STUDY OF SPATIAL DISTRIBUTION OF HUMAN TUBERCULOSIS IN ZAMBIA'S LUNDAZI DISTRICT

BY

VICTOR CHEELO

A DISSERTATION SUBMITTED TO THE UNIVERSITY OF ZAMBIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTERS OF SCIENCE IN ONE HEALTH ANALYTICAL EPIDEMIOLOGY

UNIVERSITY OF ZAMBIA

DECLARATION

I ***Victor Cheelo*** make a declaration that this research report is original and an outcome of my own effort. It is being submitted for the degree of Master of Science in One Health Analytical Epidemiology at the University of Zambia. Its contents have never been submitted before for any degree or examination either wholly or partially at this or any other University.

…… …… .....

Victor Cheelo Date

(**OHAE candidate**)

The above declaration is confirmed by:

…… … .....

Dr Musso Munyeme Date

(Supervisor)

....

Prof. B. M. Hang'ombe Date

(Supervisor)

DEDICATION

This work is firstly dedicated to my beloved parents Mr. Lazarous Munyongo Cheelo and Rachael Cheembo Cheelo. I am because of them. Secondly, I would like to dedicate this report to my wife, daughter and two sons namely; Vinita Chilala, Matimba, Chipo and Komana respectively, who were an inspiration during the time of conducting this study. They gave me encouraging words and support throughout the period I was doing this study though they were denied the fatherly love.

CERTIFICATE OF APPROVAL

This dissertation submitted by **Victor Cheelo** is approved as fulfilling part of the requirements for the award of the degree of Master of Science in One Health Analytical Epidemiology (OHAE) at the University of Zambia.

Supervisor Name: ……..

Supervisor signature: ……..

Name and Signature of Examiners

Internal Examiners

1. Name: ……Signature: ……..Date: ……..……

2. Name: ……..……Signature: ……..Date: ……..……

3. Name: ……Signature: ……..Date: ……...……

ABSTRACT

In the last decade of the 20th Century, human Tuberculosis (TB) has re-emerged with a significant impact on global public health. About 9.4 million new cases of human TB are recorded annually with approximately 1.7 million deaths, affecting mainly young adults in their most productive years. The objective of the present study was to describe the spatial distribution of TB in human population of Zambia's Lundazi district. This was a cross sectional study involving trace back of 60 TB patient participants of Lundazi district. Geographical Positioning System (GPS) coordinates were captured for the 60 sampled participants from Chitungulu, Kanyanga and Mwasemphangwe Rural Community TB diagnostic centers. This was accompanied by a semi structured questionnaire which was administered by trained interviewers to obtain information on risk factors of TB in the district.

The results obtained from this study through the use of geographical spatial referencing and mapping of human TB has demonstrated geographical clustering of human TB cases. The spatial analysis of the hotspots suggest that there were statistically significant hotspots of TB ($p < 0.0000$) and showed the existence of TB clusters in all the three diagnostic centers of the district. These hot spots were located in areas that were populated, supporting an association between population density and increased risk of TB. The degree of clustering of TB cases in the TB diagnostic centers was confirmed by Moran's *I* ($P < 0.0000$, $Z = 7.41$). Regarding the TB risk factors investigated, the disease was associated with (i) smoking and alcohol consumption i.e. the odds of developing TB was high among those respondents who were both drinking alcohol and smoking tobacco (OR = 4.6, p = 0.04, 95% CI, 1.019 - 21.485) and (ii) cigarette smoking (OR = 1.12, P = 0.020, 95% CI, 1.017 - 1.235). The ANOVA analysis also demonstrated that only History of Previous contact with TB patients ($p < 0.000$) and Smoking ($p < 0.000$), made a significant contribution to prediction.

Overall, the study has confirmed, for the first time, the existence of human TB clustering and the presence of hot spots in Lundazi district. From this study it is recommended that public health implication in relation to spatial distribution of TB, social interaction patterns, demographical factors and other multiplicative factors for transmission (i.e. co-infection with HIV) need to be considered when formulating policy action and plans.

ACKNOWLEDGEMENTS

The successful production of this dissertation would not have been possible without the scholarship from the Southern Africa Center for Infectious Disease Surveillance (SACIDS). I am also indebted to the commitment and contributions from my supervisors Dr. Musso Munyeme and Prof. Bernard Hang'ombe for their insight, guidance and wisdom that kept this work focused and plausible. This was not an easy task, as it demanded full commitment, dedication and selflessness towards the task of providing me with vital information that assisted in refining of the document.

In a special way I would like to thank my consultant Mr. Chabala Chiyaze for providing useful advice on Geographical Information System (GIS) for this project. I am also grateful to Mr. Anthony Peteni, Mr. Temwanani Nyirongo and Mr. Oliver Nkhunga who served as my Research Assistants during data collection.

My profound gratitude goes to the Ministry of Health, Provincial Health Office and Lundazi District Community Health Office (LDCHO) for the permission granted to conduct the research. Further, I may fail in my duties if I do not extend my special thanks to my family and all my other friends, colleagues and associates that were an inspiration to me.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AIDS	:	Acquired Immuno – Deficiency Syndrome
CIDRZ	:	Center for Infectious Disease Research in Zambia
DRGS	:	Directorate for Research and Graduate Studies
GIS	:	Geographical Information System
GPS	:	Global Position System
HIV	:	Human Immunodeficiency Virus
LDCMO	:	Lundazi District Community Medical Office
MDG	:	Millennium Development Goal
MOH	:	Ministry of Health
MTB	:	*Mycobacterium Tuberculosis*
MTBC	:	Mycobacterium Tuberculosis Complex
NTLP	:	National Tuberculosis and Leprosy Programme
NTM	:	Non – Tuberculosis Mycobacterium
PTB	:	Pulmonary Tuberculosis
SPSS	:	Statistical Package for Social Scientist
TB	:	Tuberculosis
UNZA	:	University of Zambia
USA	:	United States of America
VWB	:	Veterinarians Without Borders

LIST OF FIGURES

LIST OF TABLES

.

CHAPTER ONE

1.0 Introduction

Tuberculosis (TB) caused by is the leading cause of death worldwide from a single infectious agent killing about 3 million people every year (Frieden *et al*., 2009). In the last decade of the 20th Century, human TB has re-emerged with a significant impact on global public health. About 9.4 million new cases of human TB are recorded annually with approximately 1.7 million deaths, affecting mainly young adults in their most productive years (WHO, 2012). The unprecedented growth of the TB epidemic in Africa is attributable to several factors, the most important being the Human Immunodeficiency Virus (HIV)/ Acquired Immunodeficiency Syndrome (AIDS) epidemic (Chaisson and Martinson, 2008). Worldwide, the number of new cases is still rising due to an increased case load in Africa, Eastern Mediterranean and South-East Asia (WHO, 2007; Story, 2004). For example in 2007, the number of incident cases was estimated at 9.27 million, higher than the 9.24 million observed in 2006. From the 9.27 million incident cases, 1.37 million (15%) were HIV positive (WHO, 2009).

The disease burden is high, especially in sub-Saharan Africa where the number of new TB cases is on the increase due to the HIV/AIDS pandemic as compared to other regions of the World [that is 990 000 among HIV negative people and 430 000 HIV-associated TB deaths] (WHO, 2012). TB is ranked second to AIDS as the leading cause of death globally (Raviglione, 2003). TB is responsible for more deaths in people living on the

African continent than anywhere else, claiming more than 1500 lives a day despite the availability of treatment that can cure over 90% of all forms of TB cases (WHO, 2012).

Zambia was ranked among the World's top ten high TB incidence countries in Africa, having an incidence rate of 280 smear-positive TB cases per 100,000 inhabitants in 2009 (WHO, 2009). The WHO estimates the prevalence of all forms of TB in Zambia at 707 per 100,000 inhabitants. In Zambia, TB has rapidly become a major problem in the last decade. For example in 1964 alone, there were about 4, 572 cases of human TB recorded in 1964 against 58,070 cases in 2009 (Anonymous, 2009). This is in spite of the fact that there is an improved health management system in the country (Anonymous, 2009). This therefore, means that the disease is still a big problem in Zambia. It is against this background that the WHO declared TB a global health emergency in 1993 (Story, 2004). TB persists as a serious global public-health problem which needs urgent attention (Stop TB, 2002). This risk increases significantly with the advent of HIV infection (Raviglione *et al*, 1995).

The increase in TB cases is also coupled with multiple hosts including domestic and wild animals, inefficient diagnostic techniques, absence of defined national controls and eradication programs which impede the control of TB (Alexander *et al*., 2002). Further, it was highlighted that TB control has remained critical, especially in high burden settings where ongoing transmission still sustains the epidemic. TB persists as a serious global public-health problem which needs urgent attention (Stop TB, 2002). The burden of TB has stimulated much interest in research for new approaches to its management and control (Rylance *et al*., 2010).

Over the last 25 years, a substantial number of studies have been conducted focusing on various aspects of TB (Rylance *et al*. 2010). Many of these studies have concentrated on the biomedical aspect of the disease with the aim of finding the cure. On the other hand, a select few have concentrated on methods of how better to estimate the magnitude of the disease in terms of its spread over space and among certain demographic groups (Rylance *et al*. 2010). New approaches, such as the use of Geographical Information System (GIS), mapping and spatial analysis, may be of value in contributing to basic elements of TB control. The greatest potential of GIS lies in its ability to quickly show the results of a complex analysis (Pfeiffer *et al*., 2008).

1.1 Statement of the Problem

TB is a major public health problem both in Zambia and worldwide. WHO declared TB a global public health emergency in the year 1993 and since then, TB has remained a major global health problem which has caused ill-health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide, after HIV/AIDS (WHO, 2012). For example in 2011, there were approximately 8.3 to 9.0 million cases and 1.3to 1.6 million deaths from TB (Ibid, 2012). The disease burden is high especially in sub-Saharan Africa where the number of new TB cases is on the increase due to the HIV/AIDS pandemic as compared to other regions of the World (WHO, 2012).

After 20 years of declining incidence of new TB cases in Zambia, during the years 1984 – 2005, the number of new cases of TB began to increase from 100 reports of infection per 100,000 people to 580 reports of infection out of 100,000 people (Mwinga *et al*.

1998). The unprecedented growth of the TB epidemic in Africa is attributable to several factors, the most important being HIV/AIDS epidemic (Chaisson and Martinson, 2008). The majority of these cases appear in young adult population groups aged 15 to 45 years, the same age group affected by the HIV and AIDS (Anonymous, 2008).

However, despite the enormity of the task to eliminate TB in general The progress made to date is not significant as the disease is still revenging most of the poor populations in sub-Sahara Africa. Zambia has been ranked among the world's top ten high TB incidence countries in Africa having an incidence rate of 280 smear-positive TB cases per 100,000 inhabitants in 2009 (WHO, 2009).

1.2 Justification

Human TB has not been well described in Zambia's Lundazi district. Therefore, understanding the spatial patterns of TB in Lundazi among the humans will be helpful in identifying both the manner in which the disease is spread, and the potential mitigation measures. The understanding of the spatial patterns of TB will be very useful in targeting mitigation measures and appropriate resources allocation. This will mean that resources will be channeled to the areas where they are greatly needed, thus providing efficient use.

In Lundazi district, no organized studies have been conducted to investigate the spatial distribution of TB. In addition to this, information is lacking on epidemiological factors of TB in the district. Therefore, without knowledge and tools for identifying TB in the population, it is impossible to add any meaningful contribution to the effort on the global fight against TB and to achieve the Millennium Development Goal (MDG) of reducing the incidence and halting the spread of TB by 2015.

This research will attempt to understand the spatial patterns, risk factors coupled with knowledge on demographics, socio-economic, gender, and ethnic characteristics of those who are suffering from the disease and will help in gathering useful information for decision making which can be used by the Lundazi community district health office and other stakeholders. Further, communities will be more knowledgeable and be involved in control leading to reduction of spread and deaths related to TB.

1.3 Significance of the Study

Understanding the TB spatial distribution and analysis will help in the formulation of control and prevention strategies of the disease in Lundazi district. This study is significant in the sense that GIS will be the tool in identifying and supporting the claim of previous studies. The study will help in gathering useful and simplified information through maps for decision making and reducing the cost related to management of TB by Lundazi district community health office (LDCMO).

1.4. Research Questions

1. What are the risk factors associated with TB in communities living in Lundazi district?
2. What are the spatial patterns, geographical point distribution and clusters of sampled human TB cases in Lundazi District?
3. How has the trend of TB been in Lundazi district for the past five (5) years (2009 – 2013)?

1.5 Objectives

1.5.1 Main Objective

To describe the spatial distribution of TB in human population of Lundazi district

1.5.2 Specific Objectives

- To describe the TB disease trends in human population through retrospective data for the last 6 years in Lundazi district (i.e. 2008 to 2013).
- To identify factors associated with human TB in Lundazi district
- To identify and map TB patient clusters in Lundazi district

CHAPTER TWO

2.0 Literature Review

2.1 Tuberculosis

TB is an infectious disease caused by the rod-shaped, non–spore-forming, aerobic bacterium (Park, 2009). Basically there are two types of tuberculosis: **Pulmonary tuberculosis (PTB)** affects the lungs (lung parenchyma) and is the commonest form of tuberculosis. **Extra-pulmonary tuberculosis is (EPTB)** the disease that affects organs other than the lungs, such as pleura, lymph nodes, pericardium, spine, joints, abdomen or genito-urinary tract. It may affect any part of the body (Anonymous, 2008).

The disease is usually chronic with varying clinical manifestation. Kumar *et al*. (2007) demonstrated that TB affects the lungs in about 80% of cases with warning signs of cough, haemoptysis, and chest pain, shortness of breath, fever, weight loss, and drenching night sweat. People infected with TB carry live tubercle bacilli, but the bacilli may be present in small numbers and dormant (latent), in which case there may be no apparent disease (Dye *et al*., 2006). Further it was stated that TB disease occurs when the bacteria multiply, overcome immune defenses, and become numerous enough to cause damage to tissues (WHO, 2004).

2.1.1 Aetiology

The *Mycobacterium tuberculosis* complex (MTBC) is a highly related complex of bacteria composed of seven members. These include *M tuberculosis*, the primary

causative agent of human TB responsible for the vast majority of TB cases in the world (Imaeda, 1985); *M bovis*, which is responsible for bovine TB and TB in Kafue lechwe antelopes and includes the vaccine strain *M. bovis* BCG; *M africanum*, the main causative agent of TB in West Africa (Munyeme *et al.,* 2008: Kallenius *et al*., 1999); *M microti*, responsible for infecting voles and humans in rare circumstances (Van Soolingen et al., 1998). Others are *M canetti* commonly found in Somalia, characterized by smooth and glossy colonies (Pfyffer *et al*., 1998); *M caprae* isolated from goats (Aranaz *et al*., 1999); and the seal bacillus also referred to as *M pinnipedii* (Cousins *et al*., 2003).

Recent reports have identified TB in humans caused by *M bovis* in countries that are officially free from bovine TB (Aranaz *et al.,* 1999) and suggest that the true prevalence of zoonotic TB may be underestimated clinically (Benatar and Upshur**,** 2010). The association between the keeping of cattle and the occurrence of cases of both pulmonary and extra-pulmonary TB in man has been well documented (Grange and Yates, 1994). In the absence of cultural evidence, the prevalence of mycobacterial infection caused by *M bovis* in man can be judged from the number of cases of extra pulmonary TB (Kleeberg 1984). Although *M tuberculosis* is the most common infection in humans, it is believed that *M. bovis* is responsible for an increasing proportion of human TB cases (Cosivi *et al*., 1998). In contrast, *M. tuberculosis* is considered primarily a human pathogen and has been reported only in domestic or wildlife species living in close, prolonged contact with humans (Ibid, 1998). For instance, an average of 16% of the cases of TB in Tanzania involves extra pulmonary infection (National Tuberculosis and Leprosy Programme, Tanzania [NTLP], 1996).

Mycobacteria typically measure 0.5 μm by 3 μm, are classified as acid-fast bacilli, and have a unique cell wall structure crucial to their survival (Lee *et al.*, 2005). The well-developed cell wall contains a considerable amount of a fatty acid, mycolic acid, covalently attached to the underlying peptidoglycan-bound polysaccharide arabinogalactan, providing an extraordinary lipid barrier (Lee *et al.*, 2005). The main defining characteristic of the genus *Mycobacterium* is the property called acid-fastness, which is the ability to withstand decolorization with an acid-alcohol mixture after staining with carbolfuchsin or auramine-rhodamine (Ioachimescu and Tomford, 2010. Mycobacteria are primarily intracellular pathogens, have slow growth rates, are obligate aerobes, and produce a granulomatous reaction in normal hosts. In cultures, *M tuberculosis* does not produce significant amounts of pigment, has a buff-colored, smooth surface appearance, and biochemically produces niacin. These characteristics are useful in differentiating *M tuberculosis* from nontuberculous mycobacteria (Ibid, 2010).

2.1.2 Pathogenesis

M tuberculosis is spread by small airborne droplets, called droplet nuclei, generated by the coughing, sneezing, talking, or singing of a person with pulmonary or laryngeal tuberculosis (Lee *et al.*, 2005). These minuscule droplets can remain airborne for minutes to hours after expectoration. The number of bacilli in the droplets, the virulence of the bacilli, exposure of the bacilli to UV light, degree of ventilation, and occasions for aerosolization all influence transmission (Lee *et al.*, 2005). Primary infection with TB occurs on first exposure to the bacteria. The bacteria are inhaled and lodge in the terminal bronchioles where they drain to the hilar lymph nodes (WHO, 2004). Inhaled infectious droplets lodge in the alveoli, and bacilli are taken up there by macrophages, beginning a

series of events that result in either the containment of infection or the progression to active disease (Frieden *et al*, 2003). Following the uptake by the macrophages, TB replicates slowly but continuously and spreads through the lymphatic system to hilar lymph nodes (Ibid, 2003). The cell mediated immunity associated with a positive tuberculin test, develops two to eight weeks after infection (Dye and Floyd, 2006). Activated T lymphocytes and macrophages form granulomas, which limit the further replication and spread of the bacilli. When the immune response cannot suppress replication, primary infection leads to active TB (Ibid, 2007).

In general, a relatively small proportion of people infected with TB will develop TB disease; however, the probability of developing TB is much higher among people infected with HIV. TB is also more common among men than women, and affects mostly adults in the economically productive age groups (Crofton *et al*. 1999; Rieder 1999).

2.1.3 Epidemiology and Risk Factors

According to WHO (2009), globally, there were an estimated 9.27 million incident cases of TB in 2007. This is an increase from 9.24 million cases in 2006, 8.3 million cases in 2000 and 6.6 million cases in 1990. The percentage of the population infected varies from region to region: in Western Europe, around 11% are infected, mostly elderly persons, while in some tropical countries over half may be infected, including a much higher proportion of younger people (WHO, 2011). For example, most of the estimated numbers of cases in 2007 were in Asia (55%) and Africa (31%), with small proportions of cases in the Eastern Mediterranean Region (6%), the European Region (5%) and the Region of the Americas (3%) (WHO, 2008). The five countries that rank first to fifth in terms of total numbers of cases in 2007 are India - 2.0 million, China - 1.3 million, Indonesia - 0.53

million, Nigeria – 0.46 million and South Africa - 0.46 million (Ibid, 2008). Of the 9.27 million incident TB cases in 2007, an estimated 1.37 million (15%) were HIV-positive; 79% of these HIV-positive cases were in the African Region and 11% were in the South-East Asia Region. Each year, approximately 2 million persons worldwide die of tuberculosis (CDC, 2007). All regions of the world have a stable or falling number of cases of TB except for the African region where the numbers of new cases of TB continue to rise, fuelled by the HIV epidemic (Ibid, 2007). Figure 2.1 below shows the estimated rates of tuberculosis incidence by WHO region in the year 2006 and the African region is the most affected.

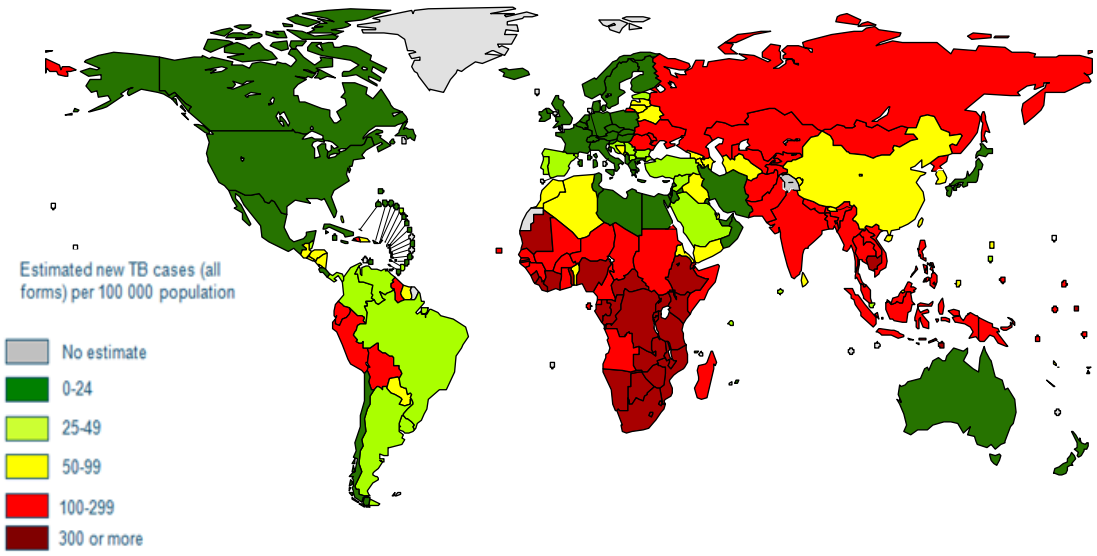


Figure 2.1: Estimated new TB cases (all forms) per 100, 000 by WHO Regions (WHO, 2006)

The prevalence of TB is continuing to increase because of the increased number of patients infected with HIV/AIDS, bacterial resistance to medications, increased international travel and immigration from countries with high prevalence, and the growing numbers of the homeless and drug abusers (Goldrick, 2004). HIV infection has,

emerged as the most important and widespread risk factor for the development of active TB (WHO, 2011).

The risk of developing TB following infection also changes with age. Infants and young children up to the age of five years who are infected with *M tuberculosis* are at relatively high risk, particularly of severe forms (mainly miliary TB and TB meningitis), because of their immature immune systems. Children between the ages of five and fifteen years are relatively resistant to TB. The risk then rises again through adolescence, remains approximately stable during adulthood, but increases again in the elderly (Crofton *et al.* 1999; Rieder 1999).

Other factors that enhance the risk of developing TB following infection include under nutrition; toxins (tobacco, alcohol, corticosteroids, immunosuppressive drugs); and other diseases (diabetes mellitus, silicosis, leukemia, renal failure, liver failure, cancers, chronic malaria, measles, and whooping cough in children); causes of lung damage e.g silicon and other industrial dusts (Crofton *et al.* 1999; Rieder 1999).

2.1.4 Public Health Significance of TB

TB is certainly a disease that affects the poor and is a major cause of poverty as it has a devastating economic impact on households in developing countries. On average, a family loses 30% of its income if a money-earner develops tuberculosis, and 15 years of income if that person dies of the disease. (Grange and Zumla, 1999). The other direct monetary costs of diagnosis and treatment are borne by health services and by patients and their families. Added to these are the indirect costs of lost income and production, incurred when TB patients are too sick to work and when young adults often parents and

householders die prematurely (WHO, 2000). Beyond these losses are enormous psychological and social costs which are associated with TB. These extra costs are less easily quantified, but they are nonetheless real.

2.1.5 Diagnosis of TB

The success of TB control programmes depends critically on the quality of diagnostic services. Diagnosis of tuberculosis is based on a high index of clinical suspicion, appropriate clinical and radiological examinations and laboratory investigations (Grange and Zumla, 2008). Several traditional methods for the diagnosis of tuberculosis are available which include tuberculin skin test, culture and sputum smear microscopy (Ibid, 2008).

The following investigations help to confirm the diagnosis and monitor treatment: The most common method for diagnosing TB worldwide is ***Bacteriological examinations*** *through* sputum smear microscopy and it includes detection of acid-fast bacilli by microscopy and culture of the causative organism from appropriate clinical specimens such as sputum, bronchial aspirates and brushings, gastric aspirates, pleural, peritoneal and pericardial fluids, cerebrospinal fluid (CSF), blood, bone marrow and tissue aspirates or biopsies (Dye and Floyd, 2006; Grange and Zumla, 2008). Sputum smear examination is a useful diagnostic and epidemiologic tool, because smear-positive TB patients are more infectious than smear-negative patients and have a higher fatality rate.

The second method is ***Imaging techniques*** - including radiology, ultrasound, computed axial tomography (CAT) scanning, magnetic resonance imaging (MRI) and radioisotope scans (Grange and Zumla, 2008). Others are: ***Molecular techniques*** **-** utilizing nucleic

acid amplification systems - the polymerase chain reaction (PCR) and related techniques; ***Haematological and biochemical investigations*** - such as haemoglobin levels, erythrocyte sedimentation rate (ESR) and liver function tests may be required in overall patient management but have limited diagnostic roles and ***Tuberculin skin tests*** and other immunological tests including interferon-γ assays (Grange and Zumla, 2008). One of the most important drawbacks of the tuberculin test is that prior vaccination with Bacille de Calmette et Gue´rin (BCG) may result in a false-positive result even many years afterwards (Wood, 2007).

2.1.6 Treatment of TB

The three aims of ant TB therapy are to cure the patient, to render the patient rapidly non-infectious and to prevent the emergence of drug resistance. Drug treatment is an individual and public health measure to reduce TB among the humans. The treatment regimens contains multiple drugs to which TB organisms are susceptible, and given for a sufficient period of time (Jamison *et al*. 2006). WHO recommends the short course directly observed therapy (DOTS). This consists of 2 months of Isoniazid, rifampicin, pyrazanimide and ethambutol given daily. This is followed by a 4 months of isoniazid and rifampicin given thrice weekly (Jamison *et al*. 2006).

2.1.6 Prevention and Control of TB

TB control means reduction in the prevalence and incidence of the TB disease in the community (Park, 2009). Since TB is an infectious disease, the basic principles of prevention and control are the same as for any other infectious diseases. Thus it follows that identifying those at increased risk of developing TB disease is strategic in TB control (Ward, 2004). The control measures consist of the following: Case finding – this involves

identifying and treating active cases and is currently the primary and most effective control measure for TB. The corner stone of this approach is the DOTs strategy. DOTS strategy entails diagnosis with a positive sputum sample, short course treatment with effective case management, regular drug supplies, and systematic monitoring to evaluate outcomes for every patient (Park, 2009; Jamison *et al*. 2006). This should be an intensive, ongoing programme. The other intervention for controlling TB is preventing infection by means of vaccination using Bacille Calmette-Guérin (BCG). According to Jamison *et al*. (2006), about 80% of infants worldwide are currently receiving a live attenuated vaccine BCG.

Other control measures, include TB preventive treatment, HIV testing and ARV therapy for TB patients, and various interventions against HIV (and therefore indirectly against TB) and general health promotion (De Cock and Chaisson 1999; Maher *et al*. 2002). Therefore, TB incidence rate is a key indicator for monitoring performance in TB control interventions, and an understanding of the epidemiology of TB forms the basis for implementing a successful national TB control programme (Rieder, 1999; Ward, 2004).

2.2 Global Tuberculosis

WHO declared TB a global public health emergency in the year 1993 and since then, TB has remained a major global health problem which has caused ill-health among millions of people each year and ranks as the second leading cause of death from an infectious disease worldwide, after the HIV/AIDS (WHO, 2012). The latest estimates included in this report were that in 2011, there were approximately 8.3–9.0 million cases and 1.3–1.6 million deaths from TB. Of all the TB cases, approximately 3.9 million cases were

sputum – smear positive, the most infectious form of the disease. This is despite the availability of treatment that will cure most cases of TB (Ibid, 2012).

In addition, 9.2 million new TB cases and approximately 2 million deaths were reported worldwide (WHO, 2008). The report revealed that all regions of the world have a stable or falling numbers of cases of TB except for the African region where the numbers of new cases of TB continue to rise, fuelled by the HIV pandemic (Ibid, 2008). For example India is among the highest TB burden country in the world and accounts for nearly 20% of the global burden of TB (WHO, 2009). This means that every year approximately 1.8 million people develop TB of which about 0.8 million are new smear positive highly infectious cases (Park. 2009). In China an exploratory spatial data and spatial cluster analysis study was conducted at a city level and it was estimated that china alone accounts for about 12% of all incident TB cases worldwide as of the year 2010 (Wang *et al*. 2012). Below is a map from WHO (2012) showing the distribution and incidence of TB worldwide:

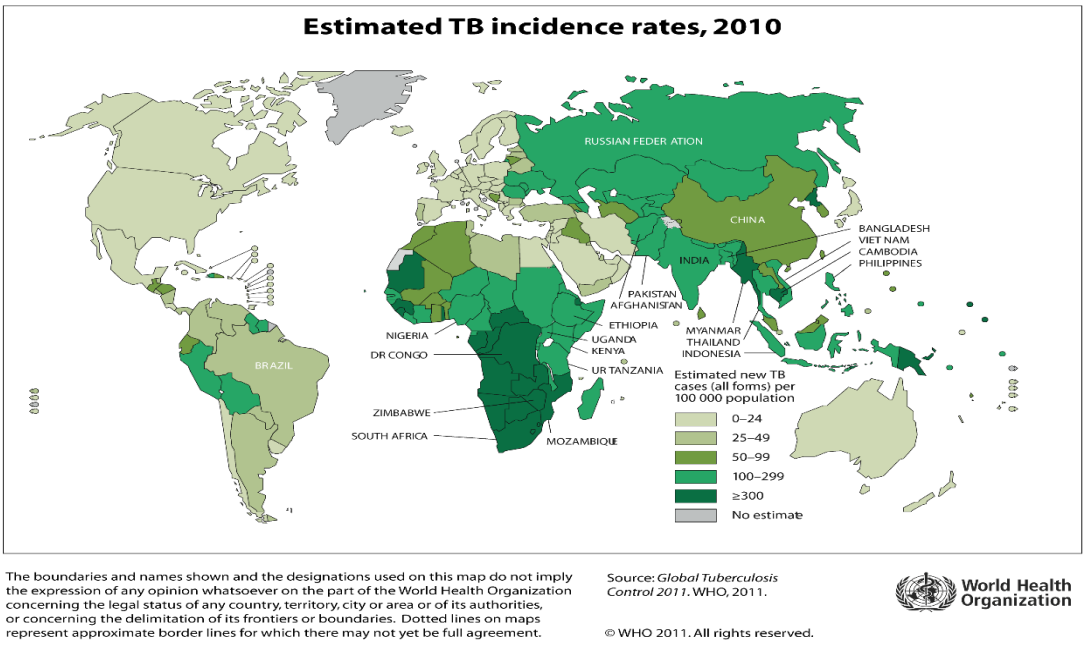


Figure 2.2: Estimated world TB incidence rate for 2010 (WHO, 2012)

This shows that the epidemiology of tuberculosis varies substantially around the world. The highest rates (100/100,000 or higher) are observed in sub-Saharan Africa, India, China, and the islands of Southeast Asia (WHO, 2009).

2.3 Tuberculosis in Africa

Africa, with 11% of the world's population, carries 29% of the global burden of TB cases and 34% of related deaths, and the challenges of controlling the disease in the region have never been greater (Richard *et al*. 2008). In addition to this, WHO report of 2012 indicated that the African Region has approximately 24% of the world's TB cases and the highest rates of TB cases and deaths per capita. It was further estimated that the average incidence of tuberculosis in African countries more than doubled between 1990 and 2005, from 149 to 343 per 100,000 populations (WHO, 2007).

To show the gravity of the disease, Regional Committee for Africa comprising health ministers from 46 Member States declared TB an emergency in the African region as a response to an epidemic that has more than quadrupled the annual number of new TB cases in most African countries since 1990 and is continuing to rise across the continent, killing more than half a million people every year (WHO, 2005). This translates into 2.5 million new infections annually. It is the only continent where incidence is increasing; primarily due to HIV/AIDS as a co-factor of transmission and active infection. This typically means that, a TB patient loses 3-4 months per year due to illness and an estimated 20%-30% of annual household income (Ibid, 2006).

It has been reported that TB kills more people in the African continent than anywhere else, claiming more than 1500 lives a day. Further, in Africa the case rate is 216 per 100 000 and the 11 countries of the Southern Africa sub-region contribute approximately 275 000 cases every year to the total case load in Africa (Fourie, 1999).

The major problem being experienced in countries is that examination of acid-fast bacilli in sputum smears, which forms the cornerstone of TB diagnosis in Africa, does not permit differentiation between *M. tuberculosis* and *M. bovis* (Anita and Michael, 2003).

2.4 Tuberculosis in Zambia

In 1964, Zambia had a TB prevalence rate of approximately 100 cases per 100,000 persons (Chanda, 2002). That figure remained constant for the next 20 years. The first case of HIV/AIDS in Zambia was diagnosed in 1984 (Anonymous, 2002). Between 1984 and the present, the prevalence of TB has risen dramatically. In 2004, the case rate of TB

was 450 cases per 100,000 and in 2005 was approaching 500 cases per 100,000. The mortality rate by the mid 2000s averaged 88.7 per 100,000 persons. The Country is among those with the highest TB infection rates on the continent of Africa (Anonymous, 2009).. The WHO, (2005) estimated an annual incidence of 64,632 of all forms of TB (i.e. 680 cases per 100,000 population) in Zambia, with 32, 129 (280 per 100,000 Population) of these estimated to be new smear positive pulmonary TB. In 2006, Zambia reported over 60,000 cases of TB, of which at least 40% were estimated to be HIV positive (Anonymous, 2008). In the year 2010 -2012, Zambia had the ninth (9^{th}) highest TB incidences worldwide (433/100,000) with particularly high risk of infection in specific subgroups such as HIV infected persons, pregnant women, children and prisoners (CIDRZ, 2012).

According to WHO (2007), TB has remained a threat in Zambia considering that the number of cases has steadily increased from 4,572 cases in 1964 to 58,070 cases in the year 2004. The majority of these cases appear in young adult population groups aged 15 – 45 years, the same age group affected by the HIV and AIDS (Anonymous, 2008). The rapid increase of TB in Zambia from 1985 onwards is mainly attributed to the HIV epidemic, but other factors like population growth, urban overcrowding and improved case detection have also contributed (Anonymous, 2008).

According to the latest WHO report published in April 2011 revealed that TB deaths in Zambia reached 2,783 or 1.63% of total deaths. The age adjusted Death Rate was 30.18 per 100,000 of population and ranked Zambia as the number 56^{th} in the world.

2.5 Spatial Analysis of Tuberculosis

The objective of spatial epidemiological analysis is the description of spatial patterns, identification of disease clusters and explaining or prediction of disease risk (Pfeiffer *et al*. 2008). GIS is a very important tool for use in disease mapping, as well as public health surveillance activities to assist in identifying high-risk groups. GIS software for mapping has an embedded relational database component, which makes the management and analysis of public health surveillance data very organized for determining spatial and time trends. Disease cases can be viewed in their surrounding social context and patterns of their geographical distribution can be analyzed by using various spatial statistical methods that account for differences in location characteristics (e.g.; latitude and longitude). In addition, mapping and spatial analysis maybe of value in contributing to basic elements of TB control for determining spatial and time trends (Morrison *et al*. 1998). GIS can be used in epidemiologic and health research in identifying specific spatial structures that include the individuals affected and how they are connected in communities, as well as the dynamics of these communities and their organization into larger units (Goodchild, 1987; Moore and Carpenter, 1999).

Therefore, GIS mapping can also allow researchers to examine many different types of questions involving the particulars of a specific location, the distribution of certain phenomenon, the changes that have occurred since a previous analysis, the impact of a specific event, or the relationships and systematic patterns of a region (Bernhardsen, 1999).

Several studies have shown that spatial analysis was useful in the detection of clusters of a variety of different diseases in a range of settings (Hjalmars *et al*. 1996; Walsh & Fenster 1997; Kistemann *et al*. 2002; Kulldorff *et al*. 2005; Tiwari *et al*. 2006). The detection of these clusters may be useful in the regular surveillance of TB while it may assist in identifying factors behind the spread of the disease. These approaches could further enhance the development of suitable policies and allocation of resources for TB control. However, many epidemiologic studies in the past have failed to examine the role that spatial patterns play in the development of trends in disease in ways that go beyond descriptive methods (Moore and Carpenter, 1999)

Recently, the use of GIS in the study of infectious disease and, more specifically TB, has been less documented than that of chronic or environmentally related illness although some believe that there is little appreciation amongst public health professionals of the value in mapping communicable diseases or associated risks (Atkinson and Molesworth, 2000).

CHAPTER THREE

3.0 Materials and Methods

3.1 Study Area and Research design

The study was conducted in Zambia's Lundazi district (latitude 12^{o} $30^{'}$ $00^{''}$ South and longitude 32^{o} $45^{'}$ $00^{''}$ East) as presented in figure 3.1 below. The district has a human population of approximately 353,032 and an annual growth rate of 2.9% (CSO, 2010).

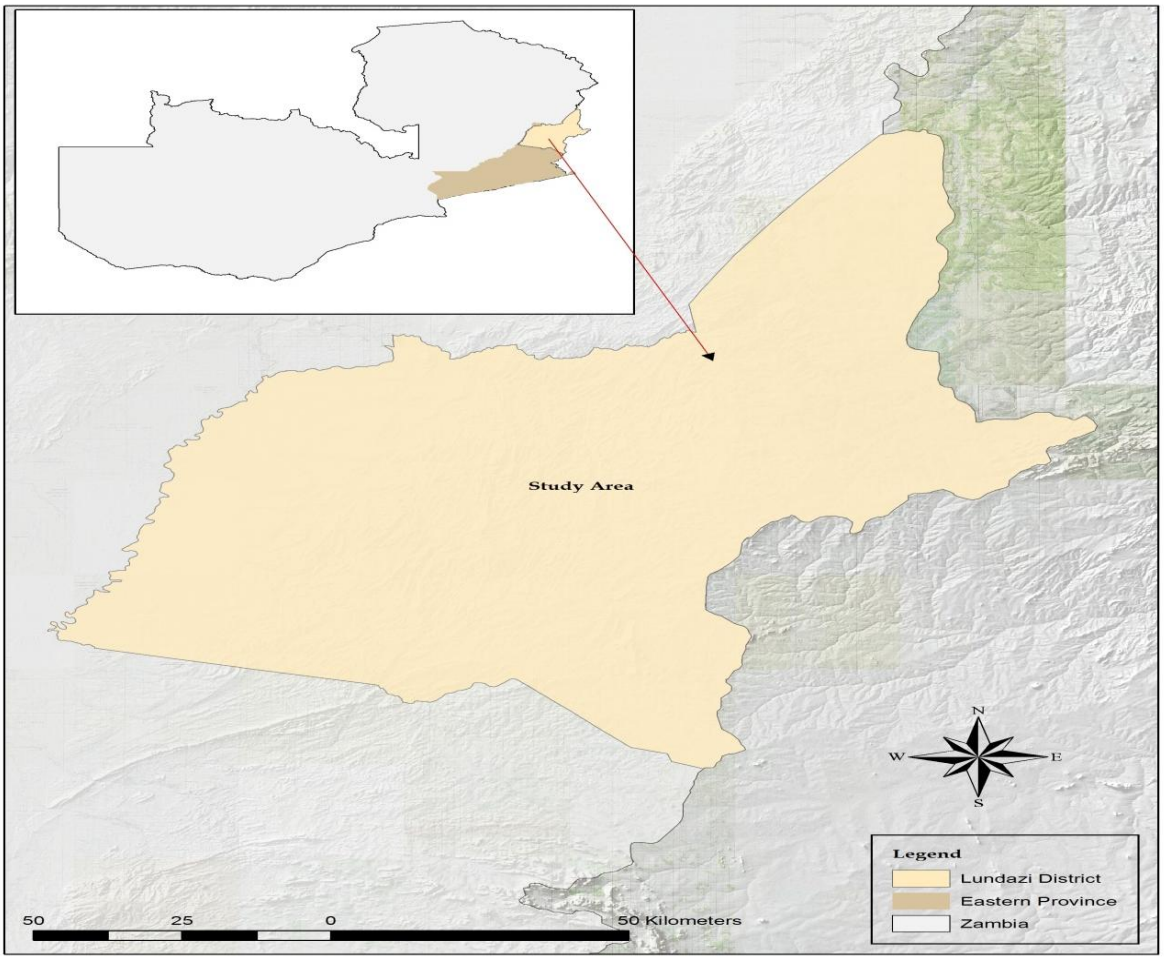


Figure 3.1: Geographical Location of Lundazi District

This was a cross-sectional study of spatial analysis of human TB carried out between November 2014 and February 2015 involving three peripheral TB diagnostic centers (Chitungulu, Kanyanga and Mwasemphangwe Health Facilities) of Lundazi district situated in the Eastern Province of Zambia. Both retrospectively and prospectively data was collected from the TB diagnostic centers. Thereafter, patient trace back was done to the areas of their origin to obtain GIS information and administer a questionnaire. The district occupies an area of 14,058 square km with two well defined geographical features; which are the valley and the plateau. The plateau is estimated to have 88.3% of the total population whilst the valley has 11.7% of the total population (CSO, 2010). Due to fertile soils and easy communication network in the plateau, it favours many economic activities such as animal and crop agriculture, and small business enterprises.

3.2 Study population, sampling and sample size

Lundazi district is served with 42 government health facilities with six TB diagnostic centers. Of the 42 health facilities, the valley is served by six government health facilities with a single TB diagnostic center situated at Chitungulu Rural Health Center. A list of all TB diagnostic health facilities in Lundazi was made. From the list of TB diagnostic centers, two health facilities out of five were randomly selected, whilst Chitungulu health Center was selected purposely as it was the only health facility in the valley area. The drawn in peripheral diagnostic centers included Chitungulu, Kanyanga and Mwasemphangwe Community Health Centers.

The study population was all TB patients diagnosed between 2008 and 2013 from the three diagnostic health centers. The sample size was estimated according to Krejcie and Morgan (1970).

The following is the calculation of the sample size using the formula. The degree of accuracy will be at 0.05 whilst the prevalence of TB in Lundazi is estimated at 0.5% of the total population.

$$S = \frac{x^2 NP(1-P)}{d^2(N-1) + x^2 P(1-P)}$$

Where

S is the Required Sample Size

N is the given population size of the TB patients in Lundazi

P is the Prevalence

d^2 is the degree of accuracy

X^2 is 1.96 Confidence level

$$S = \frac{1.96^2 * 150 * 0.5(1-0.5)}{0.05^2(150-1) + 1.96^2 * 0.5(1-0.5)} = 95.4118 = \mathbf{108}$$

Therefore, a minimum sample size of **108** TB patients was calculated. However, ERES Converge ethics committee recommended a sample size of ***60 study participants*** **(See Annex 10).** In each diagnostic center, Lundazi participants aged between 18 years and

above who met the inclusion criteria were randomly selected. The flow diagram on how the actual sampling of the study participants was done is shown by Figure 3.2 below.

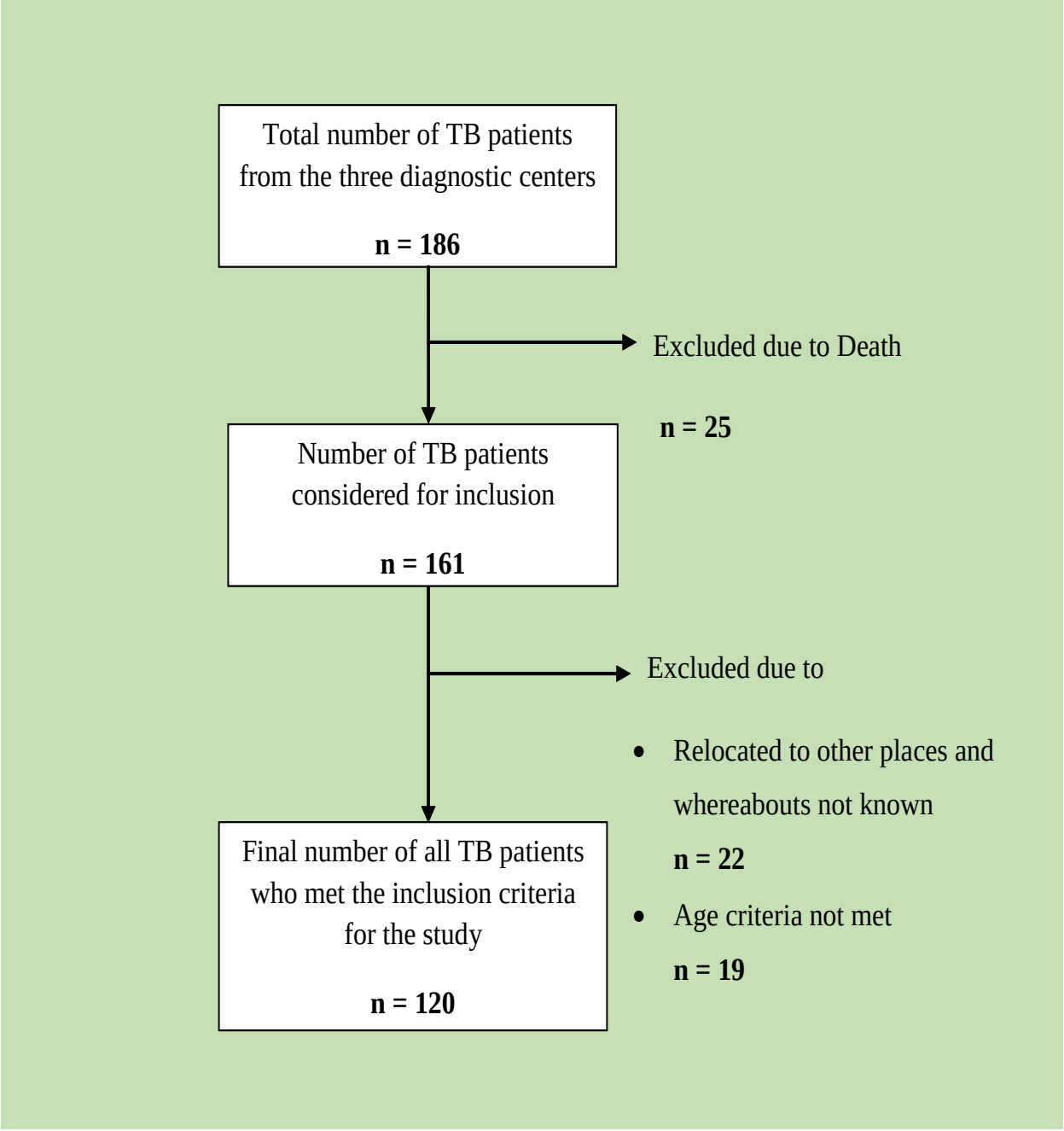


Figure 3.2: Flow diagram showing the sampling of study participants for inclusion in the study

Patients who did not meet the following inclusion or selection criteria were excluded from the study.

i. Subjects who did not sign an informed consent and those below the age of 18 years
ii. Any subject or person who was not a permanent resident of Lundazi district for whom TB was diagnosed and those that died prior to the research
iii. A TB patient without complete data on the register or TB card
iv. Subjects who were on special treatment or care for mental illness or retardation or required special treatment for a major physical disability

3.3 Data collection

The study used both primary and secondary data. Primary data was obtained from the structured questionnaires and GPS readings. The structured questionnaire was pre-tested to ascertain the validity of the questions. Thereafter, necessary modifications and corrections were incorporated. Interviews were also conducted with the sampled participants to ascertain the risk factors. The questionnaire was used to collect information on the basic socio-demographic characteristics, prior history of TB, housing status or condition, socio-cultural factors (e.g. alcohol intake, smoking status, and access to diagnostic center and history of contact with animals. The questionnaire was to understand the risk factors that were contributing to the disease transmission.

The data collected from the GPS was the geographical coordinates for the household sampled. The latitude, longitude and elevation from the three health facilities and the place of residence of all trace back TB cases from households were collected by use of a

hand held GPS, (Triamble Navigation, and Sunnyvale, CA, USA) receiver with a positional accuracy of within 4 meters. The trace back was made possible by the records as compiled from the TB diagnostic centers. The TB cases were geo-coded and matched to the area of occurrence using the software ArcGIS 10.1 (ESRI, Redlands, CA, USA). Thereafter, density maps were produced showing the distribution of all selected TB cases to identify spatial point patterns.

Furthermore, the secondary data was obtained from the diagnostic centers by extracting information from the TB register and other documents in use for TB control. The information that was extracted from the secondary reports include but not limited to TB case number, identification No. for the patient, address, sex, date of registration, date treatment started, Health facility, diagnosis type, type of tuberculosis, type of patient and the treatment outcome.

3.4 Data Analysis

In this study, GIS was used to characterize space – time distribution of TB cases. TB infection, and colour mapping was generated to depict the TB case load of each diagnostic health facility. After data collection, all questionnaires were assigned a serial number. The data collected was double entered, cleaned, validated and coded into a database using word software excel. Data analysis was done using SPSS version 17 for windows (SPSS Inc, Chicago, IL, USA) and STATA 12 *(Prionics®-Check PrioSTRIP™)* for comparison of results. The analysis involved descriptive statistics. Pearson Chi-square (x^2) and p-value were used to compare differences in categorical variables. All statistical tests were considered significant at the $p < 0.05$ and above the x^2 of 3.64. Further, the

coordinates were entered into micro software excel and uploaded into the ArchGIS software for analysis and production of maps on the spatial distribution of human TB. All coordinates (Latitude and Longitude) were converted into figures easily processed by the ArchGIS software for visualization of the exact points on the maps.

Moran's *I* Analysis

The global spatial autocorrelation statistical method was used to measure the correlation among the neighbouring observations to find the pattern and the levels of spatial clustering among the neighbouring TB cases. The presence of spatial as well as space – time clusters of TB cases and identification of their possible locations were determined with spatial autocorrelation Moran's *I* coefficient to test whether TB cases were distributed randomly. Calculations were based on the location of features (point of cases and Location) as well as their attribute values such as income, sex, age and distance to health facilities. The study evaluated whether the distribution of cases with the associated variables was clustered, dispersed or random. The formula for Moran's *I* statistic for spatial autocorrelation used was:

$$I = \frac{n \sum_i \sum_j W_{ij}(Z_i - \bar{Z})\left(Z_j - \bar{z}\right)}{\left(\sum_i \sum_j W_{ij}\right)(Z_k - \bar{Z})^2}$$

Where *N* is the number of spatial units indexed by *i* and *j*, W_{ij} is the measure of closeness of areas *i* and *j* or is an element of a matrix of spatial weights, *Z* is the variable of interest and $\bar{Z}$ is the mean of Z.

The Z_I score for the statistics is computed as

$$Z_I = \frac{1-E\,[I]}{\sqrt{V(I)}}$$

Where

$$E\,[I] = -1/(n-1)$$

$$V = E\,[I^2] - E\,[I]^2$$

The Z score and the P values were also calculated to evaluate the significance of the features and associated variables.

Hot – Spot Analysis

Further, the study calculated the hot spots for the three sampled areas to detect the potential spatial or temporal clusters. The local $G_I^*(d)$ statistics was used to test the statistical significance of local clusters (Getis and Ord 1992; 1996) This was useful for identifying individual members of local clusters by determining the spatial dependency and relative magnitude between an observation and neighbouring observation. The *Gi(d)* statistics were computed as follows (Ding and Fotheringham, 1992; Getis and Ord 1996; and Kitron et al., 1997):

$$G_i^* = \frac{\sum_{j=1}^{n} \omega_{i,j}^2 X_j - \bar{X} \sum_{j=1}^{n} \omega_{i,j}}{S\sqrt{\frac{\left[n \sum_{j=1}^{n} \omega_{i,j}^2 - \left(\sum_{j=1}^{n} \omega_{i,j}\right)\text{^}2\right]}{n-1}}}$$

Where the X_j is the attribute value for feature *j*, $\omega_{i,j}$ is the spatial weight between feature *i* and *j* is equal to the total number of features as shown below:

$$\bar{X} = \frac{\sum_{j=1}^{n} X_i}{n}$$

$$S = \sqrt{\frac{\sum_{j=1}^{n} X_j^2}{n} - (\bar{X})^2}$$

By comparing local estimates of spatial autocorrelation with global averages, the Gi(d) statistics identifies 'hotspots' in spatial data. Note that the $G_i(d)$ statistics was the z score with no further calculations.

In addition to mapping, logistic regression analysis was conducted to the collected data. The ANOVA tables were generated to test the significance of the variables in terms of R – square and *P* values.

3.5 Ethical Clearance

Ethical clearance for the study was obtained from the ERES Converge ethics committee I.R.B. No. 00005948, F.W.A. No. 00011697 under approval ***Reference number: 2014 – May - 004*** following approval by the University of Zambia, Directorate of Research and Graduate Studies (DRGS) through the Assistant Dean- Post Graduate school of Veterinary Medicine. Further, official permission was obtained from the Permanent Secretary Ministry of Health, Provincial Health Office, Lundazi District Community Medical Office, and the local Health Centers including community leadership (Headmen and civic leaders). On the other hand, written informed consent was obtained from the study participants before enrollment. Detailed explanation of the study was done to participants which enabled them to participate voluntarily. Confidentiality and privacy was strictly maintained at all times to protect the rights and dignity of people involved in the study.

CHAPTER FOUR

4.1 Descriptive Results

A total of 60 clinically diagnosed TB cases from the three diagnostic centers (Chitungulu, Mwasemphangwe and Kanyanga) were enrolled in the study and the data was collected between November 2014 and February 2015. More than half of the study participants (31) were males, representing 51.7% (95% CI 38.6 - 64.7%) while 29 were females, representing 48.3% (95% CI 35.3 - 61.4%). The mean age was 46.4 years with a standard deviation (SD) of 17 years while those in the age group 41 – 50 years and those aged > 61 years were the majority (16) at 26.7% for each group followed by the 31 – 40 years (13) at 21.7%, with 12 (20%) being in the age group 18 – 30 and the least (3) being those between 51 – 60 years at 5%. See also annexes on maps showing the sex, marital status, education, occupation and age distribution of the participants.

The majority of the participants 39 (65%, 95% CI 52.6 - 77.4%) were married or had a partner; 8 (13.3%, 95% CI 4.5 - 22.1%) were single; and another 8 (13.3%, 95% CI 4.5 - 22.1%) were widowed whilst the remaining 6 (8.4%, 95% CI 0 - 10.6%) were either divorced or separated. The majority of participants 25 (41.7%, 95% CI 28.8 - 54.5%) attained primary education 14 (23.3%, 95% CI 19.8 - 33.1%) had attained junior secondary education whilst 11 (18.3%, 95% CI 14.7 - 26.6%) were without any formal education. Those with senior level and tertiary education were only 5 (8.4%, 95% CI 7.5 - 12.5%). In terms of occupation, 41 (68.3%, 95% CI 57.5 - 79.8%) were farmers 9 (15%, 95% CI 9.5 - 19.9%) were unemployed, 6 (10%, 95% CI 6.5 - 12.9%) were housewives or homemakers, 3 (5%, 95% CI 1.5 - 5.8%) were working under government while 1

(1.7%) working as a fishermen. Table 4.1 shows the distribution of the demographical variables of the participants.

Table 4.1: Distribution of Demographic Variables for the Participants

Variables and Category	Total	Percentage (%)
Gender		
Male	31	51.7
Female	29	48.3
Total	**60**	**100**
Age Distribution (Years)		
18 – 30	12	20
31 – 40	13	21.7
41 – 50	16	26.7
51 – 60	3	5
> 60	16	26.7
Total	**60**	**100**
Marital Status		
Single	8	13.3
Married	39	65.0
Divorced/Separated	6	8.4
Widowed	8	13.3
Total	**60**	**100**
Education Level		
None	11	18.3
Primary	25	41.7
Secondary	19	31.7
Tertiary	5	8.3
Total	**60**	**100**
Occupation Status		
Farmer	41	68.3
Unemployed	9	15
Homemaker	6	10
Employed (Employed)	3	5
Fishing	1	1.7
Total	**60**	**100**

4.2 Trend analysis results of Tuberculosis cases between 2008 and 2013

A trend analysis of pulmonary versus non – pulmonary TB cases between 2008 and 2013 cases was conducted.

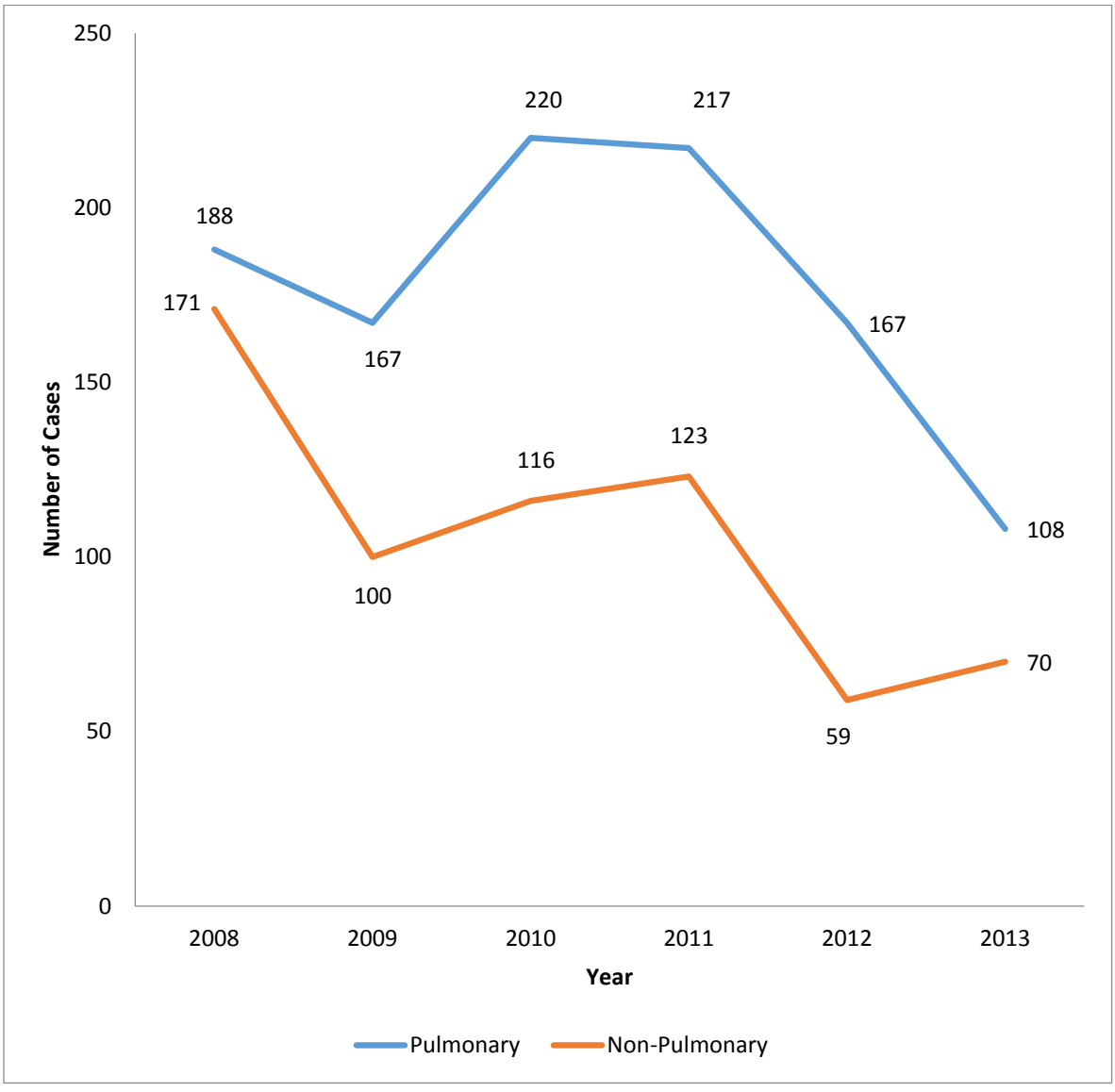


Figure 4.1: Line graph showing the TB trends in Lundazi district from 2008 to 2013

The distribution of TB trends shown by Figure 4.1 was generated from the analysis of TB case load per 100 000 for each of the years. The study revealed that the smear positive (Pulmonary) cases from 2008 were significantly higher ($P < 0.001$). The year 2010 and 2011 had the highest number of pulmonary cases with the subsequent years showing a downward trend for both the pulmonary and non – pulmonary cases (Figure 4.1).

4.3 Spatial analysis results of human tuberculosis cases in Lundazi district

The dot map, Figure 4.2 below shows the 60 analyzed TB cases and suggests that the cases were concentrated in all the three diagnostic centers and were distributed around the clinic catchment area of the health facilities. The samples were drawn as follows: 13 were sample from Chitungulu, 23 from Mwasemphangwe and 24 from Magodi diagnostic centers. These areas where samples came from are characterized by poor communities, poor housing and high densities of people.

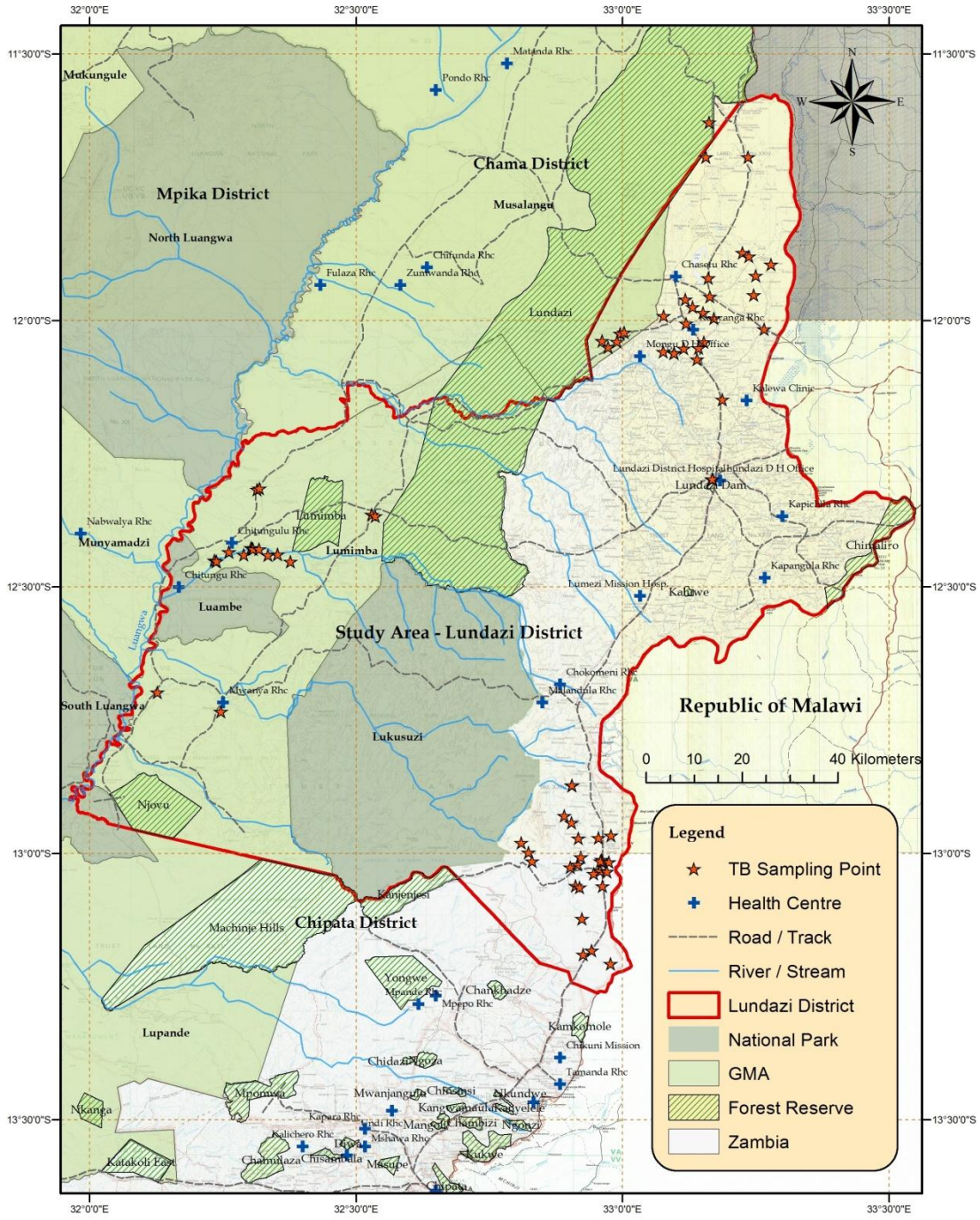


Figure 4.2: Spatial distribution of Human TB cases (Lundazi district)

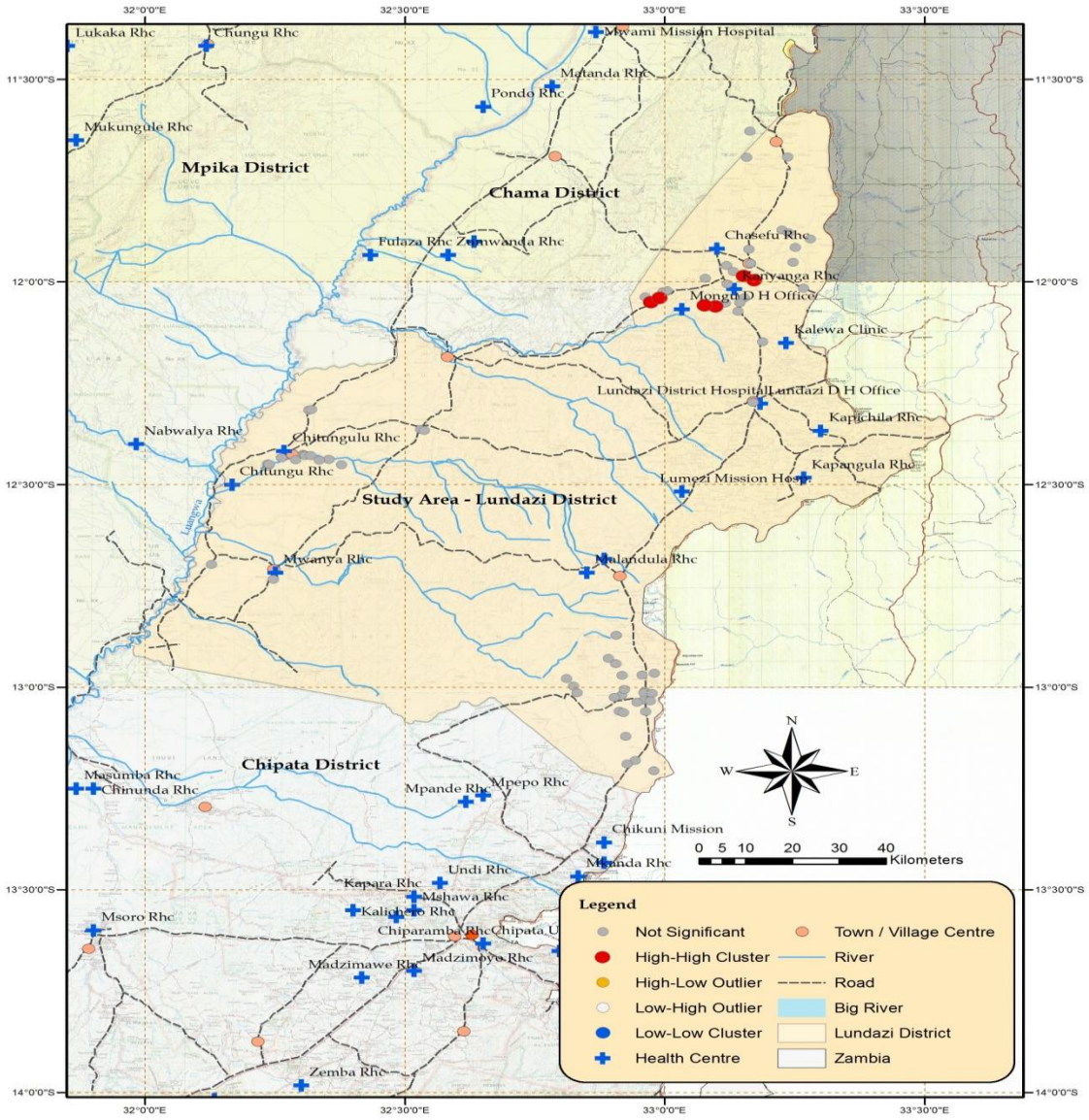


Figure 4.3: Spatial pattern of TB cluster analysis

The map above shows the most likely cluster in the sampled areas and the spatial distribution of cases displayed distinct patterns with Kanyanga diagnostic center demonstrating high – high clustering of TB cases (See Figure 4.3). The solid red dots in the map indicate areas with high – high clustering among the analyzed cases whilst the other two areas the clustering was not significant.

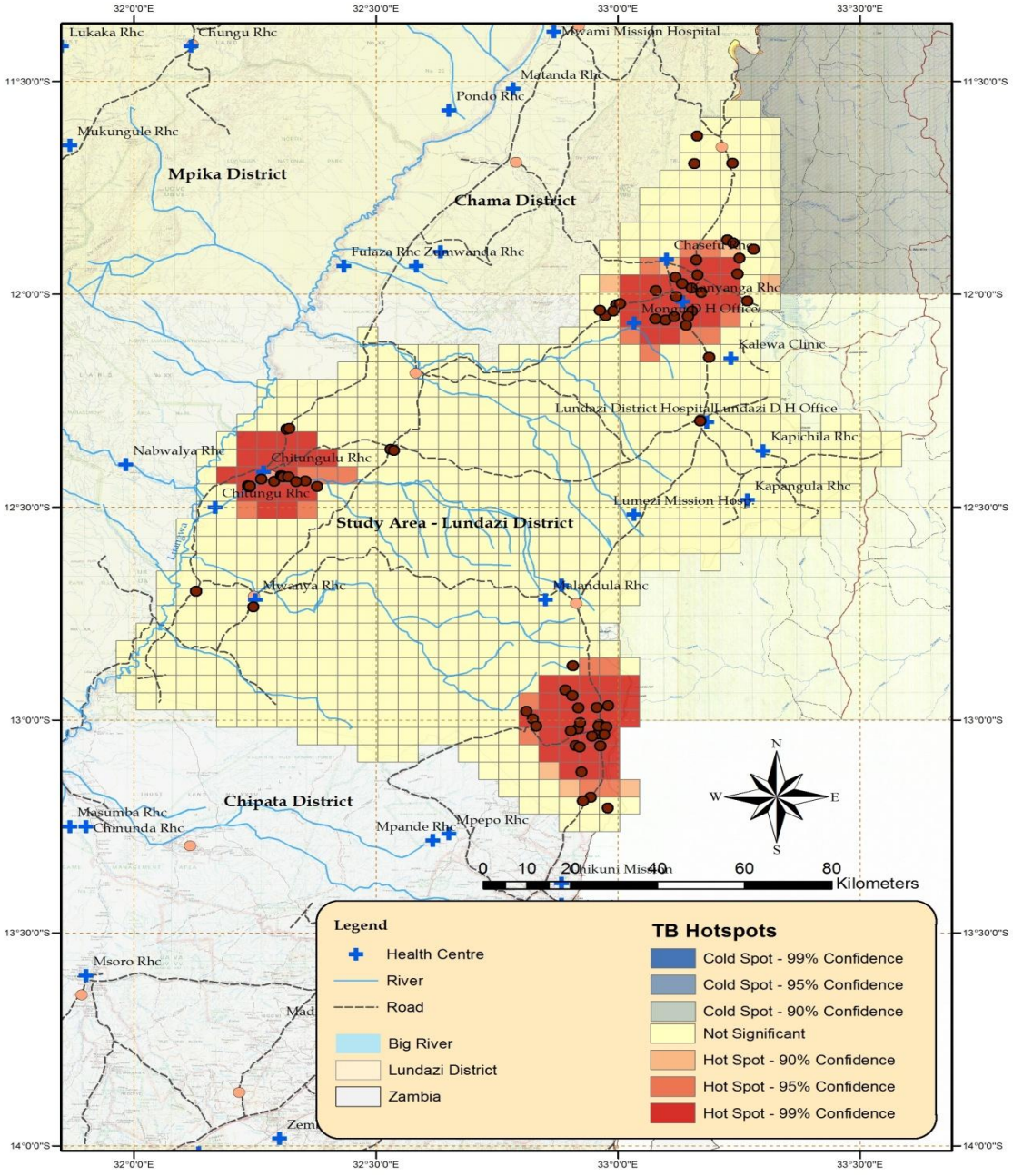


Figure 4.4: TB Hotspots

Figure 4.4 shows hotspots of TB in the sampled areas which displayed an evident pattern of clustering particularly in the areas surrounding the TB diagnostic centers with the red colour representing the hot spots. It is apparent that cases of "hotspots" were largely

located in areas that were densely populated, supporting a positive association between population density and increased risk of TB exposure.

To identify the degree of spatial clustering of TB cases, distance and location analysis were done using the Moran's *I* test. This method derives the nearest neighbor index, which provides an approximation of whether points are more clustered or dispersed than would be expected on the basis of chance. A highly spatial cluster of TB cases was observed given the Z – score of 7.411590 as it was above Z > 2.58 with the Moran's I of 0.277733 (Refer to Table 4.2 and Figure 4.5). There is less than 5% likelihood that this cluster pattern observed could be the result of random chance. The Z score reached its peak at 14 km and exhibited considerable cluster in terms of distance. Results are summarized in Table 4.2 (ArcGIS 10.1.)

Table 4.2: Moran's I Results

Global Moran's I Summary	
Moran's Index:	0.277733
Expected Index:	-0.011236
Variance:	0.001520
z-score:	7.411590
p-value:	0.000000
Dataset Information	
Input Feature Class:	TB Sampling Point
Input Field:	DISTANCE_F
Conceptualization:	FIXED_DISTANCE
Distance Method:	EUCLIDEAN
Row Standardization:	False
Distance Threshold:	13560.6256 Meters
Weights Matrix File:	None
Selection Set:	False

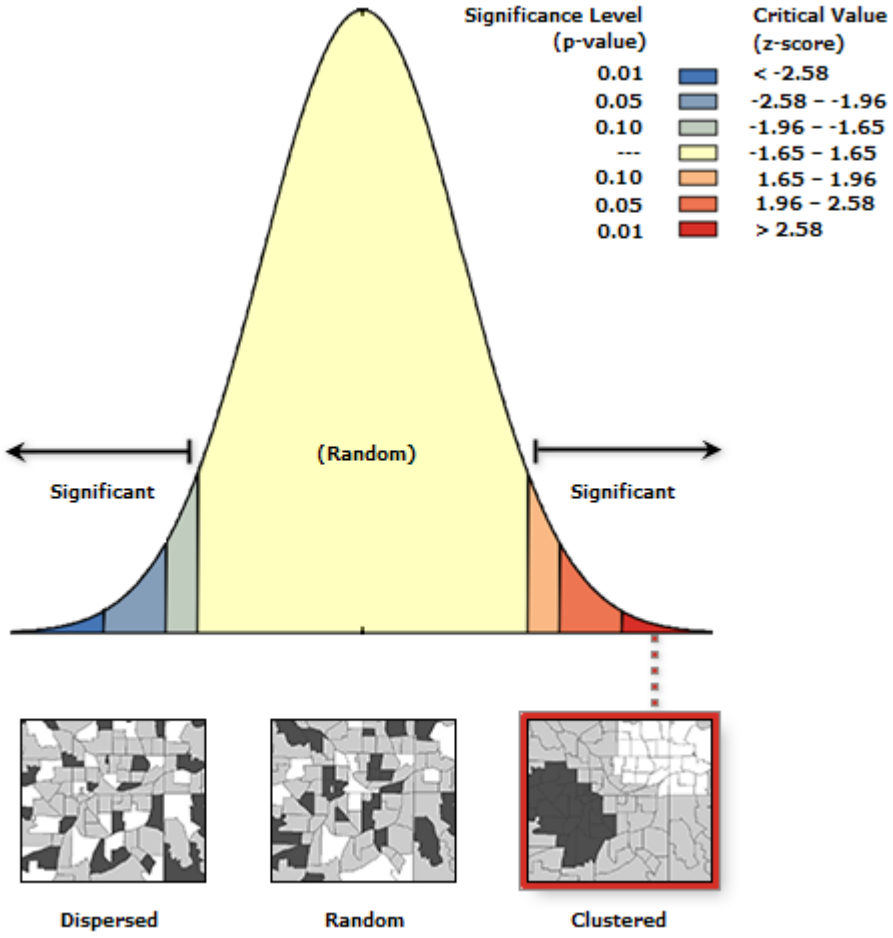


Figure 4.5: Moran's *I* analysis showing Z-Scores

Further, analysis was done in connection with the age distribution of the participants whether there was any clustering in comparison with how the cases were distributed. The investigation revealed that there was clustering ($p < 0.05$).

Logistic Regression Analysis for Risk Factors

The logistic regression analysis was done to identify significant risk factors of TB in order to consolidate the spatial analysis. Table 4.3 below shows the multiple logistic regression analysis results:

Table 4.3: Summary of Logistic Regression Analysis of Risk Factors

Variable	**Odds Ratio (OR)**	**P - Value**	**95% Confidence Interval (CI)**
Age	0.8164	0.76	0.227 – 2.941
Average Income	1.7	0.54	0.313 – 9.240
Cigarette Smoking	1.116	0.02	1.017 – 1.235
Education	1.2765	0.23	0.854 – 1.909
Gender	1.865	0.29	0.592 – 5.872
Marital Status	0.8705	0.60	0.518 – 1.464
Milk Consumption	1.913	0.32	0.537 – 6.808
Number of Rooms	0.9249	0.73	0.5967 – 1.434
Number Sleeping in a Room	1.556	0.11	0.889 – 2.725
Occupation Status	0.6261	0.19	0.311 – 1.259
Patients who Drink and Smoking	4.680	0.05	1.019 – 21.485
Window Type	0.670	0.47	0.226 – 1.983

From results of table 4.3, most factors did not seem to have any associations with the occurrence of TB. However, the disease was associated with smoking and alcohol consumption as the odds of developing TB was high among those respondents who were both drinking alcohol and smoking tobacco (OR = 4.6, p – = 0.04, 95% CI, 1.019 – 21.485) and cigarette smoking was found to be significant as the p = 0.020 (OR = 1.12, 95% CI, 1.017 – 1.235). However, likelihood of having TB for people who sleep in the same room was about 1.6 times more.

Further, the multiple regression analysis by the use of ANOVA table was conducted to predict the interaction of various variables and table 4.4 below shows the outcome of the analysis.

Table 4.4: ANOVA Analysis

Model		Sum of Squares	df	Mean Square	F	Sig.
	Regression	57.184	5	11.437	341.871	.000
	Residual	1.673	50	.033		
	Total	58.857	55			

a. Predictors: (Constant), age of respondent, previous contact with TB patients, age when started smoking, number of rooms in the house, cigarette smoking, number of cigarettes you smoke per day.

b. Dependent Variable: Total number of participants who suffered from TB in the household.

Table 4.5: Model Summary

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.986	.972	.969	.18290

a. Predictors: (Constant), age of respondent, total TB patients, age first smoked, number of rooms in the house, number of cigarettes smoked per day

b. Dependent Variable: Total number who suffered from TB in H/H

A multiple regression analysis was conducted to predict total number of cases of TB occurrence for 60 patients using number of cigarette smoked, age first smoked, and previous contact with TB patients and number of rooms in the house as predictors. A test of the full model against a constant only model was statistically significant (p = 0.0000), indicating that the predictors as a set reliably distinguished between number of TB cases and TB risk factors. These variables statistically significantly predicted the total number of TB cases F (5, 50) = 341.87, P = 0.000, df = 5 (Refer to table 4.4).

R = 0.986 and Nagelkerke's R square of 0.972 indicate a very strong relationship between prediction and grouping (See table 4.5 above). All the five variables added statistically significantly to the prediction, P – value < 0.05.

The wald criterion demonstrated that only history of previous contact with TB patients (p = 0.000) smoking (p-value = 0.000), made a significant contribution to prediction while the rest of the risk factors were not significant predictors (Annex 7). EXP (B) values indicate that smoking had an OR of 9.4, Alcohol consumption OR of 4.2 and the

combination of both alcohol consumption and smoking gave an OR of 4.5 while the number of people sleeping in one room OR was 9.6.

Limitations of the Study

A number of limitations in this study have to be recognized. For example, income did not show a significant effect, but the measure was possibly subject to underestimation because data was collected using a face to face questionnaire. Due to this method and the inherent belief by the respondents of receiving help if they are proved to be poor. The participants may have not reported the actual income and may have prevented the detection of the actual picture of the economic status of the community. Secondly, due to the limited sample size, it will be difficult to generalize these results outside the study area. Thirdly, the sample collected was based on the TB diagnostic center population; hence, it could have missed out those patients who did not present themselves to the center thereby biasing the study.

However, despite these shortfalls, this study demonstrated internal and external validity given the congruency of the findings with other similarly formulated studies in other places.

CHAPTER FIVE

5.0 Discussion

Over the last few decades, the application of spatial analysis in the context of epidemiological surveillance and research has increased in an exponential fashion (Pfieffer *et al*. 2008). The transmission of infectious diseases, especially those of a chronic nature such as TB is closely linked to the concepts of spatial and spatial-temporal proximity, as transmission is more likely to occur if the at-risk individuals are close in a spatial and a temporal sense. This epidemiological scenario is key in describing the nature and distribution of chronic diseases such as TB and it is against this background that this present study was formulated. The main thrust of this study was to determine the spatial distribution of human TB and its potential risk factors.

The findings generated from this study through the use of geographical spatial referencing and mapping of human TB, have demonstrated geographical clustering of TB cases and has provided useful information on the prevailing epidemiological distribution situation of TB in Lundazi district with associated risk factors. One important point worth noting is the spatial distribution and disease burden in areas where people live in either very close proximity or relatively high density communities. This was evident when compared, the clusters of the local G_i^* in all the three TB diagnostic areas sampled displayed a significant high risk pattern of TB clustering particularly in areas surrounding the TB diagnostic centers. The use of statistically robust geographical analysis methods to predict the spatial distribution of human TB cases and the application of geospatial

analysis in the study conferred considerable advantages over the traditional, frequent modeling approaches, in that the spatial analysis was able to demonstrate clustering through the use of maps.

The study has helped to geographically stratify the three sampled areas by intensity of clustering and risk of human TB. The distribution of human TB in Lundazi district explains, at least in part, the high case load of TB in the high population density areas around the TB diagnostic centers. This clustering of TB cases in the areas around the TB diagnostic centers may suggest that the transmission of the bacteria is widespread and there is a possibility of active open TB cases going on in these areas. These data are consistent with the study that was conducted in China by Chan-Yeung *et al*. (2005) which demonstrated that overcrowded homes and environments increase the risk of contact and therefore enhances the transmission of the disease. In the present study, one of the major risk factors of TB was found to be alcohol consumption and associated cigarette smoking, both of which usually draw people close together.

Further, the study has demonstrated the applicability of the map overlays, using GIS in understanding human TB distribution and how that relates to risk factors. GIS has emerged as an important component of many projects in public health and epidemiology (Boscoe *et al*. 2004 in Sara *et al.* 2005). The created maps can therefore act as a reference point to help gain insight into how the disease occurrence changes by looking at the changes in the maps which can be created later. Fluidity in maps over a period of time can give very good information with regards to the changes in the disease pattern and possible spread. stated that Epidemiology generally centers on understanding how the occurrence of a disease relates to potential explanatory factors (Mary and Eric, 2004).

Similarly, Bellec *et al*. (2006) demonstrated that the use of GIS with spatial autocorrelation and hot-spot analysis has been applied to the spatial pattern analysis of a variety of diseases. For this reason, it is not uncommon to include for consideration when available the spatial locations at which cases of the disease occur. They posit the most fundamental tasks in spatial epidemiology as they differentiate the spatial distribution of disease through the creation of maps. That is what the research project achieved by coming up with the human TB clustering maps.

The spatial analysis of the hot spots suggest that there were statistically significant hotspots of TB and showed the existence of TB clusters in all the three TB diagnostic centers of the district. Thus, these clusters are basically the hotspots of TB transmission. According to Dowdy *et al*. (2012), the importance of the hotspot to city-wide TB control depends strongly on the extent of TB transmission from the hotspot to the general community and it was elucidated that high-incidence hotspots may play an important role in propagating TB epidemics. Hence, the importance of disease "hotspots" cannot be overlooked as they have a role in the transmission of diseases. From our current study, this provides some evidence that the three statistically significant hot spots at Chitungulu, Kanyanga and Mwasemphangwe TB diagnostic centers may have persisted over time and are likely to be the major hotspots for TB occurrence and transmission in the area. These hot spots were located in areas that were populated supporting an association between population density and increased risk of TB. For instance, a model of TB transmission in Rio de Janeiro revealed that a subpopulation (hotspot) comprising 6.0% of the city's population accounted for a disproportionate amount of ongoing TB transmission, where each case of active TB in the hotspot caused 0.5 secondary transmissions in the general

community, and that each secondary transmission within accounted for 35.3% of all TB transmission in the city (Dowdy *et al*. 2012).

Further, these findings are in line with the study conducted in India by Tiwari et al. (2006) which demonstrated that purely spatial and retrospective space time analysis were used to find significant hotspots of TB in Almora District. Therefore, the mapping of the disease helps in confirming the location of the "hotspots" but does not indicate whether transmission has occurred at that particular location. This is so because each particular case is not a static point, but a living human being, with possibilities of moving between various locations that are very hard to detect retrospectively despite intensive contact tracing and interviewing (Munch *et al*. 2003). As a result of mobility, people, are able to transmit the disease to other people. Other studies have also shown clustering of TB cases for example, a multi-country comparison of geographical and temporal distribution of TB (Uthman, 2008), suggested that 25 countries in the African continent were at increased risk of TB and ten countries could be grouped as 'hotspots'. The pattern of clustering that has been observed in Lundazi district could mean sustained transmission of TB within the communities.

The global spatial autocorrelation of the overall clustering of the human TB in Lundazi district was supported by the statistically significant Moran's *I* (0.2777, Z = 7.411590, P = 0.0000) indicating a strong correlation and a highly spatial clustering of the TB cases in the study area. Taken together, these data suggest that there was spatial clustering of human TB in the area.

The study has particularly demonstrated the usefulness of spatial analysis in describing the geographical distribution and the density analysis of point events of TB cases in Lundazi district. The results have provided useful information on the prevailing epidemiological situation of human TB. The new knowledge about the presence of hotspots of TB in the district can help the district TB unit to intensify their remedial measures in the identified areas of high TB prevalence for more effective disease control.

Due to the above observations, this particular study also investigated the risk factors of human TB in Lundazi district to try and understand what sustains the disease in the District. The logistic regression analysis of TB's risk factors revealed that tobacco smoking, alcohol consumption, and previous contact with TB patients were significant risk factors.

The study established that smoking was associated with an increased risk of active TB ($p = 0.020$, OR = 1.12, 95% CI, 1.017 – 1.235). Smoking as a risk factor may contribute to the increased susceptibility of individuals developing TB infection. Therefore, TB patients who smoke at home are making their condition more vulnerable and are also placing their families at a greater risk of TB infection. In addition, exposure to passive smoking may also increase the risk for both TB infection and development of active TB disease for people who are non smokers (Bates et al. 2007; Lin et al. 2007). The finding that smoking is an important risk factor for TB in Lundazi has obvious TB control implication.

Other studies have also pointed to an association between tobacco smoking and active TB cases. For example, a 14-year prospective cohort study carried out in South Korea from

1992 to 2006 revealed evidences that smoking increases the incidence of TB, the mortality rates, and TB recurrence (Jee *et al*. 2009). Further, the study conducted by Lienhardt *et al*. (2005) revealed that the risk of TB was two times higher in current smokers compared with the non smokers. These and other results are in accordance with those reported by Harling *et al*. (2008), in South Africa which confirmed that cigarette smoking was an independent risk factor, and with a another study conducted in Taiwan (Lin *et al*. 2009) which revealed that tobacco smoking was associated with a two – fold increased risk of active TB.

The study did not explore further the mechanism by which smoking influences the susceptibility to TB. However, according to Altet *et al*. (1996), smoking may affect many organ systems, but the lungs suffer by far the most damage. Smoking damages the lungs and compromises the body's immune system, making smokers more susceptible to TB infection. The occurrence of TB has been shown to be linked to altered immune response and multiple defects in immune cells such as macrophages, monocytes and CD4 lymphocytes (Altet *et al*. 1996). Other mechanisms, such as mechanical disruption of cilia function and hormonal effects, could also appear secondarily to smoking (Buskin *et al*. 1994). Chronic exposure to tobacco impairs the normal clearance of secretions on the tracheobronchial mucosal surface and may thus allow the causative organism, *M. tuberculosis,* to escape the first level of host defenses, which prevent bacilli from reaching the alveoli (Houtmeyers *et al*. 1999).

The study has further, established that there was an association between those respondents who consume alcohol and smoke at the same time ($p = 0.047$, OR = 4.6, & 95% CI 1.02 – 21.49) and the risk of TB. The association between alcohol use and TB

could be explained by specific social mixing patterns, which may increase the risk of exposure to people with TB in public gatherings settings.

Previous contact with TB patients ($p < 0.0000$, 95% CI 0.931 – 1.034) was also found to be one of the TB risk factors. Some of the participants were able to recall the previous contacts that they stayed with. The significance in the regression analysis of previous contact with TB patients as a risk factor for TB may indicate prolonged contact periods at home or at social meeting places where transmission of infection may have occurred by people who come in contact with each other. The social mixing patterns may increase the risk of exposure to people with infectious TB.

This study has shown no association between age and the risk of TB. The possible reason for the lack of association may be that there are other factors that are masking the age as a risk factor, for example the low HIV/AIDS prevalence in the population. However, other studies found that an increasing age was reported as a strong risk factor for TB incidence in Guinea-Bissau study (Gustafson *et al*. 2004).

The male gender has been reported as an independent risk factor in other studies in sub-Saharan Africa (Gustafson *et al*. 2004). However, in this study gender was not an important risk factor, even though there were more men who had TB. The underlying reason for this outcome can be attributed to the increase in risk behaviours such as alcohol and substance abuse for both men and women. This means that the risk of exposure to TB infection is the same for both sexes. Even though, other studies have also found that more men than women are diagnosed with TB worldwide (Rieder, 1999; Uplekar *et al*. 1999; Uplekar *et al*. 2001).

Marital status which was not found to be a risk factor by this study has long been associated with the risk of developing TB in Africa (Lienhardt *et al*. 2005). The plausible reason for this finding is the rate of cohabiting among the unmarried individuals. Cohabiting is common for single individuals who live together like married couples. This makes the risk of exposure to TB infection for both married and the unmarried the same.

Education background did not seem to significantly play a major role as a risk factor of the disease among patients. The explanation for this outcome could be that the majority of the participants were living closer to the TB diagnostic centers and were more likely to reflect a more peri-urban type of setting. This helped them to access information on TB and the associated risk factor. This contradicts the findings of M'Imunya *et al*. (2012) who found that different educational and counseling interventions may increase successful treatment completion. Another study found low education as a risk factor for TB (Shetty *et al*. 2006).

The study established that income of the participants was not a significant risk factor. The possible explanation was that information on income was difficult to obtain owing to the fact that participants were expecting financial benefits, hence, they did not give the actual income they had. On the other hand, income generating activities were seasonal and variable in time thereby making it difficult for participants to recall or reflect on the overall income which they might have accumulated over time which subsequently led to recall bias

The number of people sleeping in one room (Crowding), number of rooms in a house, type of windows and type of roof (Housing conditions) were not significant risk factors

The plausible reason for these findings is that the majority of the households had meet the minimum housing standards. The type of housing in these areas is improved as people who live closer to the clinic are likely to reflect a more peri-urban type of setting and were more likely to have improved housing structures. However, the possible reason in the transmission of TB in these households was the previous contact with a TB patient as it is related to the mixing patterns of members of the household who usually come in close contact with the patient excreting the bacilli. For example a study conducted by Gustafson *et al*. (2004) found that increasing age ($p < 0.0001$), gender (male sex) OR 2.58, adult crowding OR 1.68, family structure and poor housing conditions were important risk factors for TB.

Ownership of cattle and consumption of raw milk were found not to be risk factors in this study. This finding may be attributed to the majority of the participants who do not own cattle and rarely consume contaminated milk and carcasses. Hence, there is less likelihood of exposure to TB through both contaminated milk and carcasses. However it has been established that TB can be transmitted to humans if contaminated milk or contaminated carcasses are consumed (Philips *et al*., 2003).

CHAPTER SIX

6.0 Conclusion and Recommendations

6.1 Conclusion

In conclusion, the findings of this study demonstrated the importance of GIS as a useful tool for identifying clusters and hot spots of TB. Therefore, the combination of spatial analysis and risk factors were important in understanding the epidemiology of the disease. The results obtained from this study through the use of geographical spatial referencing and mapping of human TB have demonstrated, for the first time, the existence of geographical clustering and the presence of hot spots of human TB cases in Lundazi district. Further, the finding that clustering is likely to occur in high density localities is very important for policy formulation and disease control. In addition, the spatial analysis of the hotspots suggests that there were statistically significant hotspots of TB and showed the existence of TB clusters in all the three TB diagnostic centers of the district. These hot spots were located in areas that were populated supporting an association between population density and increased risk of TB. Identifying these geographical patterns was very important for understanding how geographical phenomenon behavior in time and space. Our findings are relevant as they have shown that there were hot spots where TB seems to be transmitted and sustained at a much high rate.

Cigarette smoking, alcohol consumption and previous contact with TB patients were identified as important risk factors associated with human TB.

Finally, these findings will help in targeting specific areas of the district with more efficient educational programmes and control measures.

5.2 Recommendations

The evidence obtained in this study has identified the areas that need attention in terms of control and prevention of human TB in Lundazi district. Therefore, the following are the recommendations:

i. From this study, it's recommended that public health implication in relation to spatial distribution of TB, social interaction patterns, demographic factors and other multiplicative factors for transmission (co-infection with HIV) need to be considered when formulating policy action and plans.

ii. The local District Health Information Officers to integrate the use of GPS in data analysis as it can provide officials with necessary tools and feedback on different disease trends over time as well as identifying and targeting preventive treatment to high risk populations to reduce the foci where TB can easily be transmitted.

iii. The District Community Health Office should concentrate their efforts on active TB case identification or detection in these areas as it suggests that there may be a sustained transmission of TB cases due to the presence of clustering coupled with cases that are not reported by the community to the health facility. There is need to intensify active case finding in the community.

iv. Health workers treating the Tuberculosis patients should ensure that patients are educated on the dangers of smoking.

References

Alavi S.M. and Ershadian S. (2009), *Association between cigarette smoking and pulmonary tuberculosis,* Pakistian Journal of Medical Sciences; 25:912-915

Alexander K.A., Pleydell E., Williams M.C., Lane E.P., Nyange J.F.C. and Michel A.L. (2002). *Mycobacterium tuberculosis*: *an emerging disease of free-ranging wildlife*, Emerging Infectious Diseases; 8; 592–595.

Altet M.N., Alcaide J., Plans P., Taberner J.L., Salto E., Folguera L.I. and Salleras L.I. (1996), *Passive smoking and risk of pulmonary tuberculosis in children immediately following infection*: A case-control study. Tuberculosis Lung Diseases; 77:537–44

Anonymous (2002), *Zambia National HIV/AIDS/STI/TB Policy*, Lusaka, Zambia

Anonymous (2008), *The current TB/HIV situation in Zambia*, Lusaka, Zambia

Anonymous (2009), *The National Tuberculosis and Leprosy Programme TB Manual Zambia*, Lusaka, Zambia.

Aranaz A., Liebana E., Gomez-Mampaso E., Galan J.C., Cousins D., Ortega A., Blazquez J., Baquero F., Mateos A., Suarez G. and Dominquez L. (1999), *Mycobacterium tuberculosis subsp. Caprae subsp. Nov.: a Toxonomic Study of a New Member of the Mycobacterium tuberculosis Complex Isolate from Goats in Spain*; International Journal of Systematic Bacteriology 49, 1263-1273

Atkinson P and Molesworth A. (2000), *Geographical Analysis of Communicable Disease Data,* In Spatial Epidemiology: Methods and Applications. Eds. Elliott P, Wakefield JC, Best NG, Briggs DJ. New York: Oxford University Press, Inc.

Bates M.N., KhalaKdin A., Pai M., Chang L., Lessa F. and Smith K.R. (2007), *Risk of Tuberculosis from Exposure to Tobacco Smoke: A Systematic Review and Meta-analysis.* Archives of Internal Medicine; 167: 335–342.

Bellec S., Hemon D., Rudant J., Goubin A. and Clavel J. (2006), *Spatial and space–time clustering of childhood acute leukaemia in France from 1990 to 2000*: a nationwide study. British Journal of Cancer; 94:763–770.

Benatar S.R., and Upshur R., (2010), *Poverty and Tuberculosis: What could (and should) be done?* International Journal of Tuberculosis and Lung Disease; 14: 1215-1221

Boscoe F.P., Ward M.H. and Reynolds P. (2004), *Current practices in spatial analysis of cancer data: data characteristics and data sources for geographic studies of cancer*. International Journal of Health Geography; 97: 14041-3

Brosch R., Gordon S.V., Marmiesse M., Brodin P., Buchrieser C., Eiglmeier K., Garnier T., Gutierrez C., Hewinson G., Kremer K., Parsons L.M., Pym A.S., Samper S., Van Soolingen D., and Cole S.T., (2002). *A New Evolutionary Scenario for the Mycobacterium Tuberculosis Complex*, Proceedings of the National Academy of Sciences USA; 2002; 99:3684–3689

Buskin S.E., Gale J.L., Weiss N.S. and Nolan C.M. (1994), *Tuberculosis risk factors in adults in King County, Washington, 1988 through 1990.* American Journal of Public Health; 84:1750-6.

Centers for Disease Control and Prevention [CDC] (2007), *Trends in tuberculosis incidence, United States, 2006.* MMWR (Morbidity and Mortality Weekly Report); 56:245–250.

Central Statistics Office [CSO] (2011), Zambia 2010 *Census of population and housing:* Preliminary Population figures, Government Printers, Lusaka, Zambia.

Chaisson R.E. and Martinson N.A. (2008), *Tuberculosis in Africa – Combating an HIV Driven Crisis.* The New England Journal of Medicine; 358:1089-1092

Chanda D. (2002), *A study to determine the High Prevalence of Tuberculosis among Nurses in the University Teaching Hospital.* Lusaka, Zambia.

Chan-yeung M., Yeh A.G., Tam C.M., Kam K.M., Leung C.C., Yew W.W., and Lam C.W. (2005), *Socio-demographic and geographic indicators and distribution of tuberculosis in Hong Kong*: a spatial analysis. International Journal of Tuberculosis and Lung Disease; 9:1320–1326.

Cleaveland S., Hess G.R., Dobson A.P., Laurenson M.K., McCallum H.I., Roberts M.G., and Woodroffe R.,(2001). *The Role of Pathogens in Biological Conservation*. In: Hudson P.J., Rizzoli A., Grenfell B.T., Heesterbeek H., Dobson A., (Eds.).*The Ecology of Wildlife Diseases*. Oxford, UK: Oxford University Press; 139–150.

Cook G.C. and Zumla A. (2008), Manson's Tropical Disease, 22nd Edition, WB Saunders, London, UK.

Cosivi O., Grange J.M., Daborn C.J., Raviglione M.C., Fujikura T., and Cousins D., (1998). *Zoonotic tuberculosis due to Mycobacterium bovis in developing countries*. Emerging Infectious Diseases Journal.1998; 4:1–14.

Cousins D.V., Bastida R., Cataldi A., Ouse V., Redrobe S., Dow S., Duigan P., Murray A., Dupont C., Ahmed N., Collins D.M., Butler W.R., Dawson D., Rodrigez D., Loureiro J., Romano M.I., Alito A., Zumarraga M. and Bernardelli A. (2003), *Tuberculosis in Seals caused by a Novel Member of the Mycobacterium Tuberculosis Complex: Mycobacterium pinnipedii sp. Nov*: International Journal of Systematic and Evolutionary Microbiology; 53, 1305-1314

Crofton J., Horne N. and Miller F. (1999), *Clinical Tuberculosis,* 2nd ed. London: Macmillan Education.

Dowdy D. W., Golub J. E., Chaisson R. E. and Saraceni V. (2012), *Heterogeneity in tuberculosis transmission and the role of geographic hotspots in propagating epidemics*, Rio de Janeiro, RJ 22441-030, Brazil

Dye C, and Floyd K (2006), *Tuberculosis.* In Disease control priorities in developing countries Jamison D.T, Breman J.G, Measham A.R, Alleyne G, Claeson M, Evans D.B, Jha P, Mills A, Musgrove, Editors. Washington, DC: Oxford University Press.

Dye C., Harries A.D., Maher D., et al., *Tuberculosis*. In: Jamison D.T., Feachem R.G., Makgoba M.W., et al., Eds. (2006),.*Disease and Mortality in Sub-Saharan Africa*. 2nd Edition. Washington (DC): World Bank; 2006. Chapter 13. Retrieved on 18th June 2013 from: http://www.ncbi.nlm.nih.gov/books/NBK2285/

Fotheringham, A.S. (1992), *Exploratory Spatial Data Analysis and GIS, Environment and Planning;* 24: 1675-1678.

Fourie P.B. and Donald P.R. (1999), *Epidemiology of Tuberculosis*. In: Donald P.R., Fourie P.B., Grange J.M. (Eds.). *Tuberculosis in Childhood*. Proteria: Van Schaik's Publishers.

Frieden T., Sterling T.R., Munsiff S.S., Watt C.J. and Dye C. (2003), *Tuberculosis*. Lancet; 362: 887-99

Frieden T.R., Teklehaimanot A., Childeya S., Farmer P, Kim J.Y. and Raviglion M.C. (2009), *A Road Map to Control Malaria, Tuberculosis, and Human Immunodeficiency Virus/AIDS*. Archives of Internal Medicine; 169: 1650-1652.

Getis A. and Ord J.K. (1996), Local Spatial Statistics: An overview. In Longley P.and Batty M. (Eds), Spatial Anlaysis: Modeling in a GIS Environment, Geoinformation International, Cambridge. UK.

Getis A., Morrison A.C., Gray K. and Scott T.W. (2003), *Characteristics of the spatial pattern of the dengue vector, Aedes aegypti, in Iquitos, Peru***.** American Journal of Tropical Medicine and Hygiene; 69:494-505.

Getis, A. and Ord J.K. (1992), *The Analysis of Spatial Association by Use of Distance Statistics*, Geographical Analysis; 24, 189- 206.

Goldrick B.A. (2004), *Once dismissed, still rampant: tuberculosis, the second deadliest infectious disease worldwide.* American Journal of Nursing; 104:68–70.

Goodchild M.F. (1987), *Application of a GIS benchmarking and workload estimation model*. Papers and Proceedings of Applied Geography Conferences; 10: 1 - 6.

Gopinath K., and Singh S. (2010), *Non-tuberculosis Mycobacteria in TB-endemic Countries: Are we Neglecting the Danger?* PloS Neglected Tropical Diseases; 4: 615

Grange J.M. and Yates M.D. (1994), *Zoonotic Aspects of Mycobacterium bovis Infection*. Veterinary Microbiology Journal; 40: 137-151

Grange J.M. and Zumla A. (1999), *Tuberculosis and the Poverty – disease Cycle*. Journal of RS Med; 92: 105 – 107

Grange J.M. and Zumla A. (2008), *Bacterial Infections*, In Cook G.C. and Zumla A. Manson's Tropical Disease, 22nd Edition, WB Saunders, London, UK.

Gustafson P., Gomes V.F., Vieira C.S., Rabna P., Seng R., Johansson P., Sandstrom A., Norberg R., Lisse I., & Samb B., Aaby P. and Naucler A. (2004), *Tuberculosis in Bissau: incidence and risk factors in an urban community in sub-Saharan Africa*. International Journal of Epidemiology; 33:163–172.

Harling G., Ehrlich R., and Myer L. (2008), *The social epidemiology of tuberculosis in South Africa: A multilevel analysis*. Social Science and Medicine; 66: 492–505.

Hjalmars U., Kulldorff M., Gustafsson G. and Nagarwalla N. (1996), *Childhood Leukemia in Sweden: using GIS and a spatial scan statistic for cluster detection*. Statistics in Medicine; 15: 707-715.

Houtmeyers E., Gosselink R., Gayan-Ramirez G. and Decramer M. (1999), *Regulation of mucociliary clearance in health and disease*. The European Respiratory Journal; 13: 1177–1188.

Imaenda T. (1985), *Deoxyribonucleic Acid Relatedness among Selected Strains of Mycobacterium tuberculosis, Mycobacterium bovis BCG, Mycobacterium microti, and Mycobacterium africanum.* International Journal of Systematic Bacteriology; 35: 147-150

Jamison D.T., Breman J.G., Measham A.R., Alleyne G., CLaeson M., Evans D.B., Jha P., Mills A. and Musgrove P. (2006), *Priorities in Health*, World Bank, Washington DC, United States of America.

Jee S.H., Golub J.E., Jo J., Park I.S., Ohrr H. and Samet J.M. (2009), *Smoking and risk of Tuberculosis Incidence, Mortality, and Recurrence in South Korean men and women*. American Journal of Epidemiology; 170: 1478–85

Kallenius G., Koivula T., Ghebremichael S., Hoffner S.E., Norberg R., Svensson E., Dias F., Marklund B.L. and Svenson S.B. (1999), *Evolution and Clonal Traits of*

Mycobacterium tuberculosis Complex in Guinea-Bissau. Journal of Clinical Microbiology; 37: 3872-3878

Keet D.F., Kriek N.P.J., Penrith M-L., Micheal A. and Huchzermeyer H. (1996), *Tuberculosis in Buffaloes (Syncerus caffer) in the Kruger National Park*: Spread of the Disease to other Species. Onderstepoort Journal of Veterinary Research. 63: 239-244

Kistemann T. Munzinger A. and Dangendorf F. (2002), S*patial patterns of Tuberculosis Incidence in Cologne (Germany). Social Science and Medicine 55, 7–19.*

Kitron U., Micheal J., Swanson J. and Haramis L. (1997), *Spatial analysis of the Distribution of Lacrosse Encephalitis in Illinois, using a geographic information system and local and global statistics*. American Journal of Tropical Medicine and Hygiene; 145: 558 - 566

Kleeberg H.H., (1984), *Human Tuberculosis of Bovine Origin in Relation to Public Health*, Revue Scientifique et Technique (OIE); 3: 11−32.

Kreijcie R.V. and Morgan D.W. (1970), *Determining Sample for Research Activities:* Educational and Psychological Measurements; 30: 607 - 610

Kulldorf M., Heffernan R., Hartman J., Assuncao R. and Mostashari F. (2005), *A space-time permutation scan statistic for disease outbreak detection.* PLoS Medicine; 2: e59

Kumar V., Abbas A. Fausto K., N. and Mitchell R. N., (2007), *Robbins Basic Pathology*, Saunders Elsevier, 8th Edition, Philadelphia, Pa, USA.

Lee RB, Li W, Chatterjee D, Lee RE. (2005), *Rapid structural characterization of the arabinogalactan and lipoarabinomannan in live mycobacterial cells using 2D and 3D HR-MAS NMR*: structural changes in the arabinan due to ethambutol treatment and gene mutation are observed. Glycobiology; 15: 139–151.

Lienhardt C., Fielding K., Sillah J.S., Bah B., Gustafson P. and Warndorff D. (2005), *Investigation of the risk factors for tuberculosis: a case-control study in three countries in West Africa.* International Journal of Epidemiology; 34: 914 - 923.

Lin H., Ezzati M., Chang H. and Murray M. (2009), *Association between Tobacco Smoking and Active Tuberculosis in Taiwan: Prospective Cohort Study*. American Journal of Respiratory and Critical Care Medicine; 180: 475–480.

Lin H.H., Ezzati M. and Murray M. (2007), *Tobacco Smoke, Indoor air Pollution and Tuberculosis: A Systematic Review and Meta-analysis*. PLoS Medicine; 4: e20.

Lundazi District Community Medical Office [LDCMO], (2013), *Health Management Information System Data 2013*, Lundazi, Zambia

M'Imunya J. M., Kredo T. and Volmink J. (2012), *Patient education and counselling for promoting adherence to treatment for tuberculosis,* Cochrane Database of Systematic Reviews, (5)

Mahy B.W.J. and Murphy F.A. (1998), *Emergence and Re-emergence of Viral Infections*, In: Collier L., Balows A. and Sussman M. (Eds.). *Topley and Wilson's Microbiology and Microbial Infections*. Vol. 1. London, UK: Arnold; pp. 1011-1025

Mary M.L. and Eric D.K. (2004), *A multi-scale method for disease mapping in spatial epidemiology*, statistics in medicine; 00; 1-1

Medeiros L., Marassi C.D., Figueiredo E.E.S. and Lilenbaum W. (2010*), Potential Application of New Diagnostic Methods for Controlling Bovine Tuberculosis in Brazil*. Brazilian Journal of Microbiology; 41: 1-4

Michel A. L., (2003), *Tuberculosis Laboratory of the ARC,* Onderstepoort Veterinary Institute, South Africa

Moore, D.A. and Carpenter, T.E. (1999), *Spatial analytical methods and geographic information systems:* Use in health research and epidemiology. Epidemiologic Reviews; 21: 143-161.

Morrison AC, Getis A, Santiago M, Rigau-Perez JG and Reiter P (1998), *Exploratory space-time analysis of reported dengue cases during an outbreak in Florida*, Puerto Rico 1991-1992. American Journal of Tropical Medicine and Hygiene; 58: 287–298.

Munch Z., Van Lil S.W.P., Booysen C,N., Zietsman H.L., Enarson D.A. and Beyers N. (2003), *Tuberculosis Transmission Patterns in a high – Incidence area: a spatial analysis*. International Journal of Tuberculosis and Lung Disease; 7: 271 – 277

Munyeme M., Muma J.B. Siamudaala V. M. Skjerye E., Munang'andu H.M. and Tryland M. (2008), *Tuberculosis in Kafue Lechwe antelope (kobus lechwe Kafuensis) of the Kafue Basin in Zambia.* Preventive Veterinary Medicine Journal; 95: 305 – 308

Mwinga A., Hosp M., Godfrey-Fausset P., Quigley M., Mwaba P., Mugala B. N., Nyirenda O., Luo N., Pobee J., Elliot A. M., McAdam K. P.J.W., and Porter J. D.H. (1998). *Twice weekly tuberculosis preventive therapy in HIV infection in Zambia.* Lusaka, Zambia.

National Tuberculosis and Leprosy Programme Tanzania [NTLP] (1996), 2007, *Annual Report 1995*, Ministry of Health, Dare salaam, Tanzania

Park K., (2009), *Park TextBook of Preventive and Social Medicine*. 19th Edition, M/S Banarsidas Bhanot Publishers. India

Pfeiffer D.U., Robinson T.P., Stevenson M., Stevens K.B., Rogers D.J., and Clements A.C.A. (2008), *Spatial Analysis in Epidemiology*, Oxford University, New York, USA

Pfyffer G.E., Auckenthaler R., van Embden J.D., and van Soolingen D. (1998), *Mycobacterium canettii the Smooth Variant of M. tuberculosis, Isolated from a Swiss Patient Exposed in Africa*. Emerging Infectoius Diseases Journal; 4: 631-634

Philips C.J., Foster G.R., Morris P.A. and Teverson R. (2003), The transmission of *Mycobacterium bovis* Infection to cattle, Research in Veterinary Science Journal; 74: 1-15

Porth C.M. (2002), *Alterations in respiratory function: respiratory tract infections, neoplasms, and childhood disorders*. In: Porth CM, Kunert MP. Pathophysiology: Concepts of Altered Health States. Philadelphia, PA: Lippincott Williams & Wilkins: 615–619.

Raviglione M.C. (2003), *The TB Epidemic from 1992 to 2002.* Tuberculosis; 83: 4-14.

Raviglione M.C., Snider D.E. and Kochi A. (1995), *Global Epidemiology of Tuberculosis*: Morbidity and mortality of a worldwide epidemic. *JAMA*; 273: 220–226.

Richard E., Chaisson, M.D., Neil A., and Martinson, M.B., (2008), *Tuberculosis in Africa — Combating an HIV-Driven Crisis,* The New England Journal of Medicine; 358: 1089-1092.

Rieder H. L. (1999), *Epidemiologic Basis of Tuberculosis Control.* 1st Ed. Paris: International Union Against Tuberculosis and Lung Disease.

Rylance J., Pai M., Lienhardt C. and Garner P. (2010), *Priorities for Tuberculosis Research*: A systemic Review.

Sara P, Simon B, Hannah K, Silvio M, David M, and Allen, (2005), *Mapping the global distribution of trachoma*. Bulletin of the world health organization; 83 (12)

Schaeffer R.L., Mandenhall W. and Ott L. (1990), *Elementary Survey Sampling*, Youth Edition. Duxbury press, Belmont, California, USA.

Shajahan S. (2009), *Research Methods for Management*: 3rd Ed., Jaico Publishing House, Mumbai, India.

Shetty N., Shemko M., Vaz M. and D'Souza G. (2006), *An epidemiological evaluation of risk factors for tuberculosis in South India: a matched case control study*. International Journal of Tuberculosis and Lung Disease; 10: 80–86.

Stop T.B. (2002), *An Expected Framework for Effective Tuberculosis Control.* International Journal of Tuberculosis and Lung Disease; 6: 378-88

Stop TB Partnership and the WHO (2006), *The Global Plan to Stop TB: 2006–2015*. Geneva: World Health Organization; 2006: WHO/HTM/STB/2006.35.

Story A. (2004), *Tuberculosis – A General Introduction*. The Pharmaceutical Journal; 273: 289 – 291. Available at: www.pjonline.com

Tiwari N, Adhikari C M S, Tewari A, and Kandpal V. (2006), *Investigation of geospatial hotspots for the occurrence of tuberculosis in Almora District*, India, using GIS and spatial scan statistic. International Journal of Health Geographics; 5: 33–43.

Touray K., Adetifa I. M., Jallow A., Rigby J., Jeffries D., Cheung Y. B., Donkor S., Adegbola R. A. and Hill P. C. (2010), *Spatial Analysis of Tuberculosis in an Urban West African Setting*: is there evidence of clustering? Tropical Medicine and International Health; 15: 664–672.

United Nations Population Information Network (2003), *World Population Prospects: The 2002 Revision (Population Division)*. Accessed March 2013 at http://www.un.org/popin/data.html.

Uplekar M., Rangan S. and Ogden J. (1999), *Gender and tuberculosis control: towards a strategy for research and action*, WHO/TB/2000.280. Geneva: World Health Organization.

Uplekar M.W., Rangan S., Weiss M.G., Ogden J., Borgdorff M.W. and Hudelson P. (2001), *Attention to gender issues in tuberculosis control.* International Journal of Tuberculosis and Lung Disease; 5: 220–224.

Uthman O.A. (2008), S*patial and temporal variations in incidence of tuberculosis in Africa 1991 to 2005.* World Health and Population; 10: 5–15.

van Soolingen D., van der Zanden A.G.M., de Haas P.E.W. et al., (1998), *Diagnosis of Mycobacterium microti Infections among Humans by using Novel Genetic Markers*. Journal of Clinical Microbiology; 36: 1840-1845

Veterinarian Without Boarders [VWB] (2010), *One Health for One World:* A compendium of Case Studies; Canada

Walsh S.J. and Fenster J.R. (1997), *Geographical Clustering of Mortality from Systematic Sclerosis in the Southeastern United States 1981-90*. Journal of Rheumatology; 24: 2348-2352

Wang T., Xue F., Chen Y., Ma Y. and Liu Y. (2012), *The spatial epidemiology of tuberculosis in Linyi City*, China.

Ward H.A. (2004), *Risk Factors in the Progression from Tuberculosis Infection to Disease*, Master of Science Thesis, University of Saskatchewan.

WHO (2006), *Global Tuberculosis Control: Surveillance, Planning, Financing*. WHO Report. Geneva: World Health Organization; 2006: WHO/HTM/TB/2006.34962.

Wood R. (2007), Challenges of TB Diagnosis and Treatment in South Africa, Roche Symposium 3rd South African AIDS Conference, Durban, South Africa

World Health Organization (2004), *Global Tuberculosis Control-WHO Report*. WHO/HTM/TB/2004.331

World Health Organization (2005), *TB Emergency Declaration issued by WHO Regional Office for Africa:* WHO report, Retrieved on 25th June 2013: www.who.int/tb/featuresarchive/tbemergencydeclaration/en/indexhtml

World Health Organization (2005). *Media center fact sheet. Tuberculosis*. Retrieved from: www.who.int/mediacentre/factsheets/fs104/en

World Health Organization (2006), *The Stop TB strategy: building on and enhancing DOTS to meet the TB-related millennium development goals.* Geneva, Switzerland: WHO; Available at http://www.who.int/tb/strategy/en/index.html.

World Health Organization (2009), *Global tuberculosis control: epidemiology, strategy, financing WHO report 2009*. Geneva. Retrieved on 9th November 2013 from *http://whqlibdoc.who.int/publications/2009/9789241598866_eng.pdf*

World Health Organization (2013), *Tuberculosis Media center fact sheet No. 104*. Retrieved July 18th, 2013 from: www.who.int/mediacentre/factsheets/fs104/en

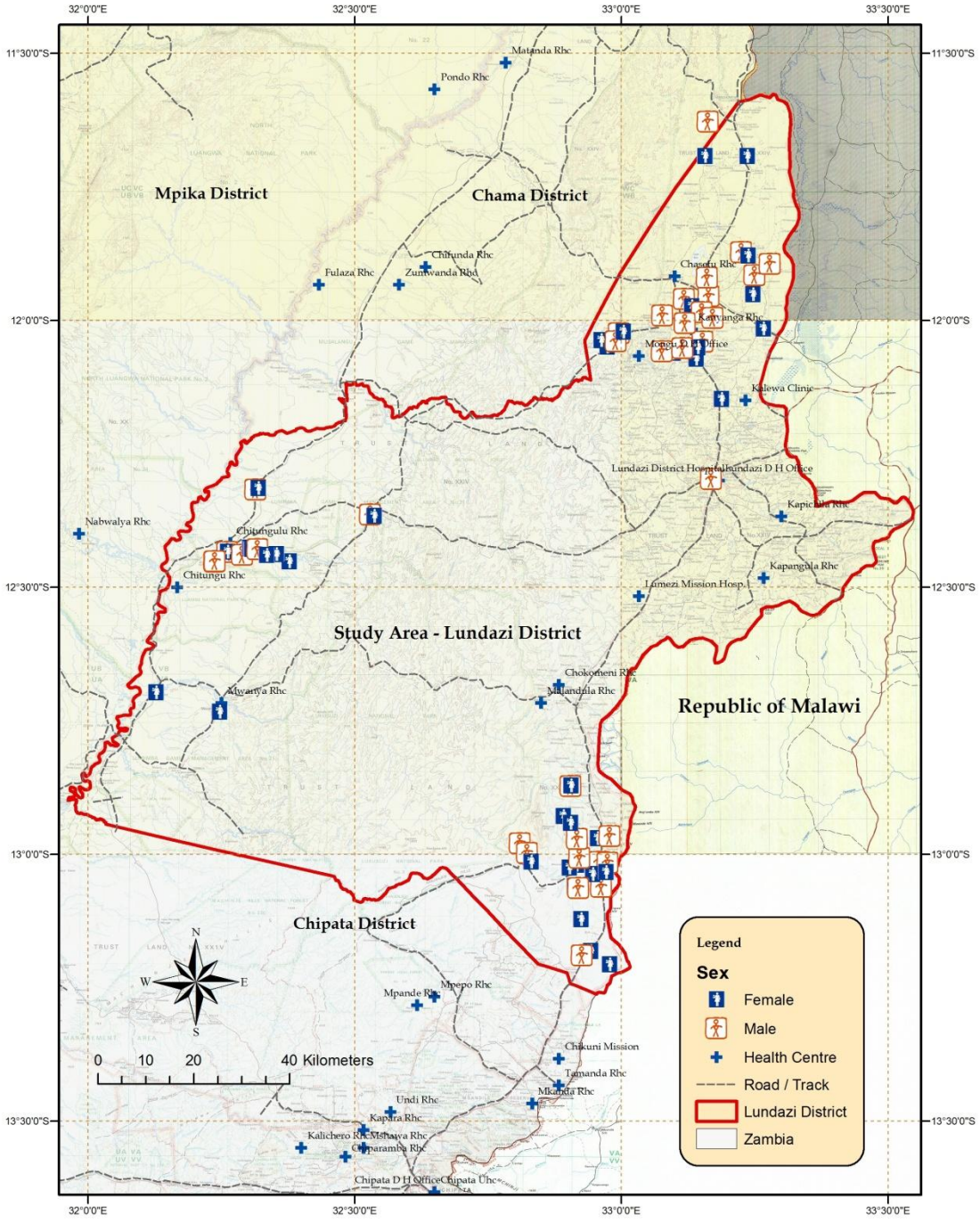
World Health Organization Report (2001), *Global Tuberculosis Control*. WHO/CDC/TB/2001.287. Geneva: WHO, 2001

World Health Organization report (2007), *Global Tuberculosis Control: Surveillance, Planning, Financing*. Geneva: World Health Organization, 2007. (WHO/HTM/TB/2007.376.)

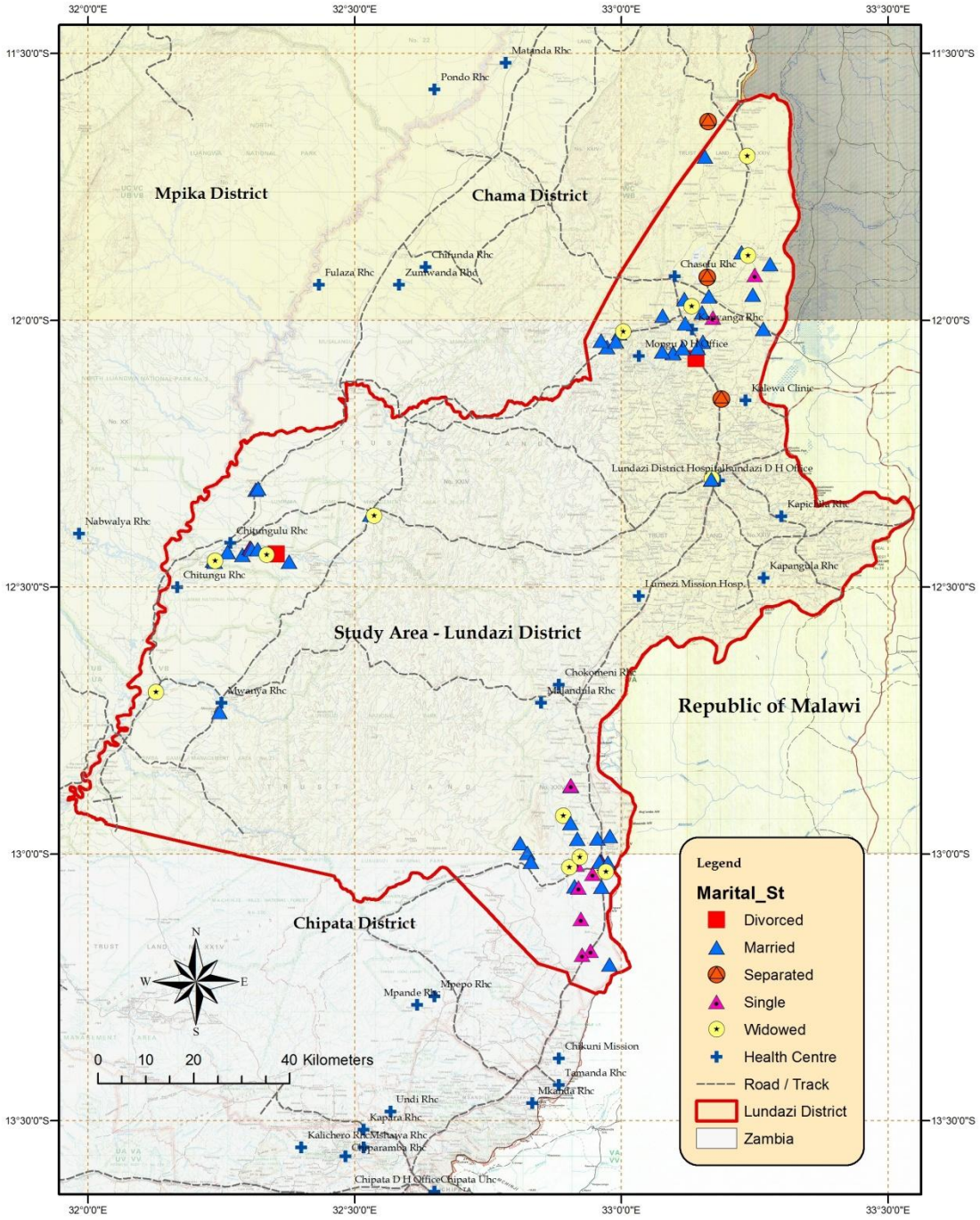
World Health Organization report (2012), *Global Tuberculosis Report 2012*. Geneva: World Health Organization, 2012. (WHO/HTM/TB/2012.6)

Annexes

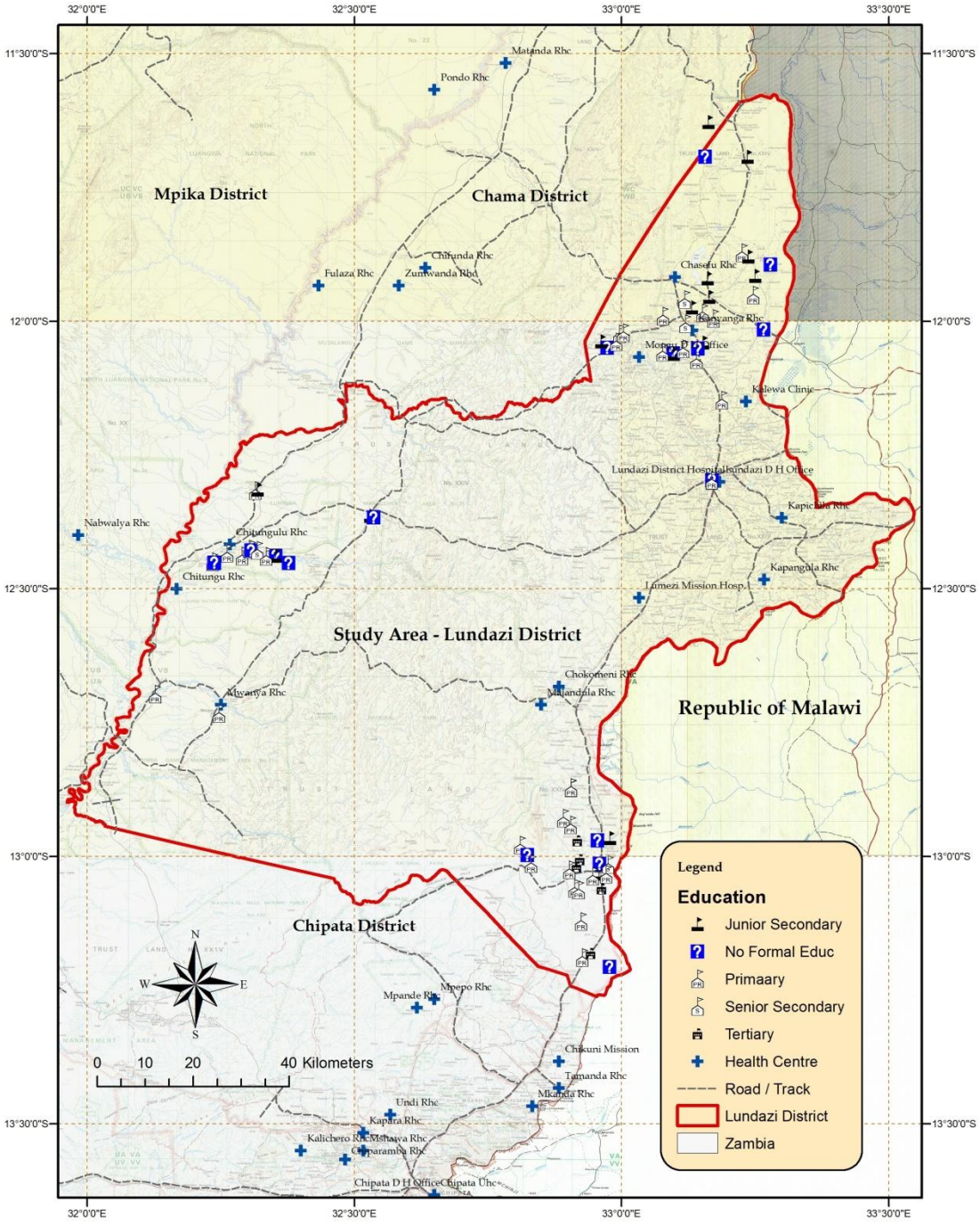
Annex 1: Spatial Distribution in Relation with Sex



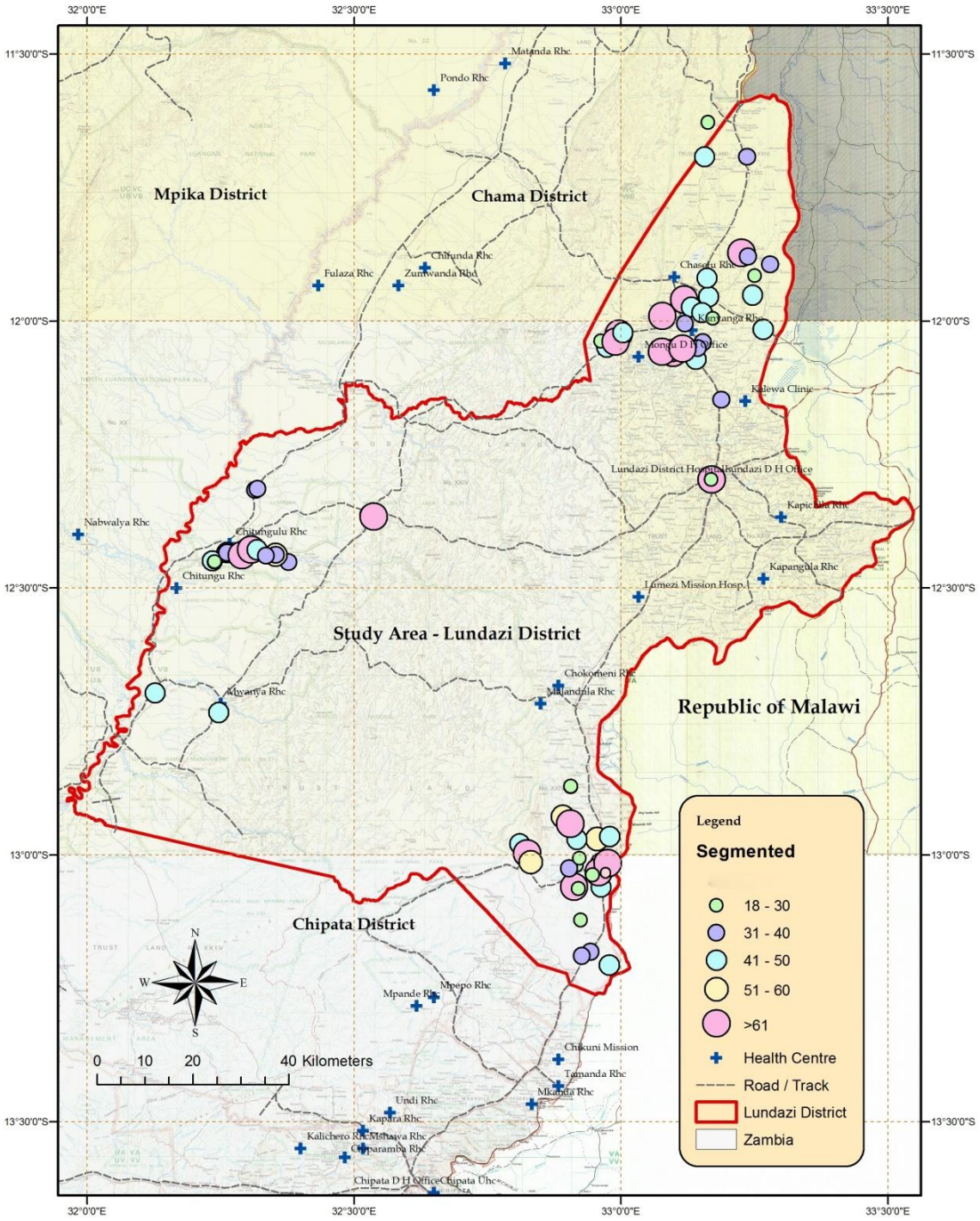
Annex 2: Spatial Distribution in Relation to Marital Status



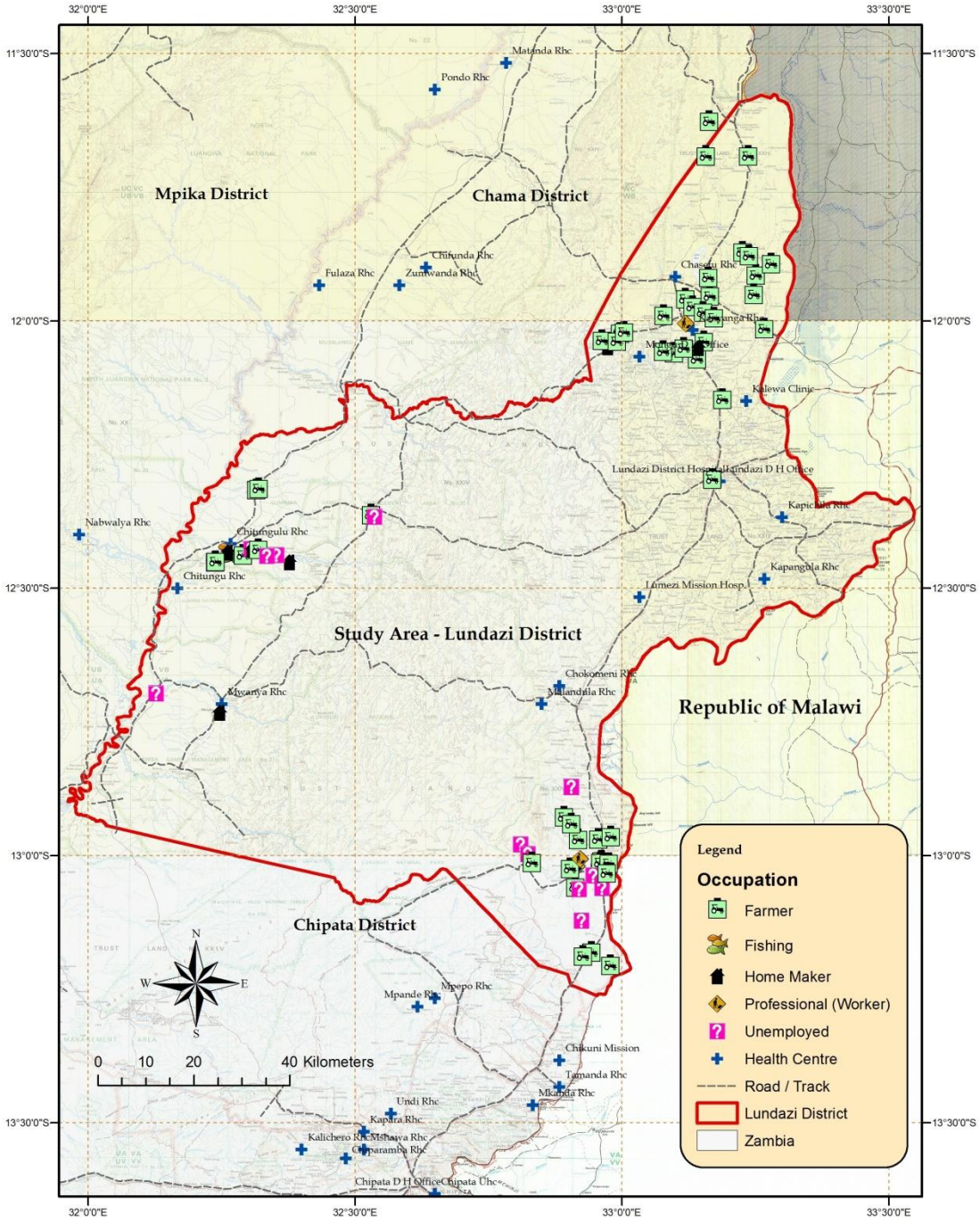
Annex 4: Spatial Distribution in Relation to Education Level



Annex 5: Spatial Distribution in Relation to Segmented Age



Annex 6: Spatial Distribution in Relation to Occupation



Annex 7: Logistic Regression Results from STATA analysis

Regression Total TB Patients: against Age, Previous contact with TB Patients, Number of Rooms, Age at start of Smoking, Number of TB Pts, and Number of Cigarettes smoked

Source	SS	df	MS	Number of obs =	56
				F(5, 50) =	327.45
Model	55.6573982	5	11.1314796	Prob > F =	0.0000
Residual	1.69974465	50	.033994893	R-squared =	0.9704
				Adj R-squared =	0.9674
Total	57.3571429	55	1.04285714	Root MSE =	.18438

TotalTBPts	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
Age2	.0046698	.0174815	0.27	0.790	-.0304429-	.0397824
Previous Cont. TB pts.	.9828219	.0259063	37.94	0.000	.9307876	1.034856
Number of Rooms	-.0331114	.0211158	-1.57	0.123	-.0755238	-.0093009
Age start Smoking	-.0032687	.0028164	-1.16	0.251	-.0089255	-.0023882
Cigarette smoking	.0761094	.0260517	2.92	0.005	.0239418	.1282771
Number of Cigarette	-.0011614	.0069181	-0.17	0.867	-.0150569	.012734
_cons	1.073062	.0722946	14.84	0.000	.9278543	1.21827

Regression Analysis on Total TB Pts and Number of cigarette smoking

Source	SS	df	MS	Number of obs = 59
				F(1, 57) = 8.54
Model	7.78767169	1	7.78767169	Prob > F = 0.0050
Residual	52.0089385	57	.912437517	R-squared = 0.1302
				Adj R-squared = 0.1150
Total	59.7966102	58	1.03097604	Root MSE = .95522

Total TB Pts.	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
Cigarette smoking	.0761094	.0260517	2.92	0.005	.0239418	.1282771
_cons	1.44265	.1394704	10.34	0.000	1.163365	1.721935

Annex 8: Questionnaire

Part I.
Semi – Structured Questionnaire on Spatial Analysis of Tuberculosis in Lundazi District

Introduction and Consent

Hello. My name is _____ and I am working with _____. We are conducting a research to get a better understanding of the distribution of tuberculosis in the community so that it can help us improve health services for TB patients and TB suspects. Your household was selected at random and I would like to ask you some questions pertaining to the care you received and the information you know about TB. The questions in the interview do not ask anything private and you can choose not to answer any question if you so wish. Let me state that your answers will remain strictly confidential and I will not take down your name or address so that no one will know who gave us these answers. There are no correct and wrong answers, each of your answers will depend on your views and situation. Participation in this research is completely voluntary.

This interview will take about 30 to 45 minutes of your time. However, I hope you will participate in the research since your views are important.

At this time, do you want to take part in this research?

RESPONDENT AGREES	:	1→ Go ahead with interview
RESPONDENT DOES NOT AGREE	:	2→ End interview

Notes to Interviewer

Remember to ask all questions unless the questionnaire tells you to skip questions or move to another section. All answers to pre-coded questions must be coded by circling

the correct response. Where you see open-ended questions, you are required to write in the response.

IDENTIFICATION		
1	Identification number of Respondent	☐☐☐
2	District:	_____
3	Chief:	_____
4	Compound/Village:	_____
5	Date of Interview:	☐☐/☐☐/☐☐
6	Name of interviewer:	_____

No	Questions and filters	Responses	For official use only
SECTION A – Background Information			
7	Gender of respondent	Male…….....1 Female…….....2 [ ]	
8	What is your age as at last birth day?	1. ..... Years 99. Refused to disclose [ ]	
9	What is your marital status?	Single……...…..1 Married…….…..2 Divorced…….....3 Widowed……....4 Separated……....5 [ ] Others (Specify).....	
10	What is your level of education?	Never went to school….....1 Primary…….....2 Junior secondary……...….....3 Senior Secondary……...…....4 Tertiary…….....…..5 [ ]	

11	What is your occupation?	Farmer……..….....1 Unemployed.....2 Home maker.....3 Business person.....4 Fishing …….....5 [ ] Others Specify.....	
12	On a monthly average, how much do you earn as an income?	ZMK ……/Month	
SECTION B – Previous Medical History			
13	Apart from you, is there any other family member who ever suffered from TB?	Yes…….1 No……..2 [ ]	
14	If yes how many are they?	…….	
Section C – Housing Condition/Standard			
15	Record type of the house the respondent lives in?	Grass and poles…….....….1 Mud and poles……...2 Mud and unbaked bricks……...3 Mud and baked bricks……...4 Cement and bricks…… …5 [ ] Other specify ……..	
16	Record Material of the Roof	Grass/thatching ……1 Iron Sheets ……2 Plastics ……..3 [ ] Others …….	
17	Record Type of windows	Type …… Approximate Size……	
18	How many Rooms are there in your house? (# of rooms)	Indicate No: ..... [ ]	
19	How many people,	Indicate Total No: …… [ ] Segregate as follows:	

	including you, live in this house?	Males:…… Females:…… Children:……	
20	Approximately how many people sleep in each sleeping space?	……..	
SECTION D – SOCIO – CULTURAL FACTORS **(Now will discuss issues of Smoking and Other Risk factors).**			
21	Have you or any of your household members ever smoked tobacco? (If no Go to Question on Alcohol)	Yes ……..1 No ……..……2 [ ]	
22	How old were you or he /she when you started smoking?	Indicate age ……..	
23	What type of tobacco do you use	Chewable Snuff Cigarettes Tradition	
24	How many cigarettes or tobacco do you use in a day? (state in terms of cigarettes)		
Section E – Alcohol use to be completed by all			
25	Have you ever consumed alcohol? (***If answer is no go to Section G***)	Yes……1 No…….2 [ ]	
26	Do you Drink alcohol now? (***If your answer is 'No' go to section G***)	Yes……1 No…….2 [ ]	
27	What type of beer do you drink? (state the actual name)		
28	Do you usually smoke more when you are drinking?	Yes……1 No…….2 [ ] Unsure …….3	

SECTION F – Access to Diagnosis			
29	How far is you home from this health facility? (Distance in terms of Time or Distance	Hours..... Kilometers…….	
30	Is there any other health facility closer to your Home??	Yes……..1 No……...2 [ ]	
31	Is the center easy to get to (Accessibility/convenience of transport)??	Yes……..1 No……...2 [ ]	
32	Do you have to pay for transportation to get to the health center?	Yes .....…….1 No .....…….2 [ ]	
SECTION G – History of Contact with Animals			
33	Do you own cattle	Yes……..1 No……...2 [ ]	
34	Have you previously owned cattle (Livestock)?	Yes……..1 No……...2 [ ]	
35	Do you usually consume raw milk from cattle?	Yes……..1 No……...2 [ ]	

This is the end and I would like to thank you very much for participating in this interview

Annex 9: GPS Data Collection Form

Consent

Before taking the GPS readings, Obtain permission from the Household head and explain the use of the GPS readings.

1. Date of Visit — Day | Month | Year

2. Record Time GPS readings Taken — :

3. Record Whether Permission was received from the Household — 1 …...…….Yes; 2……No

4. District _____ Code

5. Rural Health Center _____ Code

6. Chief/Chieftainess's Area _____

7.Village or Locality _____

8. Identification No. of Household _____

9. GPS receiver Number

10.Cluster Number

11. Way Point Number

12. Latitude *in Decimal Places* — N/S : o

13. Longitude *in Decimal Degrees* — E/W : o

14. Elevation m

15. Accuracy m

Annex 10: Ethics Approval Letter

ERES CONVERGE

33 Joseph Mwilwa Road
Rhodes Park, Lusaka
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 966 765 503
Email: eresconverge@yahoo.co.uk

I.R.B. No. 00005948
E.W.A. No. 00011697

13th August, 2014

Ref. No. 2014-May-004

The Principal Investigator
Mr. Victor Cheelo
Lundazi District Community Medical office
P.O. Box 530013,
LUNDAZI.

Dear Mr. Cheelo,

RE: Study of spatial distribution of Tuberculosis among human population in Lundazi District.

Reference is made to your corrections dated 11th June 2014. The IRB members resolved to approve this study and your participation as Principal Investigator for a period of one year.

Review Type	Ordinary	Approval No. **2014-Mar-007**
Approval and Expiry Date	Approval Date: 13th August, 2014	Expiry Date: 12th August, 2015
Protocol Version and Date	Version-Nil	12th August, 2015
Information Sheet, Consent Forms and Dates	• English.	12th August, 2015
Consent form ID and Date	Version-Nil	12th August, 2015
Recruitment Materials	Nil	12th August, 2015
Other Study Documents	Survey Questionnaire.	12th August, 2015
Number of participants approved for study	60	12th August, 2015

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- ERES Converge IRB does not "stamp" approval letters, consent forms or study documents unless requested for in writing. This is because the approval letter clearly indicates the documents approved by the IRB as well as other elements and conditions of approval.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB

Dr. E. Munalula-Nkandu
BSc (Hons), MSc, MA Bioethics, PgD R/Ethics, PhD
CHAIRPERSON