	Andara	195	179	242	171	198	162	162;165	212	140	167	157	184	101	164;172;155	155	200;203	146	170	137;184	94;100;97	121;125	218	161;158	100	268
405	11491038835	AndaraDH	Mukwe	4/27/16	-18.0599	21.44215	Kavango	Andara	198	176	258	171	201	162;159		221	125	179	157	187	129	155	165	162;191	139	173;176	171	106	121	209	149	103;107	262
406	11491038836	AndaraDH	Mukwe	5/18/16	-18.0599	21.44215	Kavango	Andara	198		227	174	204	162	165	212		179	160	184	110	155	177	200	133	194	187	113	131	215	167	113	256
407	11491038837	AndaraDH	Mukwe	5/16/16	-18.0599	21.44215	Kavango	Andara	195	173	242	171	198	162;159	165	212;206	112	160;179	160	184	101	155	177	162;200;203	139	173;170	177	125;106	134	205	170	97	262

408	1149103838	AndararaDH	Mukwe	4/27/16	-18.05999	21.44215	Kavango	Andara	202;195;192	173;167;161	227;242	171;174	201;198	165	165	212;206;215	122	170;179	160	187;184	107;91;120;101;116	164;172;155	152;177;165	197;203;191	133;149;143;146	173;179;176;191;194;197;170;182;203	137;177;171	106;103;97	118;134;128;131;125	215;212;209;205	176;161;158	113;107;100;97	244;268;271;262;250;247;256
409	1149103839	AndararaDH	Angolaother	5/12/16	-18.05999	21.44215	Kavango	Andara	195;192	170;173;167;176;161;164	227;242	165;171;174	201;198	162;156;159	162;165	212;206;215	131;128;125;122	179;182	157;154		88;94;98;110;120;101	164;155	152;177;165;168	200;203	130;152;136;143;158	206;173;152;179;176;200;191;194;185;197;170;182;203	171	106;113;122;97	134;143;128;131;125	215;221;209;205;218	173;167;152;149;161;158;155	113;103;107;100;110	235;268;271;241;247;253
410	1149103841	AndararaDH	Mukwe	5/7/16	-18.05999	21.44215	Kavango	Andara	192	188	227	171	198	165	165	212	122	179	160	187	116	172	180	212	136	188;191	165;162	106	128	215	158	103	268
411	1149103842	AndararaDH	Mukwe	5/13/16	-18.05999	21.44215	Kavango	Andara	198	173	258	171	198	162	165	215	128	179	157	184	104	155	177	203	139	173;170	184;177	97	128	221	164	103	265
412	11491038	AndararaDH	Mukwe	5/6/16	-18.05999	21.44215	Kavango	Andara	192	158	242	174	201	162	165	197	140		166		110;113	143;140;137;146	168	197	155	173	196	106	118;128	221;212;209;205	179;182	103;107;100;110	274

	43																																
413	1149103844	AndaraDH	Mukwe	5/7/16	-18.0599	21.44215	Kavango	Andara	195	167	236	171	198	162	162;165	206	122	179	157	184	91	164		187;162;191	149	194	137		118	209	158;155	122;116;119	268
414	1149103845	AndaraDH	Mukwe	4/21/16	-18.0599	21.44215	Kavango	Andara	195	158	242		198	162	165	206	122	167	163	187	110	143;134;140;137;146	168	194		176	190	116	131	215;221;212;209;205	161;158	107;100;110	286
415	1149103846	AndaraDH	Mukwe	5/17/16	-18.0599	21.44215	Kavango	Andara	189	167	258	171	198	162	165	212	128	179	157	187	101	155	171	200	139	173;176	177	91	115	218	146;173	103	259
416	1149103847	AndaraDH	Mukwe	5/14/16	-18.0599	21.44215	Kavango	Andara	192	164	242	174	201;198	162;156	165	212;197	131;128	179	160	187	110	155	152;165	215;197	152	176;194;197	184;177	110	121;128	209;218	158	103;100;97	292;280
417	11491	Andar	Mukwe	5/7/	-18.0	21.44	Kavan	Anda		170	242	171	198		162	221	125	182	160		132	155	165	194	136	173	171		131	215	158	103	262

	03848	aDH		16	599	215	go	ra																									
418	1149103849	AndaraDH	Mukwe	5/2/16	-18.0599	21.44215	Kavango	Andara	195;192	170;173;167	227;242		201;198	162;165	162;165	206	131;119;125	179	157;160	187;184	110;113;120;104;101;116	155	152;155	200;203	149;143	182	174;177	125;119	115;131	215;221;209;218	197;161;164	103;100	271;253;259
419	1149103850	AndaraDH	Mukwe	5/8/16	-18.0599	21.44215	Kavango	Andara	195;192	161;158	242	171	201	162;165	165	206	119;125	179;182	166;169	187	123;110;113;120	143;140;149;146	152	203	155	182	177	113;119;88;91	115	215;221;218	191;200;197;194;170;179;182;161;164	103	286;268;271;250;253
420	1149103851	AndaraDH	Mukwe	5/4/16	-18.0599	21.44215	Kavango	Andara	189;195	170;161	276	171	201;198	162;159	162	206;209	134;122	182	157	187;184	110;101	164;155	165;168	215;159;218;197	152;146	179;176;170	174;168;150;199	100;116	128	205;218	136;179;149;161;158;155	103;107	277;253;259
421	1149103856	AndaraDH	Mukwe	5/14/16	-18.0599	21.44215	Kavango	Andara	189	176;185	227		201	165;159	165	206	131;125;122	179	157	184	101	164	168	206	143	197	181	110;122	140	209;205		103;100	286

422	1149103857	AndararaDH	Mukwe	5/3/16	-18.0599	21.44215	Kavango	Andara	189;195	167	242	171	201;198	162	162;165	224;206;227;209	134;137	170;157;182	157	190;187	129;101	155	152;155	200;203	139;146	173;152;170;182;203	181	94;110;103	121	215	136;185;146;194;167;149;182;155	103	268;253
423	1149103858	AndararaDH	Mukwe	5/8/16	-18.0599	21.44215	Kavango	Andara	195	164;158	242	171	201;198	162;165	165	224;212;221;215	125	179;182	157;160	187	82;107;110;91;101	155	165;168	194;197	136;146	179	181;184	94;106;91	121;128	209	161	107	256
424	1149103859	AndararaDH	Mukwe	5/13/16	-18.0599	21.44215	Kavango	Andara	187;198;195;192	158	227;242	171;174	201;198	168;162;156;159	165	212;206;203;215;209	134;131;128;125;122	179;182	166;160;175;178;169	190;187;184	129;113;120;104;101	143;134;140;137;146	152;177;165;168;155	215;197;200;203;206;212	136;146;155	173;179;176;185;170;182	174;181;171	110;100;106;113;119;91	134;121;128;115;131;125	221;212;209;205	143;170;167;152;161;158;155;164	103;107;100;110	268;274;277;262;250;247;253;265;256;259
425	1149103860	AndararaDH	Mukwe	5/3/16	-18.0599	21.44215	Kavango	Andara	198;192	167;176	227;242	171	201;198	168;162	165	206;209		179;167	157	190;187	120;101	164;155	152;165	200;203;206	136;139	173;179;215;221	187;171	119;91	128;131	221;209	170;173;161;158;155	103	250;256;259
426	11491038	AndararaDH	Mukwe	5/5/16	-18.0599	21.44215	Kavango	Andara	195;192	158	227;242		198	156	165	212;203;215	122	179	166;169;172	184	113;104;101	134;137	177;165;168	200;212	155	170	174;181	110;100	115;131	215;209;202	143;140	103	268;271

	61																																
427	1149103862	AndaraDH	Mukwe	5/8/16	-18.0599	21.44215	Kavango	Andara	198;195;192	167;176	258;242	171	201;198	162;156	165	212;221	125;122	179	157	187;184	129;120	164;155	165;168	162;200	136;139;146	176;170	171	110;100;106;103	115;131	209	136;143;146;149	103;107	262
428	1149103863	AndaraDH	Mukwe	5/2/16	-18.0599	21.44215	Kavango	Andara	192	167	242	174	201	162	165	212	134	179	157	184	110;120;116	164	168	200	146	188;191	184	103	128	215	167	103;107	286
429	1149103865	AndaraDH	Mukwe	4/28/16	-18.0599	21.44215	Kavango	Andara	198;189;204	173;167;176	258;242	171	201;198	162;165	162;165	212;206;215	131;128	179;182	157	187;184	110;101	164;155	171;177;168;155	194;200;203	136;139;149;143	209;179;176;188;200;197	174;181;184	110;88	121;131;125	209;218	170;179;182;161;158	103;100;97	286;268;274;262;247;259
430	1149103866	AndaraDH	Mukwe	5/11/16	-18.0599	21.44215	Kavango	Andara	189	173	242	171	198	162	165	215	128	179	157	187	110	155	171;168	194	149	179	174	110	131	218	182	97	247
431	11491	Andar	Mukwe	5/15/	-18.0	21.44	Kavan	Anda	198	158	258	171	201	165;156	162	212	128	179	166;175	187	101	143;140	168	200		209;197	184	110	121	218	170	103	286

	03867	aDH		16	599	215	go	ra																									
432	1149103868	AndaraDH	Mukwe	5/5/16	-18.0599	21.4215	Kavango	Andara	192	173	273	171		165	162	197	128	179	160;154		113	164	168	203	136	176	171	116	131	218	170	103	265
433	1149103869	AndaraDH	Mukwe	4/3/16	-18.0599	21.4215	Kavango	Andara	195;192	164	258	171	201	162	165	206	140	179	157;160	187	120	155	180	197	152;143	194	171	97	125	218	158;155	103;100	274
434	1149103870	AndaraDH	Mukwe	5/9/16	-18.0599	21.4215	Kavango	Andara	198;192	167;176;161	242	171	201;198	162	155;162	206	125	179	157	187;184	88;107;91	155	152	224;200	136;143	173	165	100;106	121;128	205;218	146;170;167;161;164	107;100	271
435	1149103871	AndaraDH	Mukwe	5/8/16	-18.0599	21.4215	Kavango	Andara	195	176	227	171	198	162	165	215	131	179	160	184	101	155	155	200	136	182	171	106	134	221	161		247

436	1149103872	AndaraDH	Mukwe	5/15/16	-18.05999	21.442215	Kavango	Andara	192	167	258	171	201	165	165	209	122	179	157	187	110	155	155	194	158	176	168	82;116;91	131	209	149	107	253
437	1149103873	AndaraDH	Mukwe	5/2/16	-18.05999	21.442215	Kavango	Andara	198	182	227	174	204	162	162;165	212	128	179	160	184	110	155	177;165	200	133	194	187;184	110;113;116	131	215	167;164	113;103;110	256
438	1149103874	AndaraDH	Mukwe	5/17/16	-18.05999	21.442215	Kavango	Andara	192	170	227	171	198	153;150	165	221	131	182	157	187	120	155	152	197	139	179	187;140	110	121	209	176	100	271
439	1149103875	AndaraDH	Mukwe	5/8/16	-18.05999	21.442215	Kavango	Andara	189	167	227;258	171;174	201;198	162	162;165	212;197;203	128	179;167	157	187	88;94;91;101	155	171;158;177;165	200	139;158	176;185	177;162	103;91	128;115	221;218	146;173;149;161;158	103;100	268;259
440	11491038	AndaraDH	Mukwe	5/9/16	-18.05999	21.442215	Kavango	Andara		173	258	174		162	162	212		179	157	187	101	155	155	206	139	176	190	100	121	221	164	100	292

	81																															
441	1149103882	AndaraDH	Mukwe	4/28/16	-18.0599	21.44215	Kavango	Andara	192	167	242	171	201	162		221	122	182	157	187	120	155	168	194	158	173;176;170	165	110	121	205	176	103	271
442	1149103883	AndaraDH	Mukwe	5/7/16	-18.0599	21.44215	Kavango	Andara	195	158	242	179;171	198	162	168;165	212	122	182	160	187;184	94;98;91;101	155	152;165	200;191	146	179;176;185;182	168;184;177;171	106;113;116;91	115;137	209	161;158;164	103;107	268
443	1149103884	AndaraDH	Angolaother	5/19/16	-18.0599	21.44215	Kavango	Andara	187;198;195;192	170;167;176;164	227;242	171;174	198	168;162;165;156	165	212;206;215;209	131;128;125	179;167	157;160	190;187;184	110;120;101	164;155	152;165;155	194;197;200;206	152;136;139;146	173;179;176;197;170;182;215	168;187;171	94;110;100;106;119;91	134;121;128;131	221;209;205;218	170;167;149;161;158;155;164	103;107;100	244;271;250;247;256;259
444	1149103885	AndaraDH	Mukwe	5/16/16	-18.0599	21.44215	Kavango	Andara	192	173	242	174	198	162	165	206	137;128	179	157;160	184	139;110	164;155	168	203	143	176	184;177	100;103	128;125	205;218	152;149;155	103;107	268;277;256
445	11491	Andar	Mukwe	5/13/	-18.0	21.44	Kavan	Anda	195	164	242	171	201	162;159	165	212	137	179	157	187	110	155	152	162;200	136	179;176	187	119	121	209	161	103;107	256

	03886	aDH		16	599	215	go	ra																								
446	1149103888	AndaraDH	Mukwe	5/12/16	-18.0599	21.44215	Kavango	Andara	204	158	258	174	201	165	162	206	131	179	166	184	110	134;137	177	200	155		184	116	121	209	158		274
447	1149103892	NyanganaDH	NdongaLinena	3/27/16	-18.00337	20.67655	Kavango	Nyangana	195	173	242	174	201	159	165	212;206;197;203	140	173;182	157	187	113	172;155	168	197	136;143	173;170;146	171	113	121;125	212	155	103;100	265
448	1149103900	NyanganaDH	NdongaLinena	5/4/16	-18.00337	20.67655	Kavango	Nyangana	192	173	242		201	162;156	162	206	125;122	179;182	157	184	110	155	165	194;206	136;149	173;176	143;171	88;103	115;112	215	158	103;100	253
449	1149103902	NyanganaDH	NdongaLinena	5/4/16	-18.00337	20.67655	Kavango	Nyangana	195	170	242		198	156	165	215	125;122	160;182	157	187	129	164	174	203;206	133	173;170	174	113	128	218		103;100	268;265

450	1149103903	NyanganaDH	Ndiyona	5/3/16	-18.00337	20.67655	Kavango	Nyangana		170;176	227;242	171		162	165	206	122		157;163	184	101;116	164;155	152	200;206	133;149	179;182	174;171	110;100;103	125	209	170;167;161;164		271;265
451	1149103910	NyanganaDH	Ndonga Linena	3/30/16	-18.00337	20.67655	Kavango	Nyangana	198	173	242	171	198	162	165	215	122	179	157	187	120	181	171;168	194	136	179	174	110	131	209	109;182	97	241
452	1149103911	NyanganaDH	Ndonga Linena	3/28/16	-18.00337	20.67655	Kavango	Nyangana		161;164	242	171	201;198	159	165		134;131	157;167	157;154	184	107;91;101	155	152	159;194;197	136;133	173;176;170	168	113	128	199		103;100	265
453	1149103916	NyanganaDH	Ndonga Linena	3/30/16	-18.00337	20.67655	Kavango	Nyangana	204	173	248	171	198	165		227	137	179	157	184	107;110	164	155	200	143	173	165	94;122	112	215	188	103	268
454	11491039	Nyangana	Ndonga Linena	3/30/16	-18.003	20.67655	Kavango	Nyangana	198;192	170;173	242	171;174	201	168;165	162	212;206	119;125	160;179	157;160	187;184	98;101	164;155	165;168	194;197	139;143;146	209;173;152;188;170	177	110;100	118;121	209	197;158	103;100	268;271

	17	DH			37																												
455	1149103924	NyanganaDH	Ndonga Linena	5/11/16	-18.00337	20.67655	Kavango	Nyangana	195	173	273	171	201	162	165	197	128	179	160	178	101	164	168	218	143	176	174	100	125	221	179;158	103	277
456	1149103925	NyanganaDH	Ndonga Linena	4/29/16	-18.00337	20.67655	Kavango	Nyangana	198	176	227	171	198	162	162	218	128	179	157	184	88	155	152	224	139	173	165	113	125	205	109;158;155	100	271
457	1149103926	NyanganaDH	Ndonga Linena	5/2/16	-18.00337	20.67655	Kavango	Nyangana	198	185	227	174	198	162	168	197	122	182	160	184	101	164	155	200	152	191	177	103	118	209	188	103	256
458	1149103927	NyanganaDH	Rundu Rural W	5/11/16	-18.00337	20.67655	Kavango	Nyangana			242;233	171;174		168;162	165	206	122	185;179	160;154	184	110;104	155	171	197;203;206	136;133;143	194;185;197;182;203	143;168;184;177	113	128;131;125		173;167;179;149;176;161;158		244;271;262
459	11491	Nyang	Ndonga Li	3/26/	-18.0	20.67	Kavan	Nyang	208	170	245	171	198	162;159	162	221	122	182	160	184	110	155	155	162;197;200;203	139	179;182	190;184	125	121	221	152;149	110	241

	03934	anaDH	nena	16	0337	655	go	ana																									
460	1149103935	NyanganaDH	NdongaLinena	3/29/16	-18.00337	20.67655	Kavango	Nyangana		173	242	171;174	201	168;162	162	206	140	179;182	160	187;193	123;104;101	155	168	197;206	139	173;176	177	110;100;97	121;131	209;218	136;134	103;107;100;97	268;271
461	1149103940	NyanganaDH	NdongaLinena	4/14/16	-18.00337	20.67655	Kavango	Nyangana	198;189	170;167	227;242	171	198	165;159	165	209	131	179;167	157	187;184	82;129;110;120	164;155	171;168	200;203	139;143	173;152;179;200;191;170	174;187;181	110;106;113;116	143;125	221;218	170;173;152;179;182;161;164	94;103	274
462	1149103941	NyanganaDH	NdongaLinena	4/14/16	-18.00337	20.67655	Kavango	Nyangana	198;189	170;167	242	171	201;198	162;165	165	209	131	179;167	157	187;184	129	164;155	171;158;168;155	203	139;143	173;152;179;212;188;200;191	181	113	143;125	215;221;218	170;173;152;179;182;161;164	94;103	268;274;250
463	1149103948	NyanganaDH	NdongaLinena	4/28/16	-18.00337	20.67655	Kavango	Nyangana	192	179	242	171;174	201	165;159	165	206	122		160	187	110	155	171	200	136	173;164;191;194	184	106;113	131	212	170;173;179;176	103	280;262;265;259

464	1149103949	Nyangana DH	Rundu Rural W	4/28/16	-18.00337	20.67655	Kavango	Nyangana	198;195;192	158	242	174	198	162	165	212;218	122		166	184	113	134;137	155	197	155	170	187	113	118	221	161	103;107	247
465	1149103951	Nyangana DH	Ndonga Linena	5/6/16	-18.00337	20.67655	Kavango	Nyangana	198;195;192	173	227	171	201	165	165	206	128	179	160	187	139	155	168	212	146	182	177	106	121	221	161	103;100	268
466	1149103959	Nyangana DH	Ndonga Linena	4/8/16	-18.00337	20.67655	Kavango	Nyangana	187;189	158	242	171;174	198	153;150;162	162;165	212;206;221	140;131;125	173;179;182	184;172	187	110;120	143;137;146	165;168	200;203;206	155	206;173;194;185	143;177	110;103	121;115	212	170;152;161	103;107	232;271;250;256;259
467	1149103964	Nyangana DH	Ndonga Linena	4/11/16	-18.00337	20.67655	Kavango	Nyangana	208;198	170;173	261;242;245	171;174	219;201;198	162;165	168;162;165	212;215;209	125;122	157;182	157	187;184	123;94;82;98;110;113;91;104;101	155	165;168	200;206	152;139	173;179;176;197	174;181;177	110;103	118;128;131	209;205	176	113;103;107;100	274;253;256
468	11491039	Nyangana	Ndonga Linena	3/26/16	-18.003	20.67655	Kavango	Nyangana	198;189		227	171	198	162;165	162;165	218;215	119;128	179;182	157	187;184	88;113	164;155	152;155	224;197	139;143	173;200;170	165;181	113	134	215;205	161;158	103;100;97	271;250

	65	DH			37																												
469	1149103973	NyanganaDH	Ndiyona	5/4/16	-18.003337	20.67655	Kavango	Nyangana	187;189;195;192	173	242;236	171	201	168;165	165	206	119;122	179;182	160;154	187;184	129;110;120	164;172	152;165	194;203	130;136	179;182	205;165;177	94;110;91	134;125	215;221;224	170;176;158;164	107;100	268;250;253;265
470	1149103982	NyanganaDH	NdongaLinena	4/10/16	-18.003337	20.67655	Kavango	Nyangana	202;192		242;273	174	201;198	162;165	165	212;218	134;131	179	157;160	184	120	155	158;165	197;200	143;158	179;188	181	100;119;103	137;125	215;227;221;224	176;161;155;164	103	223;241
471	1149103983	NyanganaDH	NdongaLinena	4/10/16	-18.003337	20.67655	Kavango	Nyangana	198	170	258	171	201	162	165	200	125	167	175	187	120	164	140	215	136	173	171	110	121	218	161	100	274
472	1149104178	RunduDH	RunduRuralE	4/14/16	-17.91639	19.78028	Kavango	Rundu	198;195	170;173	242	171	198	162;165;156	168;165	212;206;215	131;119;122	179;167	157;160	187;184	94;107;98;110;113;120;101;116	164;155	152;177;165;168	194;197;200;203;209;212	136;139	173;152;179;200;170	165;181;184;171	110;106;113;116	118;134;121;128;125	215;221;209;218	170;161;158	113;103;107;100	235;268;250;247;253;265
473	11491	Rundu	RunduRur	4/25/	-17.9	19.78	Kavan	Rundu	189;192		242	171		168;162	165	206	131;128	179;182	157	187;184	110	155	168;155	203;206	143	176;182	190;177	113;103	121;125	209	143;161;158	103	271

	04201	DH	alE	16	1639	028	go																										
474	1149104202	RunduDH	RunduRuralE	5/4/16	-17.91639	19.78028	Kavango	Rundu	195	170;173;179	242	171		162;156	165	212;206;197;203	131;119;128	179	157	187;184	126;107;110;120;101	164;155	152;168	200;203;209	136	173;170	181;184;171	94;110;106;113;116	134;121;128;125	215;221;218	134;170;161;164	113;103;107;100	235;247;253;265
475	1149104203	RunduDH	Angolaother	5/23/16	-17.91639	19.78028	Kavango	Rundu	198;189;195;192	170;173;167;176;164	242	171	201;198	162;165;156;159	168;165	212;206;203;215	119;128;125	179;182;167	157;160	187;184	126;113;91;120;101;116	164;155	177;165;168;189	194;197;200;203;206;209	136;139;143	173;152;179;185;170;182	187;181;184;171	94;110;100;106;113;122;116	134;121;128;125	215;221;212;218		113;100	268;271;262;250;247;253;265
476	1149104224	RunduDH	RunduUrban	4/11/16	-17.91639	19.78028	Kavango	Rundu	198;189;195	170;173;167;176;164;185	258;242	171	198	162;165;156	168;165	212;206;215	134;140;131;119;128;125;122	188;179;167	157	187	126;110;120;101	164;155	152;177;165;168	194;197;200;209	139	173;152;179;176;200;194;185;170	181;184;171;199	94;110;100;106;113;116	134;121;128;125	215;221;212;218	170;173;167;149;161	113;103;107;100	268;271;298;300;262;250;247;253;265
477	1149104225	RunduDH	RunduRuralE	4/7/16	-17.91639	19.78028	Kavango	Rundu	198;189;195		227;242	171;174	219;201;198	153;168;165;156	162;165	200;206	134;128;122	185;179;182;167	157	187;184	107;110;113;104;101	164;155	171;152;165;168	197;203;191	130;152;136	173;152;179;176;200;221	137;165;181;171	110;100;113;103;116	128;131;125	215;209;218	185;167;161;164	103;107;100	241

478	11491042226	RunduDH	RunduRuralE	4/5/16	-17.91639	19.78028	Kavango	Rundu	198;195	170;173;176;164	258;242	171	201;198	162;165;156	168;165	212;206	134;131;119;128;125	179;182;167	157;160	184	126;91;120;101	164;155	152;177;165	197;200;209	136;139;143	173;152;179;170	181;177;171	94;110;100;106;113;116	134;121;128;125	215;221;218	170;173;167;152;161;158;164	113;103;107;100	268;271;262;250;247;253
479	11491042227	RunduDH	Calai	4/11/16	-17.91639	19.78028	Kavango	Rundu	198;189;192			171	204;201;198	162;165;156	168;162;165	206;215	131;119;128;125;122	179;182;167	157;160;175			155	152;177;165;168;155	194;197;200;203;209	152;136;139;143	173;179;176;188;185;197;170		110;100;106;113;116	134;121;128;115;131;125	215;221;212;209;218		113;103;107	268;283;271;274;280;262;250;247;253;265;256
480	11491042448	RunduDH	RunduUrban	4/5/16	-17.91639	19.78028	Kavango	Rundu	187;198;195	170;173;167;176;161;179;164	227;242	174	201;198	162;165;156	168;162;165	212;206;209	131;119;128;125	179;182	157;160	184	126;132;110;113;91;120;104;101	172;155	152;177;165;168;155	197;200;209	152;136;139;133;146	176;185;182	187;181;184;177;171	110;100;106;113;122;116	140;128;115;131;125	221;212;209;205;218	170;173;167;149;158;164	103;100;110	268;274;277;250;247;253;265;256
481	11491042449	RunduDH	RunduRuralE	5/6/16	-17.91639	19.78028	Kavango	Rundu	187;198;189;195;192	170;173;167;161;164	227;242	171;174	201;198	162;165;156	168;162;165	212;206;209	119;128;122	179;167	157;160	187;184	126;107;98;110;113;91;120;104;101	164;172;155	152;165;155	194;197;200;209	136;139;133;146	155;173;152;179;176;185;170;182;149;161	177;171	110;100;106;116	121;115;125	215;212;209;205;218		113;107;100;110	268;262;250;253;256
482	11491042	RunduDH	RunduUrban	4/5/16	-17.916	19.78028	Kavango	Rundu	198;189;195;192	173;167;176;161;164	227;258;242	171;174	201;198	162;156		212;206	119;128;122	179;182;167	157	187;184	126;110;113;91;120;101	164;172;155	152;165;155	194;197;200;203;209	136;139	155;173;152;164;185;170;182;149;158;161;146	181;171	110;100;106	134;121;128;115;131;125	215;212;209;205;218	161;158;164	113;103;107;100;110	268;271;274;262;250;253

	50				39																												
483	1149104251	RunduDH	Rundu Urban	3/19/16	-17.916339	19.78028	Kavango	Rundu	187;198;189;195;192	170;173;167;176;161;179;164	227;242	171;174	201;198	162;165;156;159	168;162;165	200;212;206;215;209	119;128;125;122	179;182;167	157;160	187;184	123;126;132;107;129;110;113;91;120;101	172;155	152;177;165;155	162;194;197;200;203;209	152;136;139;133;146	155;173;152;179;176;191;194;185;170;182;161	174;187;181;177;171	110;100;106;116;97	134;121;140;128;115;131;125	212;209;205	170;173;167;152;179;149;176;161;158;164	103;100	268;262;250;256
484	1149104272	RunduDH	Rundu Urban	4/14/16	-17.916339	19.78028	Kavango	Rundu	198;189;195;192	161;158	227;242	171;174	198	162;165	168;162;165	212;206;209	131;119;128;125	179;182	160;163;175;178;172	187;184	123;126;110;113;91;120;104;101	143;134;140;137;146;152	152;177;165;168;155	197;200;209	155	155;173;152;179;176;185;170;182;161	174;187;181;177;171	110;100;106;116	121;140;128;115;131;125	215;221;212;209;218	170;173;167;152;179;149;176;161;158;155;164	113;107;100;110	268;274;277;262;253;256
485	1149104466	NyanganaDH	Ndonga Linena	4/13/16	-18.003337	20.67655	Kavango	Nyangana	195	173;193	242	165;174	201;198	156	162	212	134;140;122	179	157	187	110;120;116	164;155	168	200;209	143	194;197	174;181;177	110	128;125	215;218		113;100;97	271
486	1149104467	NyanganaDH	Ndonga Linena	4/10/16	-18.003337	20.67655	Kavango	Nyangana	198	176	227	171	198	162	162	218	128	179		184	88	155	152	224;221;227	139	173;170	165;184	110;113;116	125	205	161;158;155	100	268;271
487	11491	Nyang	Ndonga Li	4/13/	-18.0	20.67	Kavan	Nyang	202	173;161	258	165;1	201	156	162	212	122	179;182	160	187	110	164;181	177	200	136;146	200;197	174	136;88	115;125	215;205	161;164	113;100	271;253

	04468	anaDH	nena	16	0337	655	go	ana				71																					
488	1149104469	NyanganaDH	Ndonga Linena	4/14/16	-18.00337	20.67655	Kavango	Nyangana	192	173	227	174;188	201	168	162	209	128	157;182	157;160	184	110;113	172	149	194;197	133	188;191;185	171	100;103	125	218	194		271
489	1149104470	NyanganaDH	Ndonga Linena	4/11/16	-18.00337	20.67655	Kavango	Nyangana	198;195;192	173;161;164		165;171	201;198	156	165	206;215	131;122	179	157		101	155	152;165	197;200;203	136;143	173;179;176;170		113;97;91	134;125	221;209;205	161	103	247
490	1149104471	NyanganaDH	Ndonga Linena	4/14/16	-18.00337	20.67655	Kavango	Nyangana	202;195	173	258	165	201	156	162	212	122	182	160	187	107;110	164	177	200	143;146	197	174	136	125	215	164	113;110	271
491	1149104485	NyanganaDH	Ndonga Linena	3/26/16	-18.00337	20.67655	Kavango	Nyangana	195	167	242	174	219	162	165	212	128	182	157	184	110	164	177	162;218	143	176	184	100;103	128;131	218	155	113;103;100	256

492	1149104486	NyangananaDH	Ndonga Linena	4/13/16	-18.00337	20.67655	Kavango	Nyangana	192	161	242	174	201	168	165		143	179	160	184;181	120;116	155	155	168;194;191	146	179;182	168;165;184	100;106;103	131	209	170;173	103;107	271
493	1149104487	NyangananaDH	Ndonga Linena	4/12/16	-18.00337	20.67655	Kavango	Nyangana	189	176	242	174	201	162;156;159	165	206	122	182	157	184	120;116	155	168	162;194;197	143	173;170	177	119;116	121	221	109;158;155	103	247
494	1149104488	NyangananaDH	Ndonga Linena	4/13/16	-18.00337	20.67655	Kavango	Nyangana	195	170	227	171	198	153	162		140	179	163	184	120	164	168	194;197	139	173	184	100	128	218	161		268
495	1149104489	NyangananaDH	Ndonga Linena	4/11/16	-18.00337	20.67655	Kavango	Nyangana	195;192	167;179	227	174	198	162	162	212;206	125;122	157;167	157;160;154	187	88;110;91;104	155	152;155	197;200	143;158	173;176;170	181;177	110;125	121;131	227;218	167;161	103;100	283;280;277;262
496	11491044	Nyangana	Ndonga Linena	3/28/16	-18.003	20.67655	Kavango	Nyangana	195	167	242	174	219	162	165	212	128	182	157	184	110	164	177	215;162;221;218	139;143	173;176	184	100;106;103	131	218	109;152;155	103	256

	90	DH			37																												
497	1149104491	NyanganaDH	Ndonga Linena	4/8/16	-18.00337	20.67655	Kavango	Nyangana	192	173	233	171	204	168;165	165	206	125	182	160	187	110	155	155	203;206	130	194	171	110	134	224	185;188;182	107	268
498	1149104492	NyanganaDH	Ndonga Linena	4/10/16	-18.00337	20.67655	Kavango	Nyangana	198	167	227	171		162	162			179	160	187	120	155	152	191	136	173;176	181	110;106;103	115	205	170;173;167	103	271
499	1149104493	NyanganaDH	Ndonga Linena	4/9/16	-18.00337	20.67655	Kavango	Nyangana	195	176	258	174	219	162	165	209	128		157	187	82;129;110;91;120;101	155	177	218	136	173	184	100	131	218	152	103;100	271;274;256
500	1149104494	NyanganaDH	Ndonga Linena	4/12/16	-18.00337	20.67655	Kavango	Nyangana	192	161;158	242	174	198	159	165	212	125	182		187	113	164	155	159;206;209	136;139	194	168	106	128	209		113;110;116	244;247
501	11491	Nyang	Ndonga Li	4/8/	-18.0	20.67	Kavan	Nyang	195	176	242	191;1	201	156	162	221	128	179	157	184	101	164	168	156;200;203	146	173;188;191;194	168	94;97	131	212	143;146;140	103	250

	04495	anaDH	nena	16	0337	655	go	ana				71																					
502	1149104508	NyanganaDH	NdongaLinena	4/13/16	-18.00337	20.67655	Kavango	Nyangana	187;192		227;242	171;174	201;198	165;159	165	212;206		179;182	157;160	187	113	164;155	155	197;209	139;143	200;194	168;177	106;91	128	209	197;170;173;179;176;161	113;100	247
503	1149104510	NyanganaDH	NdongaLinena	4/10/16	-18.00337	20.67655	Kavango	Nyangana	189;195;192	176;179;164	227	171	198	162;165	165	206;221	128	179;167	157;160	187	98;101	155	165	197;200	139;133	173;152;176	143	110;122	131;125	209;205	161	113;103;100	268;271;277
504	1149104511	NyanganaDH	NdongaLinena	4/11/16	-18.00337	20.67655	Kavango	Nyangana	195	176	242	174	198	162	165	218	122	179	160	184	107;104	155	152	197	133	182	171	106	125	215	173;164	110	247
505	1149104512	NyanganaDH	NdongaLinena	3/26/16	-18.00337	20.67655	Kavango	Nyangana	192	167	242	174	198	165	165	212	122	182	160	184	110	155	155	197	136	170	187	122	131	221	152	107;110	241

506	1149104513	NyanganaDH	Ndonga Linena	4/12/16	-18.00337	20.67655	Kavango	Nyangana	195	170	227	171	198	159	162	206	125	167	157	184	91	155	155	159;197	146	170	177	110;106	125	209	167;164	107	271
507	1149104514	NyanganaDH	Ndonga Linena	3/28/16	-18.00337	20.67655	Kavango	Nyangana	198	167	248	171;174	201		165	206	122		160		101		168		133	185;182	147	113;88	125;146	205	158	103	253
508	1149104515	NyanganaDH	Ndonga Linena	3/25/16	-18.00337	20.67655	Kavango	Nyangana	195;192	173;176;182	258;242	171	204;201	174;162;159	165	221;215	128;125;122	179;182	157	187;184	110;101	164;155	155;189	197;206	130;136;149	179;176;200;197	174;190;187;177	113;116	128;115;131	215;209	167;161;158;155;164	103;107	262;250;253;259
509	1149104516	NyanganaDH	Ndonga Linena	4/8/16	-18.00337	20.67655	Kavango	Nyangana	195	173	242	171	198	165		221	116	160;182	160	187	129	164	217	165;203;206	136	191	165	91	121;125	218	173;167		
510	11491045	Nyangana	Ndonga Linena	4/10/16	-18.003	20.67655	Kavango	Nyangana	189	179	258	171	198	165	162;165	221;215	122	179	160	187;184	98	164;155	165;155	215;218	139;133	173;176;170	174;187;184;171	125;122;119	131;125	209;205	170;167	113;103;110	274

	17	DH			37																													
511	1149104518	NyangananaDH	Ndonga Linena	4/8/16	-18.00337	20.67655	Kavango	Nyangana	195	158	258	174	219	162	165	209	128	130	175	187	129	134;137	177	218	155	173	184	100	131	218	152	103	274
512	1149104532	NyangananaDH	Ndonga Linena	4/9/16	-18.00337	20.67655	Kavango	Nyangana	195;192	173;158	227;258	171;174	198	156;159	162	212;206	128;122	179	160	184	129;120	164;155	165;168	197	136;146	173;176	181;184;171	110;116	134;128	215;221;212;218	143;140	103	268;271;250;253;256
513	1149104533	NyangananaDH	Ndonga Linena	4/10/16	-18.00337	20.67655	Kavango	Nyangana	198	176	227	171	198	162	162	218	128	179	160	184	88;85	155	152	224;221;227	139	173	165;184	113	125		158;155	100;97	271
514	1149104534	NyangananaDH	Ndonga Linena	4/10/16	-18.00337	20.67655	Kavango	Nyangana	192	167	242	171	198	165	165	212	122	182	160	184	101	155	177	197	143	173	181	106	128	209	185	103	283
515	11491	Nyang	Ndonga Li	4/12/	-18.0	20.67	Kavan	Nyang	195;192	167;161	258;242	171	198	165;156		212;221	143;122	179;182	160;163	184	120;101	155	177;165;168	194;197	149;143	173;152;179	190;187;181;184	106;113	121	212;209	149	103;97	250;253

	045335	anaDH	nena	16	0337	655	go	ana																									
516	1149104536	NyanganaDH	Ndonga Linena	4/8/16	-18.00337	20.67655	Kavango	Nyangana	192	179	242	171	201	162	165	206	122	179	160	187	110	155	171	200;203;206	136	194	184	110;113;116	131	212	173;179;176		262
517	1149104538	NyanganaDH	Ndonga Linena	3/26/16	-18.00337	20.67655	Kavango	Nyangana	192	176	242	171;174	198		162	212;221	125	179;182	157	187	120;101	164;155	168	194	136;143	200;191	190;187	110	121	215	161;158;164	113;100;110	271
518	1149104539	NyanganaDH	Ndonga Linena	4/12/16	-18.00337	20.67655	Kavango	Nyangana	189	173	242	174	219	162	165	218	131	182	157	187	129	164	177	218	139	203	187;184	110;106	125	218	161;158	103	262
519	1149104540	NyanganaDH	Ndonga Linena	4/13/16	-18.00337	20.67655	Kavango	Nyangana	192	173	227	179;171		162	165	206	134	179	157	187	91	155	155	212	149	179;176	187;171	94;97	128	218	185;188;182	97	274

520	1149104541	NyanganaDH	NdongaLinena	4/9/16	-18.00337	20.67655	Kavango	Nyangana		176	258	174	219	162;159	165		128;125	157;179	157;154	187	126;82;129;110;91;120;101	155	177	215;162;221;218	136;133	173;170	184	100	131	218		103;100	274
521	1149104542	NyanganaDH	NdongaLinena	3/31/16	-18.00337	20.67655	Kavango	Nyangana	192	161	242	174	198	165	165	212	122	182	166	184	110	134;137	155	197	155	170	187	100;122	131	221	152	107;110	241
522	1149104543	NyanganaDH	NdongaLinena	4/13/16	-18.00337	20.67655	Kavango	Nyangana	202	161	242	171	201	174;162	162	212	125		160	187	110	134;137;152	155	200		179;176	184	110;125;103	115	205	179	100;110	247
523	1149104556	NyanganaDH	Angolaother	4/8/16	-18.00337	20.67655	Kavango	Nyangana	187;192	167;161;164	258;242	165;171		162;165	162;165	212;215	122	179;182	157;160	187;184	110;120;101	155	165;168	197;200	133;143;158	173;185;170;182	171	113;103	134;125	209;205	167;176;161;158	113;103;107;100	244;232;235;247
524	11491045	Nyangana	NdongaLinena	4/13/16	-18.003	20.67655	Kavango	Nyangana	192	164	242	179;171	201	156	165	206	131	179	157	187;184		146;155	189	156;194;197	152	179;176	184;202	100;103	134;128	215	161;155	103;107	256

	57	DH			37																												
525	1149104558	NyanganaDH	Ndonga Linena	4/1/16	-18.00337	20.67655	Kavango	Nyangana	198;192;204		242	171	201;198	162;165	165	206;215	128;125;122	185;179;182		187;184	110;113	164;155	171;158;152;168	197;191;212	136;139;146	173;179;176;185;182	177	94;106;113	128;115;131;125	215;212;205;218	143;152;161;158;164	103;100	268;241;247;253;265
526	1149104559	NyanganaDH	Ndiyona	4/14/16	-18.00337	20.67655	Kavango	Nyangana	202;192	164	242	171	198	162;159	165	206	131;125	167	157	187	110;113;104;101	155	168;140	197;209	152;143	179;191;194;182	177;196	94;113	131;125	205	170;167;149;176;161	103;100	286;283;271;280
527	1149104560	NyanganaDH	Ndonga Linena	3/26/16	-18.00337	20.67655	Kavango	Nyangana	198;195	158	227	171	201	159	165	215	131;125	130	160;163		129;120	134;140;137		200	155	173	187	100;113	128	209;205	176;158	103;107	280;262;247;253;265
528	1149104567	RunduDH	Rundu Urban	4/23/16	-17.91639	19.78028	Kavango	Rundu	198;189;192	170;161;185	227;242;248;245	171	198	162;165	162;165	212;206;221;215	131;128;125;122	185;179	157;154	187;184	139;91;120;101	164;172	171;152;177;165;168;155	215;194;197;203;209	152;136;139;149;143;155	179;176;200;185;197;170	174;187;184;177;171	110;100;106;122;97	134;121;128;115;125	215;221;209;205	161;155;164	103;107;97	286;268;277
529	11491	Rundu	Rundu Ur	4/4/	-17.9	19.78	Kavan	Rundu	198	167	227;242;288	171;1	198	162	162;1	215	134;131;119;128;	179	157	187;184	91	155	152;168	194;197	152;139	170;167	187;184;171	110;113;103;116	134	205	191;185;188;167;179	103;100	271

	04568	DH	ban	16	1639	028	go					74			65		125;122																
530	1149104569	RunduDH	RunduUrban	4/29/16	-17.91639	19.78028	Kavango	Rundu	189;192		242	171	201;198	162;165;156	165	212;206;221;215	128;122	185;179;182	157	184	94;110;113;91	164;155	152;165	197	152;139;143	173;176;194;197	174;165	110;106	140;128	215;212;209;218	146;167;152;149;158;155	103;100	268
531	1149104570	RunduDH	Mashare	5/5/16	-17.91639	19.78028	Kavango	Rundu	189;195	167	227	174	198	156	165	212;206	122	179	157;154	184	110;113;104;101	155	152	203	133;146	173	137;181	116	118;128	209;218	109;121;158;155	103	280;262
532	1149104571	RunduDH	Mashare	4/29/16	-17.91639	19.78028	Kavango	Rundu	208;198;195;192	170;173;167;176	227;242;248	171	201;198	168;162;165;159	165	212;206;221;215	131;128;125;122	179;167	160;154	190;187;184;178	110;91;120	164;172;155	152;177;165;168;155	194;197;200;203	152;139;149;143	206;173;179;200	174;147;168;187;181;177;171;140	110;106;113;122	134;121;128;131;137;125	212;209;205	118;170;173;179;161;158;164	103;107	268;274
533	1149104572	RunduDH	RunduRuralE	5/8/16	-17.91639	19.78028	Kavango	Rundu			242	174	198	162;165	162;165		122	157;167	157;154	190;184	91;104	164;155	152	197;200	136	176;182	193	100;106	128;125			107;116	268;271

534	1149104574	RunduDH	RunduUrban	5/11/16	-17.91639	19.78028	Kavango	Rundu	198	164	242	174	201	162		212	131	179	157	187	82;139	155	152	197	139	173;170	171	110	125	205	170	103;100	271
535	1149104575	RunduDH	RunduUrban	4/19/16	-17.91639	19.78028	Kavango	Rundu	195	173	227	171	201	162	165	206	131;119;125	179	175	184	110;104	155	152	212	130	173;191	168	113	128;131		173	107	286
536	1149104576	RunduDH	Angolaother	4/27/16	-17.91639	19.78028	Kavango	Rundu	208;195;192	170;167	227;242;248	165;174	198	168;162;156;159	162;165	212;206;221	137;122	179;182	157;160;154	190;187;184;178	110;91;120;101	164;172	152;177;165;168;155	194;197;200	152;136;139;149	206;179;176	174;137;147;187;184;171;140;162	110;100;106;113;122;103;97	121;128;115;131;137;125	209;205	118;179;161;158;155	103;107;100;110	268;247
537	1149104577	RunduDH	RunduUrban	4/4/16	-17.91639	19.78028	Kavango	Rundu	208;198;195;192	182;185	242	171	219;201;198	168;162;165	165	224;212;206;230;215	128;122	182;167	160;175;178	187	110;101	155	152;165;168;155	224;197	130;152;139	173;152;221	137;171	110;103	134;128;131;125	215;209;205	185;173;161;158	103;107	268;274
538	11491045	RunduDH	RunduUrban	3/29/16	-17.916	19.78028	Kavango	Rundu	202;189	167;164	242	171;156	198	162;165	162;165	212	128	179	157	187	110	155	171	206;209;212	136;149	155;152;158	187;171	100;122;119;116	121	212	167;161;155	110	268;253

	78				39																												
539	1149104579	RunduDH	RunduRuralE	5/3/16	-17.916639	19.78028	Kavango	Rundu	198;192	173	242	171	201	165	162	206	131	179	157	184	101	155	189	197	152	179;182	190	100	128	221;205	167	103	268
540	1149104580	RunduDH	Kapako	6/6/16	-17.916639	19.78028	Kavango	Rundu	192	170;173	242	171;174	198	162;165	165	206	131	179	157	184	82;139;110;91;120;101	155	177	162;197;203	152;139	185;182	177	100;97	128	215	155	100	268;277
541	1149104591	RunduDH	RunduUrban	4/8/16	-17.916639	19.78028	Kavango	Rundu	189	170	258	171	201	162	165	206	128	179	160	187	123;94;113;120;104;101;85	164	165	194	152;149;155	179	181	100;103	125	221	161	103;107	271
542	1149104592	RunduDH	RunduRuralE	4/27/16	-17.916639	19.78028	Kavango	Rundu	202;198;189;192	170;173;167;158	258;242	171	201;198	153;162;165		206	131;128;122	179	157	187;184	123;91;120;104;101	164;155	165;189	194;197;203	152;136;139	173;179;176;170;182	190;187;181;184;177;140	110;100;103	128;125	199;221;212;209;205;218	118;167;161	103;107	268;271;250
543	11491	Rundu	RunduUr	4/18/	-17.9	19.78	Kavan	Rundu	202;187;198;189	167;161;158	227;258;242	171	201;1	153;168;162;1	168;16	212;206;197;209	131;128;122	179	157;160	187	123;76;126;88;98;110;9	164;172;155	152;165;189;162	194;197;206	152;139;133	173;176;191;185;170	190;168;181;184;177;	110;100;103	128;115;131;125	221;212;205;218	118;167;176;161;158	113;103;107	235;268;271;250;247;253;256

	04593	DH	ban	16	1639	028	go		;192				98	56;159	2;165						1;120;101						171;140						
544	1149104594	RunduDH	RunduUrban	4/12/16	-17.91639	19.78028	Kavango	Rundu	198;195	161;164	258	171	201	159	165	206	134;125	179	157;160	184	101	155	171;168	194	139	176	184	113	118		161	107	268
545	1149104595	RunduDH	RunduUrban	4/14/16	-17.91639	19.78028	Kavango	Rundu	208;187;189;195;192	158;155	261;227;242		198	162;156;159	162;165	212;206;221;215;209	137;122	185;179;182	166;175;169;172	184	123;110;120;101	140;149;137;146;152	152;165;155	221;194;197;200;191	155	173;179;176	174;147;168;187;171	110;106;113;88;91	121;128;115;137;125	215;227;212;209;205;218		103;107;110	268;271;247
546	1149104596	RunduDH	RunduUrban	4/5/16	-17.91639	19.78028	Kavango	Rundu	198;195	164	227;242	177;174	201	162	165	212;206	131	179	157	187	139;110	155	152	162;194;197;212	130;139	173;170	168;187;171	110;106;113	125	205	170;173;167;152	103;107;100	271
547	1149104597	RunduDH	RunduRuralE	5/1/16	-17.91639	19.78028	Kavango	Rundu	208;187;198;189;192	170;173;167;161;158	242	171	201;198		165	212;221;215	137;125;122	179;182	160;163	190;187	98;110;91;104;101	164;155	152	197;200	152;139;143	173;152;176;188	174;147;171;150	100;106;91	118;128;115;137	227;221;205;218	158	103;100;110	244;262;250;247;253;265

548	1149104598	Rundu DH	Rundu Urban	5/10/16	-17.91639	19.78028	Kavango	Rundu	208;192	170;173	242	171	198	168;159	162;165	212;221	134;137	182	160	190;184;178	110;113;101	164;155	152	197;200;191	152;139	173;152	174;147;150	110;106;113;119;116;91	128;131;137	227;221;209;205	143;152;161;158;155;164	103;100	244;268;262;247
549	1149104599	Rundu DH	Rundu Urban	4/29/16	-17.91639	19.78028	Kavango	Rundu			242	171	198	168;165	165		134;137;128;125	160;182	157;163	190	107;101;116	164	152	197;191	136;139	173;176;170	147	110;113	118;134;137	227		103	247
550	1149104600	Rundu DH	Rundu Rural E	4/29/16	-17.91639	19.78028	Kavango	Rundu	192	161	227	171	201;198	162	162	212	125	179	157;160	184	110;113;104;101	164;155	165	197;212	139;146	176	174;147	110;113	118	205	158;155	103	274;277
551	1149104601	Rundu DH	Rundu Rural E	6/6/16	-17.91639	19.78028	Kavango	Rundu	202;187;198;189	170;176;161;158	227;242	171;174	201;198	162	162;165	212;206;197;203	116	179;182	157;160	174;187;184	126;110;91;101	164;172;155	152;165;168	194;197;203	152;136;139	179;176;191;170	174;193;177;171;196	100;106;103	121;128;115;125	215;209;205;218	118;191;170;173;176;161;158	103;107	238;241
552	11491046	Rundu DH	Rundu Urban	5/10/16	-17.916	19.78028	Kavango	Rundu	202;187;198;189;195;192	170;173;176;161;179;158	227;258;242	171	201;198	153;162	162;165	212;206	131;128;125;122	179;182	157;160	187	123;126;88;129;110;91;120;101;116	164;172;155	152;165;168;162	194;197;191	152;139;143;146	179;200;191;197;170	174;165;181;171;140	110;100;106;103	121;115	199;221;209;205;218	118;191;115;176;161	103;107	268;271;262;250;253;265

	03				39																												
553	1149104604	RunduDH	RunduUrban	3/22/16	-17.916339	19.78028	Kavango	Rundu	202;189;192	170;161;158	227	171;177	201;198	153;150;162;165;156	168;162;165	206;197	131;128;122	179;182	157;160	187	123;110;91;120	164;155	152;165	194;197;200;191	152;139;146	173;152;179;176;197;170;182	187;140	110;100;106;113;103	121;128;115;125	199;212;209	118;121;115;176;161	103;107;100;110	268;271;262;253;265
554	1149104616	RunduDH	RunduUrban	5/10/16	-17.916339	19.78028	Kavango	Rundu	187	176	227	174	201;198	162	165	212	122	179	157	187	126;88;107;98;116;79	172;155	162	197	139	191;170	171	106	115	205	118;191	103;107	268
555	1149104617	RunduDH	Angolaother	5/12/16	-17.916339	19.78028	Kavango	Rundu	187	179	227;242	171	198	162;165;159	162;165	206	122	182	157	187;184	101	155	162	215;194;197	152;139	179;176	187;171	110;106	121;115;125	209;205	118;161;158;155	103;107	280
556	1149104618	RunduDH	RunduUrban	5/8/16	-17.916339	19.78028	Kavango	Rundu	187;195	167;176	227;242	171;174	201;198	162;165;159	165	206	137;119;128;122	179;182	160	174;187	110;91;101	164;172;155	152	194;197	152;139	170	177;171	113	121;128;115;125	205	118;146;161;158;155	103;107	274;262;250;253;256;259
557	11491	Rundu	RunduRur	5/27/	-17.9	19.78	Kavan	Rundu	208;187;198;189	170;173;167;176;	227;242	171;1	201;1	168;162;16	162;1	212;206;221;215	137;128;125;122	173;185;17	157;160	174;190;187;1	98;110;113;91;104;101	164;172;146;155	152;177;165;168;162	197;200;212	152;139;143;146	173;152;179;176;191;185;197;170	174;147;177;171;140	110;100;106;113;103	118;121;128;115;131;	209;205	118;173;167;152;161;158;155	103;107;100	244;268;274;277;250;247;

	04619	DH	alE	16	639	028	go		;195;192	161;158		74	98	5;159	65			9;182		84;178									137;125				265;256
558	1149104620	RunduDH	RunduRuralE	6/28/16	-17.91639	19.78028	Kavango	Rundu	208;195;192	170;167;161	227;242	171	201;198	168;162;165;159	165	224;233;212;206;221;215;209	137;128;125	173;185;179;182;176	157;160	190;187;184;178	110;101	164;181;146;155	171;152;177	197;191	152;139;143;146	173;152;176;185;197	147;177;171;153	110;106;113	118;149;128;131;137;125	209;205;218	173;152;176;161;158;155	103;107	244;268;271;262;250;247;253;265
559	1149104621	RunduDH	RunduUrban	4/24/16	-17.91639	19.78028	Kavango	Rundu		170;167;176	227;242	171	198	168;165;156;159			137;128	179;182	157;160	190;187;184;178	110;101	164;155	152;177;165;168	197;191	152;139;143	173;152;176;191;185;197;170	147;177;171;153	110;106;113;91	121;128;131;137;125	227;209;205;218		103	244;268;274;262;250;247;253
560	1149104622	RunduDH	RunduRuralE	4/30/16	-17.91639	19.78028	Kavango	Rundu	208;198;195;192	167;188	230;227;242	171;174	201;198	162;156;159	162;165	224;212;206;221;218;209	137;149;131;128	173;179;182	157;160	190;184;178	110;101	164;155	152;180;177;165;168;155	197;212	152;139;143	173;152;176;191;197;170;149;167	147;187;177;171;153	110;113;103;91	121;128;131;137;125	212;209;205;218	170;173;167;176;161;158;155;164	103;100	268;274;277;250;247;265
561	1149104623	RunduDH	RunduUrban	4/25/16	-17.91639	19.78028	Kavango	Rundu	189		242	174	201	159	165	218	125	185	160		82;129	146	168	197	143	185	171	113	137	205	188;164	100	268

562	1149104624	RunduDH	RunduRuralE	5/7/16	-17.916639	19.78028	Kavango	Rundu	195;192	173;167;161	227;258;242	171;174	201;198	159	165	212;206;215;209	128;122	173;179;182	157;160	187;184;178	110;101	164;172;137;146;155	152;177;168;155	197;200;206	139;143	209;173;152;179;176;188;197;170	184;177;171	94;110;113;103	121;131;125	215;221;209;205;218	173;152;161;158;155	103;107;100	268;271;274;277
563	1149104625	RunduDH	RunduRuralW	6/6/16	-17.916639	19.78028	Kavango	Rundu	187;195;192	161	227;242	171	201;198	162;159	165	206;215	143;128;122	179	160	187;184;178	104;101	140;137	152;155	197	155	179;176	177;171	94;106	121;115;125	209;205	143;146;149;158	107	268;280;262
564	1149104626	RunduDH	RunduUrban	4/18/16	-17.916639	19.78028	Kavango	Rundu	198	170	242	179;171	198	162	165	215	128	182	160	184	82;129;91;101	164	171	203	136	179;176	171	125	125	215	161	103;100	268;271
565	1149104627	RunduDH	RunduRuralE	4/25/16	-17.916639	19.78028	Kavango	Rundu	192		227	171		159	165	206	131;125	182	160		110;116	155	165	212	139	176	174	110;106	128;115;125			110	247
566	11491046	RunduDH	Kapako	5/3/16	-17.916	19.78028	Kavango	Rundu	195;192	170;173;176;161;158	261;227;258;242	171;174	201;198	153;162;165;156	168;162;1	206;197;215	128;125	179;182	157;160	184	123;139;129;110;113;91;120	164;172;155	158;177;165;155;162	197;200;203;191;212	139;143;146	173;179;176;200;197	174;147;165;181;177;171	110;100;113;103;116	121;128;115;131;125	215;199;221;212;209;205;218	118;170;167;161;158;155	113;103;107;110	244;232;268;271;241;250;253

	40				39										65																		
567	1149104641	RunduDH	RunduRuralW	5/7/16	-17.91639	19.78028	Kavango	Rundu	195	167	242	171	198	162	168	206	128	179	157	187	123	164	152	224	146	197	171	100	140	218	173	103	241
568	1149104642	RunduDH	RunduUrban	5/14/16	-17.91639	19.78028	Kavango	Rundu	195	167	276;227	171;174	198	162;159	168;165	206	119;128	179;182	160	184	101	164	152	162;197	139;146	173;176	177;171	110;100;113;91	140;131;125	209	173;158;155	107	256
569	1149104643	RunduDH	RunduUrban	4/12/16	-17.91639	19.78028	Kavango	Rundu		170;173;167;161;164	227;242	171	201;198	162;165;159	165		134;140;137;128;122	160;179;182	157;160	187;184;178	107;110;113;120;104;101	164;172;181;137;146;155	171;152;180;177;165;168;155	194;197;200;191	152;139;143;146	173;152;179;176;194;197;170	147;177;171;153	110;100;106;113;103;97;91	118;121;131;137;125	209;205		103;107;100;97	244;268;271;274;250;247;253;265;256
570	1149104645	RunduDH	RunduUrban	5/19/16	-17.91639	19.78028	Kavango	Rundu	195		242	171	198	159	165	209	128	173	160	184;181	107;98	155	152	197	143	173;185	174;168;184;171	110;106;113;116	121;125	209		103	268;265
571	11491	Rundu	Kapako	4/27/	-17.9	19.78	Kavan	Rundu	195;192	170;167	227;242	171;1	204;1	168;162;165;1	165	224;206;209	137;128;122	173;179	160	187;184;178	110;101	164;155	152;177	221;197	136;139;143	173;152;179;176;191;197;170;182;203	147;168;181;184;	110;97;91	128;131;125	209;205	170;173;167;179;161;158;155	103;107	268;271;262;247;253;265

	04647	DH		16	1639	028	go					74	98	56;159													177;171						
572	1149104648	RunduDH	RunduUrban	3/19/16	-17.91639	19.78028	Kavango	Rundu	195;192	170;167;161;185	227;242	171;174		168;162;156;159	168;165	224;212;206;221;215;209	140;137;128;122		157;160	190;187;178	110	164;181;155	152;177;168	194;197;200;203;191	139;143;146	173;152;176;194;197;170;161	143;147;168;177;171	110;100;106;113;91	134;121;131;137;125	209;205;224	170;173;167;152;179;176;161;158;155;164	103;107	268;271;274;262;250;247;265
573	1149104649	RunduDH	NotRecorded	4/18/16	-17.91639	19.78028	Kavango	Rundu	195;192	161	242;288	171;174	201;198	162	168;165	206	128	179	157	187;184;178		164;155		197	146	173;176;194;170	168;184;171		131	209;218;224	170;173;167;164	103;107	268
574	1149104650	RunduDH	RunduUrban	4/7/16	-17.91639	19.78028	Kavango	Rundu	198;195;192	173;167;161	258;242	171;174	201;198	162;156;159	168	206;221;197	128;125	179	157	187;184	123;110;113;120;101	164;155	152;177;165	162;200;206;209	139;143;146	176;197	181;171	100;116	121	209;205	121;170;173;161	113;103	232;241;250;253
575	1149104651	RunduDH	RunduUrban	5/30/16	-17.91639	19.78028	Kavango	Rundu	198		230	171	198	162	165	206	131	179	157;160;154	184	120	164	168	203	146	188;185	171	106	112	215	170;149	103;100	253

576	1149106490	AndaraDH	Angolaother	4/15/16	-18.0599	21.44215	Kavango	Andara	195;192	173;176	227;258	171;174	201;198		165	206;215	131			187;184	107;110;101	164;155	180;155;183				174	106;116	134;140	221;209	98	103;100	244;247;259
577	1149108242	NyanganaDH	NdongaLinena	3/29/16	-18.00337	20.67655	Kavango	Nyangana	202;204	161	242	171	201	165		212	125	179	160		101		155		136	200	181	113	118;112	218	109;179	103	277
578	1149108803	AndaraDH	Angolaother	5/25/16	-18.0599	21.44215	Kavango	Andara		176	242		201	165	168	215	119	182	157	184	110;113	155	152	200	136		177	110	134			107	
579	1149108808	NyanganaDH	Dirico	4/18/16	-18.00337	20.67655	Kavango	Nyangana		170	258	171	201	162	165		119	185	157;160	184	104	155	168	200	136;143	158	165		134			103;107;100	274
580	11491088	AndaraDH	Angolaother	5/5/16	-18.0599	21.44215	Kavango	Andara	192	167	258	171	201;198	162	165	206	122	182	157	187	101	155	155	200	136	146	174	122	131			107	250

	16																																
581	11491088829	AndaraDH	Angolaother	4/15/16	-18.0599	21.44215	Kavango	Andara	198	161	211		201	159	165	206	119;128;125;122	185	157;160		110;101	155	168	221	139		171	100	121			100	277
582	11491088841	AndaraDH	Angolaother	6/6/16	-18.0599	21.44215	Kavango	Andara		167	242		201;198		165		128;125;122	179;182	157	187;184	101	155	171;165;168;155	197;200	152;136;139;149	146	174;190	106;122	121			113;103;107;100	292;268;250
583	11491088851	AndaraDH	Luanda	4/7/16	-18.0599	21.44215	Kavango	Andara	192	167	242	171;174	198	165	165	212	122	179;182	157	187	101	155	165;155	197	136;149	146	174;190	106;122	121			107	
584	11491088852	AndaraDH	Mukwe	5/2/16	-18.0599	21.44215	Kavango	Andara		167	258	171		162	165	206	122	182	157	187	101	155	155	200	136	146	174	122;88	131			107	250
585	11491	Andar	Angolaot	5/15/	-18.0	21.44	Kavan	Anda	187	170;167;179	227;242		198	162	162;1	212;215	149;122	179;182	157	187;184	98;129;101	164;155	152;165;168	197	136;139;149	152;146	171	94;106;122;119	121;128			103;107;100	271

	08865	aDH	her	16	599	215	go	ra							65																		
586	1149108867	AndararaDH	NotRecorded	4/11/16	-18.0599	21.44215	Kavango	Andarara	195	170	242	171	198	162	165	206	128	182	157	187	101	155	168	197	139	152;200;146	165	94;106	121	209		103;107	268;238;253
587	1149108878	AndararaDH	Angolaother	6/10/16	-18.0599	21.44215	Kavango	Andarara	195;192	170;173;167	227;258;242		201;198	162;156	162;165	206	122	179;167	157;160;154	187;184	129;110;120;101	164;155	168;155	194;200	130;152;136;139	146	174;171	106;113				103;107;100	
588	1149108879	AndararaDH	Angolaother	4/12/16	-18.0599	21.44215	Kavango	Andarara	192	170;173	227;242		201;198	162	162;165	206;209	125;122	179;182;167	157;160;154	187;184		164;158	165;168;155	194;197	152;136;139;149;143;155	149;146	171	136;100;106;113;122;97	128			107;100	
589	1149109003	AndararaDH	Angolaother	4/6/16	-18.0599	21.44215	Kavango	Andarara	189	185		174	201		162	206	134;122				110;113	155	168			158;146		110	121	215;205	143;155	103	232;268;271

590	1149109006	AndaraDH	Angolaother	5/3/16	-18.0599	21.44215	Kavango	Andara	189	185	227	171	201		165	215	122			184	129;101	164;155	183			152;146	174	106	128	205	143	103;100	271;247;253;265
591	1149109016	AndaraDH	RunduUrban	4/22/16	-18.0599	21.44215	Kavango	Andara	195	161	258	171	201		165	212	125		157	187	110	172	177		136	149;146	177	100	118	227	146	103;100	268
592	1149109020	NyanganaDH	Angolaother	4/16/16	-18.00337	20.67655	Kavango	Nyangana	192	161	242		198	159	165	212	128	182	157	187	113	164	155	197;209	139		168	110;106		209		113;110;116	
593	1149109035	NyanganaDH	NdongaLinena	5/30/16	-18.00337	20.67655	Kavango	Nyangana		167;164		171	201;198		162;165		134		157		67;110	155	152		136;143	158		113;116	128	199	155	103	247;265
594	1149109090	Nyangana	Dirico	4/24/16	-18.003	20.67655	Kavango	Nyangana	192	167	258	171	198	162	162	215	149	179	157	184	110	155	155	197	143	176	181	116	134			103;100	

	40	DH			37																												
595	1149109042	AndararaDH	Angola other	5/12/16	-18.0599	21.44215	Kavango	Andara	195;192	170;173;167		171	204;198		162;165	212;206;215	125;122		157;160		129;110;113	164;155	165;168		136;139	200;149;146		100;106;119	118;121;128	209;205	143;101	103;107	271;253
596	1149109049	AndararaDH	Angola other	4/11/16	-18.0599	21.44215	Kavango	Andara	192	161	242	171	198		165	212	122		157;160	184	120	155	165		139;158	146	181	110;106	121	215		103;107	253
597	1149109056	NyanganaDH	Ndonga Linena	4/6/16	-18.00337	20.67655	Kavango	Nyangana	187	170	227	171	201		165	206	122			187	120	155	168			152;146	177	91	128	209		100;97	280
598	1149109064	AndararaDH	Angola other	5/17/16	-18.0599	21.44215	Kavango	Andara	195;192	170;167	261	171	204		162	197	134;131;125		160	184	110	155	168		136;139	200;149;158;146	171	100	118;121	215;205		103;107	253
599	11491	Andar	Angola ot	4/22/	-18.0	21.44	Kavan	Anda	192	167	242	174	198		162	212	131		157	187	120	164	155		149	155	190	100	125	218		107;97	274

	09065	aDH	her	16	599	215	go	ra																									
600	1149109070	AndararaDH	Angolaother	3/30/16	-18.0599	21.44215	Kavango	Andara	187	179	242	171	201;198		162;165	215;209	122			184	126;132;129;120	164;155				146	165;171		128;131	209		100	268;271;262;265
601	1149109090	NyanganaDH	NotRecorded	3/30/16	-18.00337	20.67655	Kavango	Nyangana	189		258			162;165	165	206				187	91		165;162	200	139		177	106;103	121	215		103;100	268
602	1149109166	NyanganaDH	NotRecorded	4/12/16	-18.00337	20.67655	Kavango	Nyangana	192		258		201	156	165	206	128	179	160	184	120		165;168	215	136		168;171	110;106;113;116	128	212		103	271
603	1149109170	AndararaDH	NamibiaOther	4/12/16	-18.0599	21.44215	Kavango	Andara	195		258		198	168;162	162;165	212	122	179			110		152;149	197;200	139		174;181;171;150;196	94;88;91	121;125	205;224			241

604	1149109173	AndaraDH	Not Recorded	4/25/16	-18.0599	21.44215	Kavango	Andara	195		248	174	201		165	212	131;128;125;122		157		98;101		152;168		139;146	185	171		121	205	149	103;100	286
605	1149109232	NyanganaDH	Not Recorded	4/11/16	-18.00337	20.67655	Kavango	Nyangana	189		242		201	162	165	206	122	182	157	184	120		168	197	143		177	119	121	221		103;100	247
606	1149109386	AndaraDH	Not Recorded	4/18/16	-18.0599	21.44215	Kavango	Andara	195;192		258		201;198		162;165	206	131;122		157		110;113;120;104;101		165		143;146	152;176			112		124	113;103;110	253
607	1149109414	NyanganaDH	Not Recorded	4/8/16	-18.00337	20.67655	Kavango	Nyangana	198		273		201	162	165	206		173	160	184	139			197	139		171		112	205		103;107;110	271
608	11491094	Nyangana	Not Recorded	4/8/16	-18.003	20.67655	Kavango	Nyangana	198		242			162	165	212	122	179	157	184	98		165;162	206	152		181	100;103;97	125	205		100;97	268;271

	37	DH			37																												
609	1149109492	AndaraDH	NamibiaOther	4/14/16	-18.0599	21.44215	Kavango	Andara	195		242		201;198		165	212	131;128;125;122	179	160		107;110;104		152		133	182	171	106	125			103;100;97	244;247
610	1149131269	AndaraDH	NamibiaOther	4/14/16	-18.0599	21.44215	Kavango	Andara	195			171	198		162		128;122	179	157	165;162	107;110;120;116		189		136	182	174		125			103;100;97	271;241;253
611	1149131274	AndaraDH	NamibiaOther	4/16/16	-18.0599	21.44215	Kavango	Andara	198;189			174	201			206	131;128	179	160		107;110				139	176			131	215	152	103	280
612	1149131303	NyanganaDH	NotRecorded	4/10/16	-18.00337	20.67655	Kavango	Nyangana	202		227		198	162	165	218		179	163	187	107;113;116		152;149	200	139			110;106	128	215;212		103;107;100	