THE EPIDEMIOLOGY OF TUBERCULOSIS IN THE BALIMO REGION OF WESTERN PROVINCE, PAPUA NEW GUINEA

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Nature of assistance	Contributions of others
Supervision and other advisement	This degree was supervised by A/Prof Jeffrey Warner (primary advisor) and Prof David Plummer (secondary advisor) for the full duration. Supervision was also provided by Prof Patricia Graves (July 2015 – October 2017) and Prof Emma McBryde (September 2016 to completion).
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Project support	Implementation of the project in Balimo was supported by Mr Daniel Pelowa, Sister Bisato Gula, and Mr Robert Dowi. Results presented in this study are the outcomes of four visits to Balimo. The first hospital visit was undertaken by myself, Prof David Plummer, and A/Prof Jeffrey Warner in April 2016, and accommodation, airport transfers, and other logistical arrangements were organised by Mr Daniel Pelowa. The second hospital visit was undertaken by myself in February 2017, and arrangements and accommodation were provided by Sister Bisato Gula and Mr Daniel Pelowa. The third hospital visit was undertaken by A/Prof Jeffrey Warner in June 2017, and arrangements and accommodation were provided by Mr Daniel Pelowa. The fourth hospital visit was undertaken by myself and A/Prof Jeffrey Warner in November 2017, and arrangements and accommodation were provided by Mr Daniel Pelowa and Sister Bisato Gula.
Chapter 3	The TB register data was recorded by health workers in Balimo, and then transcribed into Microsoft Excel by myself. Nomenclature was discussed with Sister Bisato Gula and Mr Daniel Pelowa. I undertook the data analysis, with statistical advisement from Adj/Prof Patricia Graves and Prof Emma McBryde. Further information regarding contributions to this chapter, in the form of contributions to the published manuscript, are described in the next section (Contribution of Co-Authors in Manuscript Publication).

Chapter 4	Laboratory assays were undertaken by a number of people with results reported for this study, as follows: - Sputum processing: Mr Daniel Pelowa, Dr Vanina Guernier - Smear microscopy: Mr Daniel Pelowa - DNA extraction and qPCR diagnostics: Dr Vanina Guernier I undertook the conventional PCR drug resistance characterisation assays (including optimisation) and sequencing analysis. Results of the assays were discussed with Dr Graham Burgess and Dr Vanina Guernier.
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Chapter 6	The JCU laboratory TB database was initially developed by Dr Marshall Feterl, and later maintained with results recorded by myself. The Balimo laboratory database is maintained by Mr Daniel Pelowa. Laboratory assays were undertaken by a number of people with results reported for this study, as follows: - Sputum processing: Mr Daniel Pelowa, Dr Vanina Guernier - Smear microscopy: Mr Daniel Pelowa - TB culture: Dr Marshall Feterl, Ms Sandra Pollard, Dr Vanina Guernier, A/Prof Jeffrey Warner - DNA extraction: Dr Marshall Feterl, Dr Vanina Guernier - qPCR diagnostics: Dr Morgane Moreau, Dr Vanina Guernier, Mr Munish Puri, myself - Drug resistance characterisation PCR: Dr Morgane Moreau, myself I collated and cross-matched the records in the two laboratory databases, and undertook the analyses. Advice on statistical analysis was provided by Prof Emma McBryde.

Chapter 7	Recording of participant data was undertaken by health workers in Balimo and myself. I transcribed all data into a Microsoft Excel spreadsheet. Blood samples were collected by Mr Daniel Pelowa, A/Prof Jeffrey Warner, and Sister Bisato Gula, with assistance from myself. Approximately half of the IGRA plate runs were undertaken onsite in Balimo by Mr Daniel Pelowa and A/Prof Jeffrey Warner. The remaining plate runs were undertaken at JCU in Townsville and were performed by myself, with some assistance from A/Prof Jeffrey Warner and Dr Vanina Guernier. I undertook all statistical analyses of the laboratory data. Results of the IGRA assays were discussed with A/Prof Catherine Rush, and advice on data analysis was provided by A/Prof Jeffrey Warner.
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CONTRIBUTION OF CO-AUTHORS IN MANUSCRIPT PUBLICATION

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Contributions of authors:

TD-E, PG, DPlummer and JW conceived and designed the study. TD-E collated, transcribed and analysed the data, developed figures and tables, and wrote the manuscript. PG and EM advised on statistical analyses; and PG, RD, BG, DPlummer, EM, DPelowa, and JW provided feedback on the analysis and results. All authors reviewed the manuscript and provided critical feedback on the content.

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I confirm the candidate's contribution to the above paper and consent to the inclusion of the paper in this thesis.

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DECLARATION OF ETHICS

The research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council (NHMRC) National Statement on Ethical Conduct in Human Research, 2007.

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ABSTRACT

Papua New Guinea (PNG) is a country of great diversity. The landscape and geography ranges from volcanic and mountainous highlands to coastal and floodplain lowlands, as well as more than 600 islands. There are more than 800 cultural and language groups countrywide, with more than 80% of the population living in rural areas (National Research Institute 2010). In 2016, tuberculosis (TB) in PNG was estimated to have caused more than 30,000 cases countrywide (World Health Organization 2017a), with control challenged by the emergence of drug resistance, and complicated by a resource-limited health system. The situation faced by PNG due to TB and drug-resistant TB (DR-TB) has been described as an emergency (Eccles 2016; IRIN 2010).

Research from regional settings in PNG has described a TB burden much higher than the national average, including in Western Province where this study was based (Aia et al. 2018; Cross et al. 2014; McBryde 2012). However, there is limited epidemiological data from areas outside the South Fly and provincial capital of Daru, despite evidence of a heavy TB and drug-resistant TB (DR-TB) burden (Aia et al. 2016; Furin & Cox 2016; Kase et al. 2016; McBryde 2012). The research presented in this thesis describes and characterises the distribution and determinants of TB in the rural Balimo region of Western Province. It is hoped that this study will provide epidemiological data about TB in the region that can inform local TB control strategies.

This study used retrospective data analysis and laboratory techniques to describe TB in the Balimo region. The Balimo District Hospital (BDH) TB patient register was analysed, and laboratory diagnostic results were examined. A molecular assay was used to describe genetic evidence of DR-TB, and an interferon (IFN)-ɣ release assay (IGRA) was used to investigate the population-level latent TB burden and *Mycobacterium tuberculosis* complex (MTBC) infection in TB patients.

A very high burden of TB was identified, with an estimated incidence of 727 TB cases per 100,000 people per year. Approximately 25% of TB patients were children, and more than

75% of all patients had extrapulmonary TB. Spatial analysis of TB across the region identifed a high TB burden in the area immediately surrounding Balimo. Lower rates of TB were identified in more remote areas, likely due to the challenges faced by people from rural regions in accessing health facilities. However, the spatial analysis also provided evidence of potential under-diagnosis of TB in more remote areas.

DR-TB was described in the region, based on molecular evidence of rifampicin (RIF) resistance. In addition, the challenges of diagnosing TB in this region, where laboratory facilities are limited, are highlighted based on validation of the smear microscopy method used at the BDH laboratory. In combination, these results emphasise the importance of implementing a molecular method of TB diagnosis and detection of drug resistance in the Balimo region.

Given the high burden of TB described in the Balimo region, an unexpectedly low proportion of latent TB was identified. Analysis of the IGRA results provided support for the accuracy of extrapulmonary TB diagnoses at BDH, which is important given more than 90% of the extrapulmonary TB diagnoses in the TB register analysis were made clinically based only on presenting signs and symptoms.

Overall, this study emphasises the very high burden of TB present in the Balimo region. The results highlight the critical importance of investing in the hospital at Balimo, and ensuring that resources and facilities at the hospital are of a high standard adequate for accurate diagnosis and effective management of TB in the region. This need is especially important in the context of a region where other infectious diseases are also endemic. As a broad epidemiological study of TB in the Balimo region, the results presented in this thesis contribute important contextual information and baseline data on which future TB control efforts can be based.

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ABBREVIATIONS

+LR	positive likelihood ratio
-LR	negative likelihood ratio
µL	microlitre
µM	micromolar
A/Prof	Associate Professor
ACSM	advocacy, communication, and social mobilisation
Adj	adjunct
AITHM	Australian Institute of Tropical Health and Medicine
APA	Australian Postgraduate Award
avg	average
BCG	Bacille Calmette-Guérin
BDH	Balimo District Hospital
bp	base pair
C	Celsius
CAS	Central Asian
CBMDT	Centre for Biodiscovery and Molecular Development of Therapeutics
CBTID	Centre for Biosecurity and Tropical Infectious Diseases
CHW	community health worker
CI	confidence interval
CNR	case notification rate

Cq	cycle threshold
df	degrees of freedom
DMS	degrees-minutes-seconds
DNA	deoxyribonucleic acid
DOT	direct observation of treatment
DOTS	directly observed treatment, short-course
Dr	Doctor
DR-TB	drug-resistant tuberculosis
DST	drug susceptibility testing
EAI	East-African-Indian
ECPNG	Evangelical Church of Papua New Guinea
ELISA	enzyme-linked immunosorbent assay
EMB	ethambutol
EPTB	extrapulmonary tuberculosis
FDC	fixed dose combination
FLQ	fluoroquinolone
GIT	guanidine isothiocyanate
GNI	gross national income
HC	health centre
HDI	human development index
HEO	health extension officer
HEW	health extension worker
HIV	human immunodeficiency virus

IFN	interferon
IFN-ɣ	interferon-gamma
IGRA	interferon-gamma release assay
INH	isoniazid
IS	insertion sequence
IU/mL	international units per millilitre
JCU	James Cook University
LAM	Latin American-Mediterranean
LLG	local level government
LLR	log likelihood ratio
M	molar
MDR	multidrug-resistant
MDR-TB	multidrug-resistant tuberculosis
MGIT	mycobacteria growth indicator tube
mL	millilitre
MRAC	Medical Research Advisory Committee
MTB	*Mycobacterium tuberculosis*
MTBC	*Mycobacterium tuberculosis* complex
n	number
n/a	not applicable
NAAT	nucleic acid amplification test
NAFB	no acid-fast bacilli
NCD	National Capital District

neg	negative
NHMRC	National Health and Medical Research Council
NGO	non-governmental organisation
nm	nanometre
No.	number
NPV	negative predictive value
NTM	non-tuberculous mycobacteria
OD	optical density
OR	odds ratio
p	p-value
PANTA	polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin
PC3	physical containment level 3
PCR	polymerase chain reaction
PCT	patient centred treatment
PICT	provider-initiated counselling and testing
PNG	Papua New Guinea
pos	positive
PPV	positive predictive value
Prof	Professor
PZA	pyrazinamide
QFT-GIT	QuantiFERON-TB Gold In-Tube
QFT®-Plus	QuantiFERON®-TB Gold Plus
QMRL	Queensland Mycobacterium Reference Laboratory

qPCR	real-time polymerase chain reaction
RIF	rifampicin
RIF DR	rifampicin drug resistance
rpm	revolutions per minute
RR	relative risk
RRDR	rifampicin-resistance determining region
RR-TB	rifampicin-resistant tuberculosis
RTP	Research Training Program
SDG	sustainable development goals
SIR	standardised incidence ratio
SNP	single nucleotide polymorphism
STR	streptomycin
TB	tuberculosis
TDR	totally drug-resistant
TDR-TB	totally drug-resistant tuberculosis
TNF	tumour necrosis factor
TST	tuberculin skin test
tx	treatment
UK	United Kingdom
USA	United States of America
USD	United States dollar
WHO	World Health Organization
WT	wild-type

X	Chi
XDR	extensively drug-resistant
XDR-TB	extensively drug-resistant tuberculosis
XXDR	extremely drug-resistant
XXDR-TB	extremely drug-resistant TB
YLD	years of healthy life lost due to disability
YWAM	Youth with a Mission
ZN	Ziehl-Neelsen

CHAPTER 1

GENERAL INTRODUCTION

Tuberculosis (TB) is an ancient disease, with both archaeological and molecular evidence of TB occurring in Egypt more than 5000 years ago (Cave 1939; Daniel 2006; Nerlich et al. 1997; Zimmerman 1979; Zink et al. 2003). In more recent times, but before the advent of antibiotic-based treatment, one of the best-known approaches to the management of TB was the sanatorium. These facilities promoted TB care through exposure to the open air, exercise, and a healthy diet (Murray et al. 2015). Later public health measures included the introduction of the Bacille Calmette-Guérin (BCG) vaccine in 1921 (Daniel 2006). However, chemotherapeutic management of TB was only developed relatively recently, with the introduction of streptomycin (STR) in 1944 (Daniel 2006). TB treatment is effective with a treatment success rate greater than 85% (and frequently higher) for drug-susceptible cases (World Health Organization 2017a). The development of drug-resistant TB (DR-TB) is therefore of critical importance, as it poses numerous challenges to TB control, requiring more expensive medications and longer treatment durations. Furthermore, treatment efficacy is lower for DR-TB strains, with treatment success rates in 2014 of only 54% globally for multidrug-resistant TB (MDR-TB) (resistance to rifampicin (RIF) and isoniazid (INH)) and rifampicin-resistant TB (RR-TB) (World Health Organization 2017a).

In a social context, TB is known to disproportionately affect vulnerable populations, including certain ethnic groups, prisoners, homeless people, refugees, and people living in lower socioeconomic settings (Centers for Disease Control and Prevention 2016; Figueroa-Munoz & Ramon-Pardo 2008). As a result, people living in countries with decreased socioeconomic levels, measured by indicators such as the human development index (HDI), as well as vulnerable groups situated within higher HDI countries, face an increased risk of TB. Additionally, such settings face a double challenge – control and management of drug-susceptible TB, as well as growing burdens of DR-TB.

Papua New Guinea (PNG) is a lower-middle-income country (based on 2018 fiscal year classifications), defined as having gross national income (GNI) of USD 1006 – 3955 per capita, and is ranked 154 (out of 188) on the HDI (United Nations Development Programme 2016; World Bank Group n.d.). PNG is also a high-burden country for TB, with incidence estimated to be 432 cases per 100,000 people per year (World Health Organization 2017a). DR-TB is considered to be high-burden, officially estimated to affect 3.4% of new TB patients, although other research has found higher rates in some settings (Aia et al. 2016; Gilpin et al. 2008; Simpson et al. 2011; World Health Organization 2017a). By comparison, Australia, which is one of PNG's nearest neighbours, is a high-income country (GNI of USD 12,236 or more per capita), ranked at 2 on the HDI (United Nations Development Programme 2016; World Bank Group n.d.). Australia has an estimated TB incidence of only 6.1 cases per 100,000 people per year, and DR-TB in 3.6% of new TB patients (World Health Organization 2018c). Furthermore, local transmission of TB is very low, with approximately 86% of TB patients diagnosed in Australia having been born overseas (Toms et al. 2017).

It is thought that TB became established in PNG in the late 1800s and early 1900s (Ley et al. 2014b). The first national TB control program using modern treatment was implemented in the 1950s, and management of TB across PNG became the responsibility of Dr. S. C. Wigley in 1957, who remained the specialist medical officer for TB for more than 20 years (Kennedy ca.1978; Levy et al. 1998; Ley et al. 2014b; Wigley n.d.). In the 1970s, a national policy for TB management was developed, in consultation with the World Health Organization (WHO) (Kennedy ca.1978). This policy outlined a plan for TB control and management, through case-finding, treatment, health education, contact tracing, and immunisation; and included clear and specific instructions for the recording and management of TB patients (Kennedy ca.1978).

Despite the long history of TB in PNG, there continues to be limited published data describing the TB burden in many settings. Additionally, there is limited insight into the factors that influence TB epidemics in local settings. These factors must be understood in the context of the geographic and cultural diversity that defines PNG – a country with a mainland area ranging from volcanic and mountainous highlands to coastal and floodplain lowlands, as well as about 600 islands located to the north, south, and east of the mainland

(Commonwealth 2018) (Figure 1.1). Furthermore, the country is populated by more than 800 different language groups, with 88% of people living in rural areas (Commonwealth 2018; National Statistical Office 2015). This diversity emphasises the importance of understanding TB in a local context.

Figure 1.1: The Fly River region in the South Fly District of Western Province, PNG. (Image credit: Tanya Diefenbach-Elstob)

For Western Province, a number of studies have described various aspects of the epidemiology of TB (Aia et al. 2016; Bainomugisa et al. 2018; Gilpin et al. 2008; Guernier et al. 2018; McBryde 2012; Pandey et al. 2018; Simpson et al. 2011). This data has described the substantial burden of TB in the province, with incidence higher than the estimated national average (McBryde 2012). The provincial capital of Daru is in the South Fly District, and is located on an island to the south of the mainland. Daru is the site of the referral hospital for presumptive DR-TB patients in the province (Department of Health 2011), and has a very high burden of DR-TB (Aia et al. 2016; Furin & Cox 2016; Kase et al. 2016). However, as the provincial capital, the island of Daru has an urban and more transient population. Additionally, the population dynamics and source of flow of TB patients into the

provincial capital has not been described. The lack of comprehensive studies from the North and Middle Fly districts of the province have resulted in a situation where the broader provincial TB burden is uncertain, and the impact of TB cases from other regions on Daru is unknown.

This research presented in this thesis will describe and characterise TB in the Balimo region, the urban centre of the Middle Fly District of Western Province. TB in the region is managed at Balimo District Hospital (BDH), a rural hospital with no resident physician, and limited diagnostic laboratory facilities. TB is known to occur in this region, and there is evidence that the burden of disease is substantial (McBryde 2012). Research from our group has identified potential under-diagnosis of TB, particularly for pulmonary smear-negative TB patients (Guernier et al. 2018). Furthermore, our earlier work has described numerous barriers and facilitators of TB treatment adherence, although many of these factors are influential throughout the entire TB journey (Diefenbach-Elstob et al. 2017). In this region, it is important to understand the burden of TB, but also to appreciate the context in which TB occurs, as understanding these factors will help to guide the design and implementation of TB control measures that are appropriate for this setting. This research will address the epidemiological knowledge gap of TB in the Balimo region, placing it in the context of social, cultural, behavioural, and geographic factors. Taking this broad approach is important to provide data that is setting-specific and appropriate to local TB management, and also to further understanding of the impact of TB from the Balimo region on the greater Western Province area.

CHAPTER 2

LITERATURE REVIEW – CHALLENGES FOR TUBERCULOSIS CONTROL IN RESOURCE-LIMITED SETTINGS

2.1. Introduction

Tuberculosis (TB) is a global disease epidemic. In 2016, TB was estimated to have caused about 10.4 million cases and 1.6 million deaths worldwide (World Health Organization 2017a). These numbers are despite TB being both treatable and curable in drug-susceptible cases. In Papua New Guinea (PNG), TB contributes substantially to disease burden. Incidence data from 2016 estimated TB to have caused almost 35,000 cases and more than 4300 deaths in PNG (World Health Organization 2017a), and more than 28,000 TB cases were notified (Aia et al. 2018). The country has the third-highest incidence of TB in the Western Pacific Region, behind Kiribati and Timor-Leste (World Health Organization 2017a). Increasing case notification rates, and a treatment success rate of only 74% in new cases and 63% in previously treated cases, signal an urgent need for improved TB control strategies countrywide (World Health Organization 2017a).

In 1994, the World Health Organization (WHO) first proposed the directly observed treatment, short-course strategy, known as DOTS, as a framework for TB control (World Health Organization 2006). The DOTS strategy was originally based on five key components: political commitment, a passive case-finding approach to detecting cases, standardised treatment with proper case management, a regular drug supply, and a monitoring system to enable program evaluation (World Health Organization 1999, 2006). However, the challenges facing DOTS, particularly in settings with poor support and inadequate infrastructure, led the WHO to release expanded DOTS guidelines in 2006, known as the 'Stop TB Strategy' (World Health Organization 2015c) (Figure 2.1). The 'Stop TB Strategy' reinforced and further defined the original components of DOTS, and emphasised community involvement and the need to consider increasing drug resistance (World Health Organization 2002). The 'Stop TB Strategy' was made up of six components. The first, to

pursue high-quality DOTS expansion and enhancement, included the original aims of the DOTS strategy (World Health Organization 2006). The other components included addressing increasing challenges to TB control, such as TB-HIV and drug resistance; strengthening health systems; engaging with all care providers, both public and private; empowerment of people with TB and their communities; and enhancing research activities (World Health Organization 2006). In 2014, the 'End TB Strategy' was implemented, parallel to the goal of ending TB that was included as part of the new sustainable development goals (SDG) adopted in 2015 (United Nations n.d.-a, n.d.-b; World Health Organization 2015c). The new strategy is built on three pillars: (1) integrated, patient-centred care and prevention, (2) bold policies and supportive systems, and (3) intensified research and innovation (World Health Organization 2015e).

Figure 2.1: TB treatment poster from PNG linked to the 'Stop TB Strategy' and DOTS. (Image credit: Tanya Diefenbach-Elstob)

Many resource-limited settings, including PNG, base their TB control programs on the strategies endorsed by the WHO. However, the implementation of these strategies is influenced by a variety of setting-specific factors, including limited resources, staff shortages, and funding short-falls (Dimitrova et al. 2006; Khan et al. 2016). In addition, treatment approaches often focus on the 'direct observation of treatment' (DOT) aspect that was historically part of the strategy, and which emphasised the need for patients to have their treatment observed by a health worker or treatment supporter (World Health Organization 1999). The focus on DOT can be problematic due to the economic burdens placed on patients when treatment observation requires travel, and results in lost time for work and food production activities (Diefenbach-Elstob et al. 2017; Gebremariam et al. 2010; Sagbakken et al. 2013; Tadesse et al. 2013). As a result of local challenges, TB control programs have by necessity adapted TB management strategies to accommodate their own setting-specific demands (Massey et al. 2012; Newell et al. 2006; Wei et al. 2008).

This literature review will introduce TB, and examine aspects of TB control strategies in resource-limited settings, with a particular focus on the challenges that are faced. The influence of such challenges on TB epidemiology will then be described. Finally, the burden of TB in PNG will be described, including the factors influencing TB epidemiology in this region.

2.2. Tuberculosis

2.2.1. The organism: *Mycobacterium tuberculosis*

Mycobacterium tuberculosis (MTB), the causative organism of TB, is part of the *M. tuberculosis* complex (MTBC) that comprises at least seven genetically similar species (Rodriguez-Campos et al. 2014; Wirth et al. 2008). Human TB cases are primarily caused by *M. tuberculosis*, with a smaller number of cases caused by *M. africanum,* and *M. bovis* (Forrellad et al. 2013; Rodriguez-Campos et al. 2014; Torres-Gonzalez et al. 2016). Other species responsible for disease primarily in animals include *M. bovis, M. canettii, M. caprae, M. microti,* and *M. pinnipedii*, and potentially *M. orygis* and *M. mungi* (Forrellad et al. 2013; Hershberg et al. 2008; Rodriguez-Campos et al. 2014).

Molecular analysis techniques have led to the sub-division of *M. tuberculosis* and *M. africanum* into seven lineages, which are further divided into a number of strains (Table 2.1) (Brites & Gagneux 2015; Nebenzahl-Guimaraes et al. 2016). The discovery of all lineages in Africa has resulted in an 'out of Africa' hypothesis describing the evolution and spread of TB throughout the world (Brites & Gagneux 2015; Gagneux & Small 2007; Nebenzahl-Guimaraes et al. 2016).

Table 2.1: The sub-division of *M. tuberculosis* and *M. africanum* lineages, including the geographic region associated with each lineage, and some of the well-known strains. (Sources: Blouin et al. 2012; Gagneux et al. 2006; Gagneux & Small 2007; Nebenzahl-Guimaraes et al. 2016)

Lineage	Species	Geographic region	Included strains
1	*M. tuberculosis*	Indo-Oceanic	EAI
2	*M. tuberculosis*	East-Asian	Beijing
3	*M. tuberculosis*	East-African-Indian	CAS
4	*M. tuberculosis*	Euro-American	Haarlem, LAM, T, X
5	*M. africanum*	West-African 1	
6	*M. africanum*	West-African 2	
7	*M. tuberculosis*	Ethiopian	

CAS: Central Asian; EAI: East-African-Indian; LAM: Latin American-Mediterranean

2.2.2. Transmission and infection

The transmission of MTB is predominantly airborne, via the aerosolisation of respiratory droplets from infected individuals, primarily through coughing (Pai et al. 2016; Yates et al. 2016). If an infected person's immune system has an adequate response, MTB bacilli that reach the lungs are contained in granulomas, resulting in a non-symptomatic and non-infectious latent infection (Pai et al. 2016). If the bacilli are not able to be contained, they replicate and spread throughout the body, resulting in active TB (Knechel 2009). Active TB may occur in up to 15% of people who have been latently infected (Trauer et al. 2016).

Active pulmonary TB, which is restricted to the lungs, is characterised by non-specific signs and symptoms such as fever, fatigue, weight loss, and night sweats; as well as more specific symptoms including a prolonged cough and haemoptysis (Pai et al. 2016).

Extrapulmonary TB refers to infection in any site of the body apart from the lungs. The most common sites of infection are the lymph nodes, pleura, gastrointestinal tract, bones, central nervous system, and genitourinary system (Peirse & Houston 2017). Presentation is often non-specific, and the condition may go unrecognised, with case reports describing misdiagnoses of extrapulmonary TB as conditions such as metastatic disease and Crohn's disease (Makhani et al. 2013; Riedel et al. 1989).

2.2.3. Identification and diagnosis

Phenotypic diagnosis of TB is generally performed by liquid or solid culture of clinical samples, frequently sputum. However, the slow replication time of MTB means that this technique is lengthy (Chien et al. 2000; Lawn & Zumla 2011). Furthermore, culture is expensive, carries the risk of generating infectious aerosols, and requires sophisticated laboratory infrastructure (World Health Organization 2012). In resource-limited settings, microscopy of sputum smears is the most common method of diagnosis (Figure 2.2). However, microscopy is increasingly being replaced by the Xpert MTB/RIF. The Xpert MTB/RIF is a semi-automated and rapid nucleic acid amplification test (NAAT) that can detect MTB infection, as well as rifampicin (RIF) drug resistance, based on amplification of the *rpoB* gene (Helb et al. 2010; Ioannidis et al. 2011; World Health Organization 2013c).

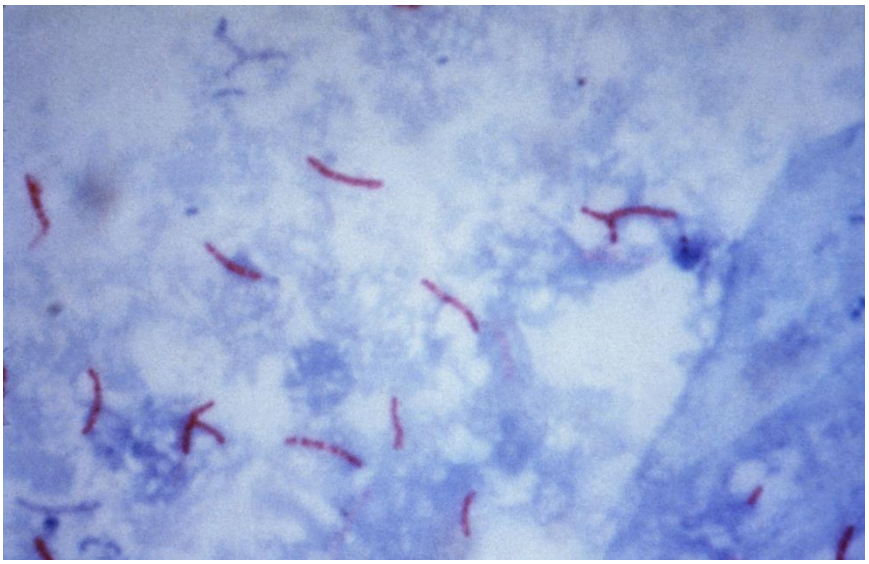


Figure 2.2: Photomicrograph of *M. tuberculosis* visualised with an acid-fast Ziehl-Neelsen stain. (Image source: CDC PHIL/ Dr George P. Kubica – Public Domain)

The diagnosis of extrapulmonary TB is frequently challenging, with the technique used dependent on the site of infection. Culture or microscopy may be used if fluid samples are available, while tissue samples may be examined using histology or cytology (Purohit & Mustafa 2015). However, the inability to collect a suitable clinical specimen, or a lack of diagnostic infrastructure even if samples are available, means that the diagnosis of extrapulmonary TB is frequently clinical, based solely on presenting signs and symptoms (Dangisso et al. 2014; Purohit & Mustafa 2015). The WHO diagnostic guidelines for TB allow for the clinical diagnosis of both pulmonary and extrapulmonary TB, using the classification of a 'clinically diagnosed TB case', based on the decision of a clinician to commence a patient on a full course of TB treatment (World Health Organization 2013a).

2.2.4. Treatment of tuberculosis

The discovery of antibiotics led to the first drug treatments for TB, beginning in 1944 with injectable streptomycin (STR) (Daniel 2006). Oral treatment followed with isoniazid (INH) in 1952, and the rifamycins in 1957 (Daniel 2006). RIF, still in use today, was discovered in 1962

(Zumla et al. 2013). The other two first-line drugs, pyrazinamide (PZA) and ethambutol (EMB), were discovered in 1954 and 1961 respectively (Zumla et al. 2013).

Six-month four-drug combination treatment regimens were introduced in the 1970s (Zumla et al. 2013), and have now been the first line of defence in TB treatment for more than 40 years. The recommended standardised treatment regimen for new TB patients consists of daily medication, with a two-month intensive phase of INH, RIF, PZA, and EMB; followed by a four-month continuation phase of INH and RIF (World Health Organization 2017b) (Figure 2.3). This regimen is often abbreviated as 2HRZE/4HR.

Figure 2.3: RIF, INH, PZA, and EMB drugs in fixed dose combinations. (Image credit: Tanya Diefenbach-Elstob)

2.2.5. The emerging crisis of drug-resistant tuberculosis

The problem of drug-resistant TB (DR-TB) emerged soon after effective antibiotic treatments were developed. Resistance to STR was first reported in 1948, only four years after the drug was first used in TB treatment (Crofton & Mitchison 1948). This pattern continued, with drug-resistant cases occurring in response to other anti-TB drugs as they were developed

and put into use (Fox et al. 1999; Keshavjee & Farmer 2012). The result of ongoing and emerging drug resistance was the implementation of the multi-drug regimens still used today.

Drug resistance can occur in response to any of the anti-TB drugs. However, DR-TB is usually described in relation to two main categories. Multidrug-resistant TB (MDR-TB) is resistance to both INH and RIF, while extensively drug-resistant TB (XDR-TB) is MDR-TB with additional resistance to any fluoroquinolone (FLQ), plus at least one of three listed injectable antibiotics (capreomycin, kanamycin, and amikacin) (World Health Organization 2013a) (Table 2.2).

Table 2.2: Definitions used to describe DR-TB (World Health Organization 2013a, 2017a). Note that the definitions of XXDR and TDR are unofficial (World Health Organization 2018e).

Type of resistance	**Associated drugs**
Mono-resistant	Any one drug
Rifampicin-resistant	Only RIF (susceptible to INH)
Multidrug-resistant (MDR)	Both INH and RIF (resistance to other drugs may also be present)
Extensively drug-resistant (XDR)	MDR, plus Any fluoroquinolone, plus Any one of capreomycin, kanamycin, or amikacin
Extremely drug-resistant (XXDR) **Totally drug-resistant (TDR)**	All first- and second-line drugs

INH: isoniazid; RIF: rifampicin

Two different pathways may lead to the development of DR-TB. Acquired drug resistance develops in a case of active drug-susceptible TB, as a result of insufficient treatment allowing the selection of resistant mutant strains (World Health Organization 2014b). Primary drug resistance occurs as a result of direct transmission of a drug-resistant TB strain (World Health Organization 2014b). For many years, management of DR-TB in TB control programs focused on acquired drug resistance and previously treated TB patients (Dheda et al. 2017). However, there is growing evidence highlighting the large proportion of DR-TB cases that

result from direct transmission of DR-TB strains (Dheda et al. 2017; Sarita et al. 2017; Zhao et al. 2012).

Globally in 2016, 4.1% of new TB cases and 19% of retreatment cases were identified as either RIF-resistant TB (RR-TB) or MDR-TB (World Health Organization 2017a). It has also been estimated that about 8.5% of TB cases have INH resistance, without concurrent RIF resistance (World Health Organization 2017a). Furthermore, it was estimated that about 6.2% of MDR-TB cases had XDR-TB (World Health Organization 2017a). Extremely drug-resistant TB (XXDR-TB) and totally drug-resistant TB (TDR-TB), while not officially recognised terms, generally refer to resistance to all of the tested first- and second-line drugs (Kanabus 2016; Udwadia et al. 2012; Velayati et al. 2009; World Health Organization 2018e) (Table 2.2). Cases of XXDR-TB and TDR-TB have been described in Italy, the United States of America, Iran, and India (Kanabus 2016).

In addition to these estimates for DR-TB, in some settings the proportion of TB drug resistance is thought to be much higher. The World Health Organization (WHO) has designated 30 countries as high MDR-TB burden countries (Figure 2.4). Of those countries in the MDR-TB high-burden list, the best estimate of MDR/RR-TB ranges from 1.3% (Kenya) to 38% (Belarus) in new cases; and from 7.1% (South Africa) to 72% (Belarus) in previously treated cases (World Health Organization 2017a). In 2014 only 54% of MDR/RR-TB cases and 30% of XDR-TB cases were successfully treated worldwide (World Health Organization 2017a). DR-TB has become a global crisis.

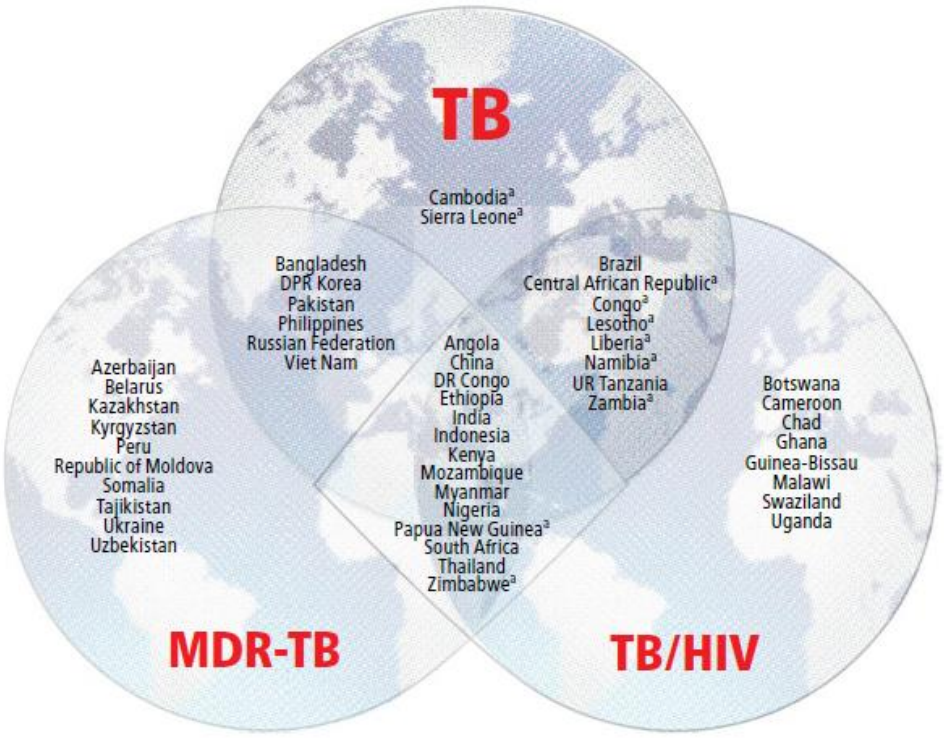


Figure 2.4: High-burden countries for TB, MDR-TB, and TB/HIV. (Image source: World Health Organization 2017a)

2.2.6. Global epidemiology of tuberculosis

In 2016, TB incidence globally ranged from a low of 0 cases per 100,000 people per year in a number of countries (British Virgin Islands, Monaco, Montserrat, Saint Kitts and Nevis, San Marino, Sint Maarten (Dutch part), Tokelau, and the Wallis and Futuna Islands) to a high of 781 cases per 100,000 people per year in South Africa (Figure 2.5) (World Health Organization 2017a). Five countries were the origin of 56% of incident TB cases; these were India, Indonesia, China, the Philippines, and Pakistan (World Health Organization 2017a). Approximately 600,000 new cases of TB were either RR-TB or MDR-TB, and of these, 47% occurred in China, India, and the Russian Federation (World Health Organization 2017a).

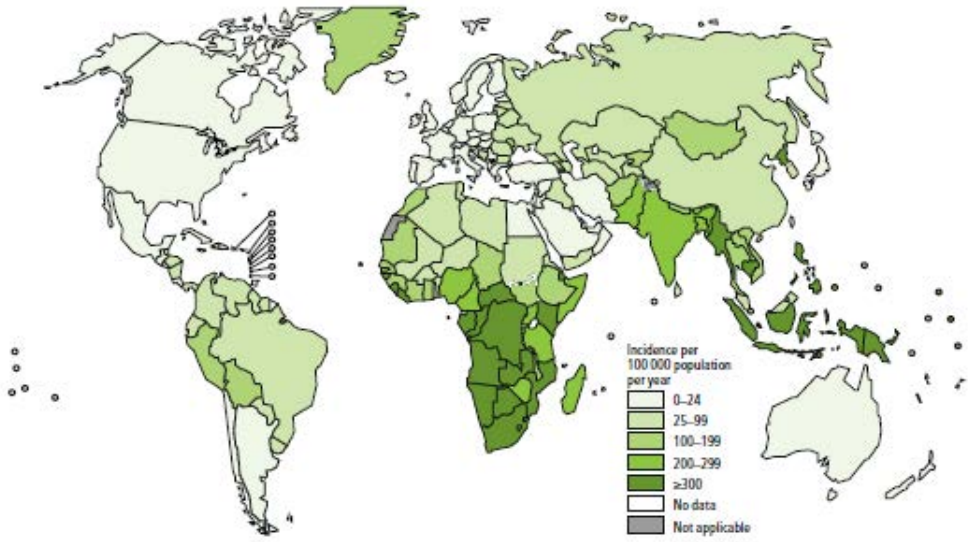


Figure 2.5: Estimated TB incidence rates per 100,000 people in 2016. (Image source: World Health Organization 2017a)

The proportion of bacteriologically confirmed cases of TB varies widely by region. For example, for pulmonary TB cases in 2016, bacteriological confirmation ranged from 38% in the Western Pacific region to 78% in the Americas (World Health Organization 2017a). Extrapulmonary TB proportions are also variable, ranging from 8% in the Western Pacific region to 24% in the Eastern Mediterranean region (World Health Organization 2017a). However, rates of extrapulmonary TB are often substantially higher in setting-specific studies. For example, studies have described extrapulmonary TB in 22.7% of TB patients in Kassala, Sudan, 49.4% of TB patients in Van, Turkey, and 24.9% of TB patients in Fiji (Abdallah et al. 2015; Pezzoli et al. 2016; Sunnetcioglu et al. 2015). In addition, the presence of TB-HIV coinfection may influence local TB epidemiology, with a number of studies describing increased risk of extrapulmonary TB in people with HIV infection (Gomes et al. 2014; Wilkinson & Moore 1996; Yang et al. 2004).

2.3. Working Towards Tuberculosis Control: Challenges in Resource-Limited Settings

Despite the implementation of the expanded ‘End TB Strategy’, there are still significant barriers to TB control in many settings; some of these barriers are described following. Local

identification of such barriers, and the adaptation of the 'End TB Strategy' to address them, is essential to ensuring that broad TB control guidelines are actually useful in the settings where they are implemented.

2.3.1. Diagnosing tuberculosis with limited laboratory infrastructure

The WHO-recommended approach to TB diagnosis is sputum smear microscopy in primary health care facilities; and culture, drug susceptibility testing (DST), and molecular detection of drug resistance in health care facilities where clinical laboratories are available (World Health Organization 2018f). However, techniques such as culture and DST are reliant on extensive laboratory networks with the necessary equipment and trained staff, as well as a national reference laboratory (World Health Organization 2006). Furthermore, access to multiple diagnostic approaches is important, to ensure that more difficult-to-diagnose cases are properly assessed, including those that are smear-negative, have HIV co-infection, and with possible drug resistance (World Health Organization 2006).

Solid and liquid culture methods, as well as DST, require moderate- and high-risk (TB-containment) laboratories (World Health Organization 2012) (Figure 2.6). However, such laboratories may not be readily accessible, with clinical samples requiring culture and DST often needing to be sent to reference laboratories that may be located in another country. The length of time required to achieve culture results is also problematic, with solid culture taking about seven weeks, while automated or semi-automated liquid culture systems such as the BACTEC 960 take about seven to eleven days (Battaglioli et al. 2013; Scarparo et al. 2004; Zhao et al. 2013). These factors mean that obtaining culture results is time-consuming. Thus, best-practice recommendations based on microscopy, culture, and DST are difficult to achieve where only limited laboratory facilities are available. These difficulties emphasise the importance of other more accessible techniques, such as the Xpert MTB/RIF platform recommended by the WHO (World Health Organization 2018f). However, as noted previously, although the platform has been rolled out across numerous settings there are limitations to its use due to cost, accessibility, the need for stable electricity and temperature control requirements, and the inability to identify resistance to other anti-TB

drugs (Boehme et al. 2011). These are important considerations in resource-limited settings, and research is important in determining the best ways to prioritise and manage Xpert MTB/RIF testing in combination with other diagnostic and screening approaches (Muttamba et al. 2018; Muyoyeta et al. 2015; Philipsen et al. 2015).

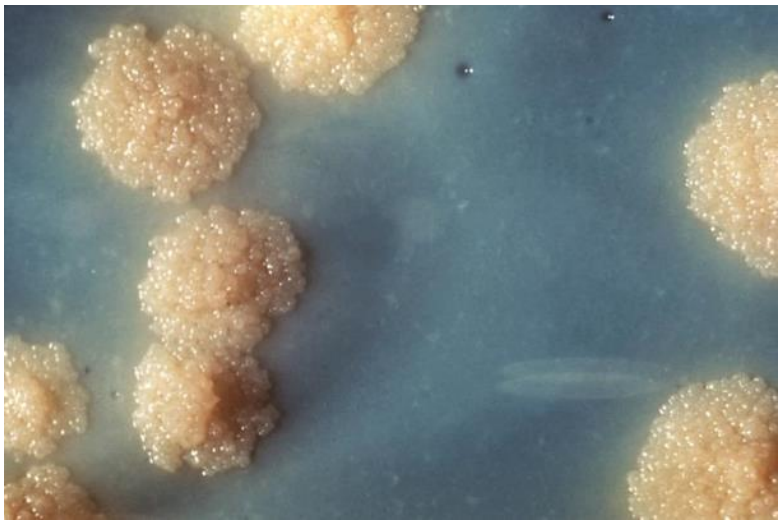


Figure 2.6: *M. tuberculosis* solid culture showing colony morphology. (Image source: CDC PHIL/ Dr. George Kubica – Public Domain)

In resource-limited settings, often sputum smear microscopy is the only technique that is readily available. However, this technique is known to have low and variable sensitivity of 31 – 98%, and poor sensitivity of the technique has been described in samples from patients who are HIV-positive (Cattamanchi et al. 2009; Elliott et al. 1993; Steingart et al. 2006a; Steingart et al. 2006b). Poor sensitivity, especially when combined with a lack of access to other bacteriological diagnostic facilities, may lead to increased false-positive clinical TB diagnoses. The challenges faced due to poor microscopy sensitivity have been described in a study from Ethiopia, where poor microscopy accuracy was noted as potentially contributing to empiric treatment of patients who did not actually have TB (Cowan et al. 2013). However, large numbers of smear-negative results may also contribute to substantial proportions of false-negative diagnoses, and potentially under-diagnosis of TB (Guernier et al. 2018). These outcomes create burdens for patients, who may be unnecessarily exposed to potentially

toxic drugs, or remain untreated despite actually having TB. Difficulties in TB diagnosis also create challenges for health workers, who may spend a considerable amount of time treating and managing misdiagnosed patients.

2.3.2. Clinical diagnosis of tuberculosis

The inability to accurately diagnose TB using laboratory-based methods may increase the likelihood of a patient receiving a clinical TB diagnosis, based solely on presenting signs and symptoms (Purohit & Mustafa 2015). This scenario may be particularly the case for presumptive extrapulmonary TB, where suitable diagnostic samples can be difficult to obtain (Norbis et al. 2014). Given the difficulties associated with bacteriological identification of TB, clinical diagnoses will undoubtedly continue to be common for the foreseeable future. Additionally, in resource-limited settings where minimal laboratory infrastructure is available, clinical diagnoses are likely to form a substantially higher proportion of the total identified TB cases.

The growing burden of DR-TB is an additional complication in the use of clinical diagnoses for TB. In clinically diagnosed TB cases, a positive response to treatment may be the only evidence available to confirm that a TB diagnosis was correct, despite this approach being problematic due to treatment failure, acquired drug resistance, and the effectiveness of some TB drugs against other bacterial organisms (Chakravorty et al. 2005; Colebunders & Bastian 2000; Ryu 2015). However, for DR-TB patients, clearly there will be non-response to first-line treatment, even if the clinical diagnosis of TB was correct. This situation may lead to prolonged periods of ineffective treatment, ongoing transmission of DR-TB strains to others, and worsening of disease.

2.3.3. Outcomes of delayed diagnosis and treatment

Poor diagnostic and treatment outcomes may occur for TB patients due to delays in the diagnosis-to-treatment pathway, including delays in either the initial diagnosis or treatment

commencement, and loss-to-follow-up between diagnosis and treatment initiation. For example, delay in a TB diagnosis may lead to loss to follow-up. A systematic review of sputum smear- and culture-positive patients found pre-treatment loss to follow-up rates ranging from 4 – 38% in low- and lower-middle-income countries (MacPherson et al. 2014). A number of studies have explored this issue further. Delays in returning the results of sputum testing were specifically identified as pre-treatment barriers in studies from Malawi and Vietnam (Buu et al. 2003; Squire et al. 2005). Distance and access to a health facility have also been noted as important. In the study from Vietnam, people from urban areas were more likely to be lost prior to treatment (Buu et al. 2003). However, in studies from India and Cambodia people located in rural areas or further from a health facility were more likely to be lost (Lorent et al. 2015; Mehra et al. 2013). In the study from Cambodia, costs associated with transport and lost time from work were also cited as barriers (Lorent et al. 2015).

Diagnostic accuracy and prompt access to diagnostic testing are particularly important in the context of case detection and follow-up of people being investigated for TB. Patients who do not receive a diagnosis on the day that they attend a health centre may be left with no choice but to return home, and subsequently may not return for diagnostic results (Squire et al. 2005). Other problems in the diagnosis-to-treatment pathway may include confusion about testing and the need to return for results, and unavailability of results even if patients do return (Skinner & Claassens 2016). Delays in receiving results may mean that patients are not traceable by health workers, due to both a lack of records and staff time constraints, and will thus remain undiagnosed and untreated (Engel et al. 2015). This challenge is particularly concerning in the context of ongoing TB transmission to others, and also in understanding the true burden of TB in settings where pre-treatment loss to follow-up occurs (MacPherson et al. 2014). Furthermore, delayed diagnosis and treatment of TB patients has been associated with transmission of MTB to patient contacts, and death and treatment failure outcomes (Gebreegziabher et al. 2016; Golub et al. 2006).

2.3.4. Treatment of tuberculosis with limited health facilities

The earlier 'Stop TB Strategy' called for standardised, short-course treatment regimens that used fixed dose combination (FDC) drugs (World Health Organization 2006). Supervision of treatment was advised, although it was stated that this should be flexible and sensitive to patient needs (World Health Organization 2006). The most recent TB treatment guidelines continue to recommend FDCs (World Health Organization 2017b). Treatment supervision is also advised, although this may be offered by a treatment supporter (friend, family, or lay person), health worker, or video observation if appropriate (Figure 2.7) (World Health Organization 2017b).

Figure 2.7: Hospital-based health workers in Balimo, PNG. (Image credit: David Plummer)

In practice, achieving treatment guidelines can be extremely difficult, particularly in resource-limited settings. Facilities that care for TB patients must contend with a lack of infrastructure and resources, and inadequate numbers of staff (Buregyeya et al. 2013; Zelnick et al. 2013). Furthermore, resource shortages may include the medications used to treat TB, with such shortages having been reported from a number of countries (Rusen et al. 2010; Stop Stockouts 2016).

In resource-limited settings, there are often inadequate numbers of health workers, with existing staff overburdened (Buregyeya et al. 2013). It has been estimated that there is a global shortage of approximately four million health workers, and that sub-Saharan Africa needs to triple its workforce (Chen et al. 2004). In many settings, the mix of providers is unsuitable, with a particular shortage of community-based workers (Chen et al. 2004). The distribution of workers can also be problematic, with excess health worker numbers in urban locations, while rural areas are under-served (Chen et al. 2004; Figueroa-Munoz et al. 2005). In PNG, where more than 80% of the population live in rural areas, in 2009 it was estimated that 44% of the health service delivery workforce (doctors, health extension officers, nurses, midwives, community health workers, and dentists) were employed in urban areas, and 56% were working in rural areas (Morris & Somanathan 2011; National Statistical Office 2015). The ageing of health workforces is also an area of concern, particularly due to the large proportion of the workforce who are approaching retirement age (Morris & Somanathan 2011). In PNG in 2009, 15.6% of the health service delivery workforce (as defined above) were aged 55 years or over, 37.7% were aged 45 – 55 years, and only 12.3% were aged under 35 years (Morris & Somanathan 2011). The challenges associated with staff shortages and health workforce distribution have the potential to compromise not only diagnosis and treatment of TB patients, but also the ability to conduct essential contact tracing and follow-up activities.

2.3.5. Treatment challenges for tuberculosis patients

Challenges presented by access factors may be substantial barriers across all facets of TB management, including treatment-seeking, diagnosis, treatment, and follow-up. Financial and travel-related burdens have been described in a number of resource-limited settings, including rural China, Nigeria, and the Solomon Islands (Massey et al. 2012; Ukwaja et al. 2013a; Wei et al. 2009; Xu et al. 2010). Access to medications in predominantly resource-limited settings such as parts of Africa is complicated by the high proportions of the population living in rural areas, where access is limited (Tetteh 2009). This challenge will also be an important factor in a setting such as PNG, where more than 80% of the population live in rural areas, only 35% live within ten kilometres of a major road, and 17% have no road

infrastructure links at all (National Research Institute 2010; National Statistical Office 2015; Ongugo et al. 2011) (Figure 2.8). It is possible that these barriers may also prevent people with TB symptoms from seeking treatment in the first place.

Figure 2.8: Travelling in a dugout canoe in the Balimo region of PNG, where there are very few roads, and travel is predominantly by boat or foot. (Image credit: David Plummer)

The economic hardship that can result from TB is also a recognised problem, as demonstrated by one of the indicators of the 'End TB Strategy', which is to have no TB-affected patients and families facing catastrophic costs due to TB by 2020 (Floyd et al. 2018; World Health Organization 2015c). Patients may face potentially devastating costs in accessing TB care. In South Africa, a study found that about 41% of costs associated with TB care were incurred before treatment had even been started, primarily due to loss of income (Foster et al. 2015). Income loss has also been described in Zambia, with TB patients reported to have delayed treatment-seeking due to money shortages, and having an average of 18 days of work lost prior to diagnosis (Needham et al. 1998). Among those patients who had ceased work due to TB, an average of 48 days of work were lost (Needham et al. 1998). In China and India, patients have described borrowing money and selling assets to cover costs (Jackson et al. 2006; Prasanna et al. 2018). In Nigeria, where TB laboratory tests and medications are free, substantial costs were still incurred for consultations with health

providers, medications and diagnostic tests unrelated to TB, as well as transportation (Ukwaja et al. 2013a). Additionally, the proportion of households who were classified as poor increased from 54% prior to the TB episode to 79% afterwards (Ukwaja et al. 2013a). In a study from India, 32.4% of households experienced catastrophic costs (TB costs that exceeded 20% of the household's annual income) during diagnosis and the intensive phase of TB treatment (Prasanna et al. 2018).

Large burdens may be placed on TB patients. For example, in some settings strict interpretation and implementation of DOT may mean that patients are not allowed to take their medications home with them, and will be expected to travel daily to a health facility to receive their treatment (Benbaba et al. 2015; Sagbakken et al. 2008). Such travel can be an insurmountable barrier for patients, with excessive transport costs, and lost time resulting in burdens in maintaining paid employment, even in settings where less frequent attendance at a health clinic is required (Benbaba et al. 2015; Chida et al. 2015). These examples demonstrate the increased burdens caused by TB diagnosis and treatment, which may disproportionately affect the vulnerable and disadvantaged populations who are least able to overcome such challenges.

For many patients, TB treatment creates disadvantage, discrimination, and perpetuation of poverty. As already discussed, patients may be unable to retain employment due to needing to attend daily at a health facility for treatment observation, resulting in economic burdens on top of existing health burdens (Gebremariam et al. 2010; Sagbakken et al. 2013). Discrimination against TB patients is prevalent in many settings, which hinders both identification and treatment (Baral et al. 2007; Gebremariam et al. 2010).

2.3.6. Achieving effective tuberculosis control despite resource challenges

Although there are numerous barriers to TB control, especially in resource-limited settings, there are many achievements from such settings that should also be acknowledged. For example, extensive research from the Solomon Islands has described treatment barriers associated with taboos and traditional beliefs, but has also detailed community-based projects aimed at overcoming such challenges (Massey et al. 2011; Massey et al. 2012;

Massey et al. 2013). Community-based systems of care have been implemented in many resource-limited settings, with such approaches often successful. For example, in Ethiopia the community-based Health Extension Program was introduced in 2004 (Ketema et al. 2014). This program offers decentralised TB services, with many TB-related activities being conducted by health extension workers (HEW) and TB supporters (Ketema et al. 2014). Where patients are unable to attend a health facility daily, medications are dispensed weekly, and treatment is observed by a HEW or treatment supporter (Ketema et al. 2014). The program found that treatment success was not significantly associated with either approach – patients had treatment success rates of 97.8% in the community and 93.5% at health facilities (Ketema et al. 2014), thus indicating the feasibility of community-based treatment support strategies. In Tanzania, a similar approach has been used, labelled 'patient centred treatment' (PCT) (Egwaga et al. 2009). This strategy uses home-based treatment observation, and patients can choose their own treatment supporter (Egwaga et al. 2009). A study of this approach in comparison to health facility-based treatment found treatment success to be significantly better for the PCT cohort (Egwaga et al. 2009).

Bangladesh provides an example of considerable efforts to deal with staffing challenges and service delivery. A Belgian non-governmental organisation (NGO), the Damien Foundation Bangladesh, has trained 12,525 informal village doctors to provide services in TB control in rural areas (Salim et al. 2006). These village doctors, known as *gram dakter*, usually practice 'alternative' and mixed forms of medicine, and are either unqualified or only semi-qualified (Salim et al. 2006). However, with more than 75% of Bangladesh's population living in rural areas, they are often the first contact that people with TB symptoms have with the health system (Salim et al. 2006). These village doctors were trained to provide TB services, including collection of sputum samples; providing treatment to patients, including DOT; and patient follow-up (Salim et al. 2006). The village doctors are in turn supervised by staff of the NGO, who visit the village doctors at least three times during each patient's treatment course (Salim et al. 2006). This system has resulted in a treatment success rate of about 90% (Salim et al. 2006). Most importantly, the success of the strategy has led to it now being incorporated into Bangladesh's national TB control policy (Salim et al. 2006). The approach used in Bangladesh provides an excellent example of how existing village health workers can

be trained and incorporated into TB control programs, to provide simple but essential services that also alleviate pressures on the health system.

Many of the barriers facing TB control in resource-limited settings are similar worldwide. Examples include inadequate numbers of health personnel, a lack of resources, and challenging geography. There are also parallels in facilitators of control in many settings, such as strong community networks, and the presence of influential organisations such as churches and community groups (Cavalcante et al. 2007; Demissie et al. 2003) (Figure 2.9). All of these factors will influence the activities that are achievable within a local TB control program.

Figure 2.9: Sister Bisato Gula providing TB awareness to a community music group involved in World TB Day activities in Balimo. (Image credit: Tanya Diefenbach-Elstob)

2.4. Tuberculosis Control in the Era of Drug Resistance

Ineffective TB control programs result in problematic management of TB patients, and can thus increase the risk of drug resistance in a number of ways:

(1) Delays in diagnosis increase the period of time that patients carry infection, and thus increase the risk of spontaneously developing drug resistance (Colijn et al. 2011). Such delays also increase the period of infectivity to others.
(2) Improper treatment, through improper drug choice, or inadequate dosing or timing of treatment, increases the selection of drug-resistant clones, and thus acquired drug resistance (Mitchison 1998).
(3) Patients with strains that are resistant to some drugs but not others will experience improper treatment even with full adherence. They are thus at risk of amplified resistance due to an incorrect combination of drugs not adequately suppressing the bacteria (Basu & Galvani 2008).
(4) Where cases are drug-resistant, the use of inadequate treatment regimens increases the risk of primary transmission of drug-resistant strains to others due to ongoing infectivity.
(5) Loss-to-follow-up of TB patients after they have commenced treatment may occur, and resultant incomplete treatment may drive the selection of drug-resistant organisms and development of DR-TB (Naidoo et al. 2017; World Health Organization 2014b).

An effective TB control program that ensures prompt diagnosis and treatment of TB patients, as well as effective contact tracing and case-finding activities, is thus essential in slowing the rise of drug resistance.

Control of DR-TB is critical, and should be specifically addressed in TB programs. However, the complexities involved in control of drug-susceptible TB are amplified for DR-TB. Access to second-line drugs can be difficult, and drug shortages can be severe. For example, waiting times of 9 – 12 months have been reported from Russia, primarily due to the limited number of suppliers who were approved and registered to sell the required drugs (Olson et al. 2011). Treatment regimens for DR-TB are not standardised, with treatment duration and the antibiotics used dependent on factors such as treatment history and DST results (World Health Organization 2016). The treatment of DR-TB is an area undergoing regular revision, with a number of new and updated guidelines having been in use over the course of this study (World Health Organization 2013b, 2014a, 2014b, 2016). Additionally, despite

increasing rates of drug resistance, as well as identification of organisms with resistance to greater numbers of drugs, there has been slow uptake of new medications such as bedaquiline and delamanid (Cox et al. 2018). The management of DR-TB is also challenged by the centralisation of treatment facilities. Patients with presumptive or confirmed DR-TB may need to travel and spend extended periods at medical facilities distant from their usual home, which creates access challenges for patients that have already been discussed (see Section 2.3.5).

Figure 2.10: Sister Bisato Gula and Mr Mauri Amadu navigating waterways in the Balimo region. (Image credit: Tanya Diefenbach-Elstob)

2.5. Building on Current Strategies in Tuberculosis Control

It is clear that in the implementation of the 'End TB Strategy' in resource-limited settings, there is no one-size-fits-all approach, and every setting will face unique sociocultural and structural challenges. Overcoming such challenges will require focused, setting-specific research, using the existing literature as a starting point and methodological guide.

Furthermore, flexibility in the approach to treatment, as well as TB outreach services, may play a role in supporting positive treatment outcomes.

The 'Stop TB Strategy' and DOTS have been extremely successful in facilitating and enabling successful TB treatment and cure for many patients (Obermeyer et al. 2008; Shargie & Lindtjorn 2005; Subramani et al. 2008). Despite this success, the challenges faced by TB patients highlight the importance of TB control programs continuing to seek ways to enable treatment and prevent disadvantage of TB patients, and to encourage the development of culturally and regionally appropriate solutions to TB control.

The extensive body of TB literature, recommendations, guidelines, and standards serve as guiding documents in the context of locally effective TB control programs. However, local conditions and resource availability mean that health care staff must often adapt these recommendations. The importance of local TB control and management highlights the importance of capacity building and research strengthening initiatives. Empowering local staff with the skills to conduct their own research will enable them to better understand and adapt control strategies to their local conditions. Furthermore, understanding of setting-specific factors will contribute to contextualising local TB epidemiology.

2.6. The Influence of Local Factors on Tuberculosis Epidemiology

Section 2.3 of this literature review described a number of the local and program factors that result in challenges for TB programs. However, it is also important to note that these same factors will play a role in the recognition and diagnosis of TB, and thus local TB epidemiology.

Costs are important in determining how and when people with TB symptoms will seek care, and the types of health care facilities at which they will present. This has been demonstrated in a study from China, where there was a high level of misunderstanding by participants about the availability of free TB services (Long et al. 2008). In addition, the most common reason given by migrants for not undergoing a recommended X-ray was cost (Long et al. 2008). Economic factors, as well as factors such as TB education and awareness, will thus play a role in actual case detection (Long et al. 2008) (Figure 2.11).

Figure 2.11: TB education posters from PNG in Tok Pisin and English. (Image credit: David Plummer)

A number of studies have linked increased distance to a health facility with delays in TB diagnosis (Bogale et al. 2017; Godfrey-Faussett et al. 2002; Lorent et al. 2015; Makwakwa et al. 2014; Mesfin et al. 2009; Tadesse et al. 2013; Ukwaja et al. 2013b). Such an association will affect TB epidemiology by resulting in under-diagnosis of TB in more distant regions. Additionally, diagnostic delay is associated with further progression of disease once diagnosis is actually achieved (Virenfeldt et al. 2014). Education can also play a role, with lower levels of schooling associated with diagnostic delay (Makwakwa et al. 2014; Segagni Lusignani et al. 2013).

Structural factors may also influence gender differences in TB epidemiology. For example, a study of people being investigated for TB in Pakistan identified more females at local facilities that were accessible by foot; and more males at facilities that were open in the evening, and that undertook more TB investigations (Khan et al. 2012). Furthermore, another study from Pakistan has reported geographic differences in rates of TB, with more females in two provinces, and more males in the other two provinces (Khan et al. 2013). A study from Peru described the lower priority that was given to women's health when

compared to men, and that women experienced economic barriers to obtaining healthcare services, despite having greater awareness of TB services (Onifade et al. 2010). These results suggest that behavioural and sociocultural factors, as well as health system availability, influence gender ratios in TB diagnoses.

The influence of sociocultural and structural factors on local TB epidemiology is difficult to assess, but will almost certainly play a role in the distributions and characteristics of local TB burdens. These factors are important to consider in the context of local TB control programs.

2.7. Health Indicators in Papua New Guinea

Data from PNG demonstrates that poor health outcomes are experienced by much of the population. In 2012, the average life expectancy was 62 years of age (World Health Organization 2015a). Although likely to be variable by region, in 2013 the under-five mortality rate was 61 per 1000 live births (World Health Organization 2015a). Lower respiratory infections are the leading cause of death, and TB and malaria are both included in the top five (World Health Organization 2015d). In earlier data, infectious diseases including TB, pneumonia, malaria, and meningitis were in the top five causes of death for both urban and rural population strata (Riley 2009), highlighting the fact that health challenges affect urban and rural populations alike. In 2017 the prevalence of HIV in adults aged 15 – 49 years was 0.9%; and in 2012 TB, HIV, and malaria combined caused the second-highest burden of years of healthy life lost due to disability (YLD) (UNAIDS 2018; World Health Organization 2015d).

One of the biggest challenges to attaining positive health outcomes in PNG is the ability of limited health infrastructure to cope with the increasing disease problem. In 2010, it was estimated that there were only 6.2 health workers (physicians, nurses, and midwives) per 10,000 people countrywide (World Health Organization Regional Office for the Western Pacific 2017b). By comparison, in 2013 Australia had 159 health workers per 10,000 people (World Health Organization Regional Office for the Western Pacific 2017a). The capacity of health facilities outside the PNG capital of Port Moresby is limited, and accessing healthcare

is challenging for much of the population, particularly in rural and remote areas (Diefenbach-Elstob et al. 2017; Ongugo et al. 2011).

2.7.1. Tuberculosis in Papua New Guinea

In PNG, TB control programs are guided by the National TB Management Protocol (Figure 2.12). This protocol is based on DOTS, and takes into consideration the WHO guidelines, as well as recommendations from the International Union Against TB and Lung Disease (Department of Health 2011). The protocol outlines the use of bacteriological methods of diagnosis, treatment regimens for various categories of patients, management algorithms for presumptive and confirmed drug-resistant patients, and guidelines for other considerations, such as infection control, TB-HIV coinfection; and advocacy, communication, and social mobilisation (ACSM) strategies (Department of Health 2011).

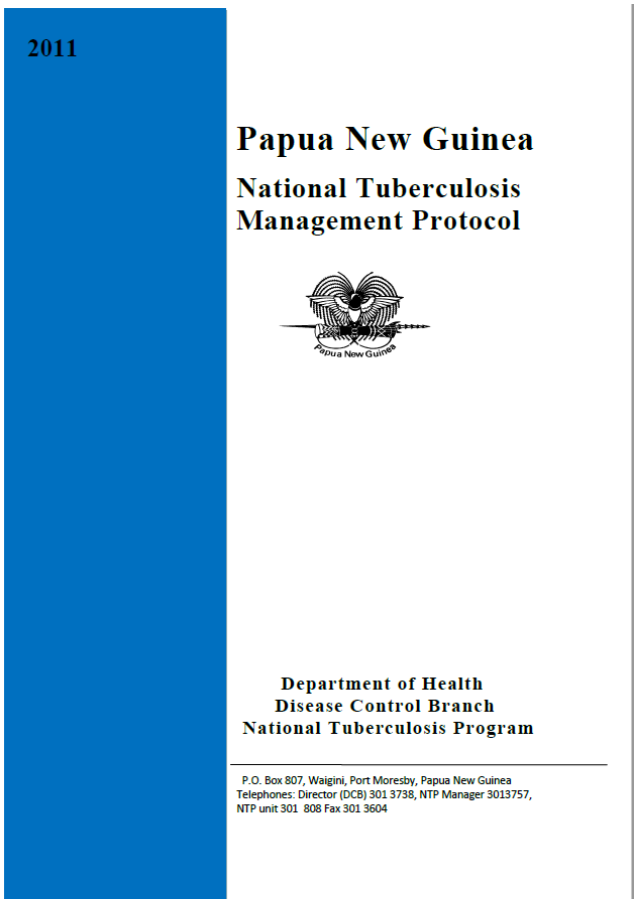


Figure 2.12: The PNG National TB Management Protocol, which outlines TB diagnosis, treatment, and reporting guidelines for the country's TB control program. (Source: Department of Health 2011)

The impact of TB on PNG's health outcomes is severe. The WHO estimates TB incidence in PNG to be 432 cases per 100,000 people each year (World Health Organization 2017a). With an estimated population of about 8.1 million people, this equates to almost 35,000 cases per year. Case notifications are lower, and in 2016 just over 28,000 TB cases were reported (Aia et al. 2018). By comparison, Australia, with a population of 24 million, has an incidence of only 6.1 cases per 100,000 people per year, equating to almost 1500 cases each year (World Health Organization 2017a).

The incidence of TB in many areas of PNG is thought to be much higher than the national estimate (Cross et al. 2014; McBryde 2012), and this assumption is supported by case notification data. In 2016 the southern region of PNG, which includes Western, Gulf, Central, Milne Bay, and Oro Provinces, had a case notification rate of 615 cases per 100,000 people

per year (Aia et al. 2018). The five provinces with the highest case notification rates were the National Capital District (1117 per 100,000), West New Britain (907 per 100,000), Western (674 per 100,000), Gulf (669 per 100,000), and Oro (592 per 100,000) (Aia et al. 2018), with four of these being located in the southern region.

Based on case notification data from 2016, 57% of TB patients in PNG had pulmonary TB, and of these 31% had bacteriological confirmation (World Health Organization 2017a). About 42% of TB patients had extrapulmonary TB, although the proportion was much higher in some provinces (78.5% in Eastern Highlands, 74.1% in Hela, 63.6% in Enga, 62.5% in Jiwaka, and 62.1% in Western) (Aia et al. 2018). With the exception of the reported case notification data, there is only one setting-specific study that describes extrapulmonary TB within a cohort of TB patients in PNG. This was a paediatric study undertaken at Modilon Hospital in Madang Province (Watch et al. 2017). Among 734 paediatric admissions with presumed TB, 384 (52.3%) had extrapulmonary TB (Watch et al. 2017).

As already discussed, diagnosis of TB can be challenging, particularly in rural and remote areas. In recent years, there was a lack of culture facilities in PNG, necessitating samples being sent to the Queensland Mycobacterium Reference Laboratory (QMRL) in Australia for culture and DST. A number of Xpert MTB/RIF machines, which can identify *M. tuberculosis* infection, as well as detect RIF resistance, are available countrywide, including at Daru in Western Province (McBryde 2012). Rural centres that have laboratory facilities rely on microscopy-based identification of MTB infection.

Diagnosis is even more challenging for extrapulmonary TB cases, where even if laboratory facilities are available, it may not be possible to collect a clinical specimen. These challenges may mean that some TB cases are missed, while some diagnosed cases may not actually have TB at all. The difficulties associated with positively identifying extrapulmonary TB in resource-limited settings are illustrated by its global epidemiology. In 2016, about 15% of TB notifications worldwide were for extrapulmonary cases (World Health Organization 2017a), although setting-specific rates are often much higher (see Appendix 1). Potentially high rates of clinical diagnoses and extrapulmonary TB cases make understanding the presentation of TB in PNG essential, and highlight the need for achieving clinician confidence in TB diagnoses.

Although DOTS has simplified TB treatment, there are still challenges to its implementation in PNG, and infrastructural factors mean that implementation must be flexible. The national management protocol states that TB treatment should be given using DOT (Department of Health 2011). However, daily travel to a health centre, or living away from home for the duration of treatment, may be prohibitively expensive for many patients, and therefore daily direct observation may not occur. This challenge is particularly relevant in a subsistence population, where patients may need to spend considerable amounts of time producing food, and income can be extremely limited (Diefenbach-Elstob et al. 2017) (Figure 2.13).

Figure 2.13: Catching fish in the Aramia River near Balimo in Western Province, PNG. (Image credit: David Plummer)

Sociocultural factors are also important, including social support, discrimination, religious beliefs, traditional healing, and the influence of witchcraft (Diefenbach-Elstob et al. 2017). There is some published literature describing the influence of sociocultural factors on health and illness in PNG (Diefenbach-Elstob et al. 2017; Lepowsky 1990; Macfarlane 2009;

Macfarlane & Alpers 2009; Ongugo et al. 2011; Whittaker et al. 2009), which provides a starting point for other PNG-based health research. As has been noted in this review, and as evidenced by prior Balimo-based research (Diefenbach-Elstob et al. 2017), such factors have a strong influence on the implementation of DOTS. Further understanding of these factors will influence the way treatment is delivered locally, particularly in rural and remote areas.

2.7.1.1. Regional tuberculosis burden in Papua New Guinea and Western Province

There is a small but growing body of published data describing the epidemiology of TB in specific settings in PNG. Of the 22 PNG provinces, those with recently published data include Eastern Highlands (Ley et al. 2014a), Gulf (Cross et al. 2014), Madang (Aia et al. 2016; Ballif et al. 2012a; Ballif et al. 2012b; Ley et al. 2014a; Luke et al. 2008; Watch et al. 2017), Milne Bay (Ley et al. 2014a), Morobe (Aia et al. 2016), and Western (Aia et al. 2016; Bainomugisa et al. 2018; Gilpin et al. 2008; Guernier et al. 2018; McBryde 2012; Pandey et al. 2018; Simpson 2011) provinces, and the National Capital District (NCD) (Aia et al. 2016; Kasa Tom et al. 2018; Uluk et al. 2013).

Cases of DR-TB have also been identified in PNG. In the Gulf Province study, three drug-resistant cases were detected using Xpert MTB/RIF (Cross et al. 2014). Two studies conducted among Western Province patients seeking healthcare on Australian Torres Strait Islands estimated drug resistance to occur in up to 25% of cases, with evidence of primary transmission of DR-TB strains (Gilpin et al. 2008; Simpson et al. 2011). However, patients presenting at these clinics may be more likely to have advanced disease. Most recently, a multi-province study undertaken in four provinces identified MDR-TB in 20/999 (2.0%) new cases, and 24/146 (16.4%) previously treated cases (Aia et al. 2016). Daru in Western Province had the highest MDR-TB burden, with 34.1% of all TB cases being MDR-TB (Aia et al. 2016). Additionally, Daru has previously been noted as a hotspot for DR-TB, and recent research has provided evidence of transmission of DR-TB strains in this setting (Bainomugisa et al. 2018; Furin & Cox 2016; Kase et al. 2016; World Health Organization Representative Office: Papua New Guinea 2016). However, the proportion of DR-TB reported from Daru and Australian health clinics in the Torres Strait will not be representative of the province-wide

burden of DR-TB, as Daru Hospital is the only referral site for presumptive DR-TB patients across Western Province (Department of Health 2011) (Figure 2.14). As a result, there is limited understanding of the extent of DR-TB in the province more broadly, as well as in specific settings outside of Daru.

Figure 2.14: The PNG and Western Province flags flying at Daru Airport. (Image credit: Tanya Diefenbach-Elstob)

Although a number of studies from Western Province have been mentioned, the majority of TB data from the region is limited to the South Fly District and Daru areas. There is one province-wide evaluation study (McBryde 2012), while other data is from Daru and the South Fly District (Aia et al. 2016; Bainomugisa et al. 2018; Pandey et al. 2018), and from PNG patients presenting at Australian health clinics in the Torres Strait (Gilpin et al. 2008; Simpson et al. 2011). Published TB data from the Middle Fly District of Western Province is limited to the province-wide evaluation study (McBryde 2012), and a molecular investigation of TB diagnosis based on sputum smear slides (Guernier et al. 2018). Th evaluation study

included two sites in the Middle Fly District, estimating TB incidence to be 330 cases per 100,000 people per year at Balimo, and 381 cases per 100,000 people per year at Awaba, suggesting a substantial burden of TB disease in the district (McBryde 2012). The province-wide estimate of TB incidence was 549 cases per 100,000 people per year (McBryde 2012). This preliminary data provides a foundation for examining the TB burden in Western Province more closely. Furthermore, the data signals an urgent need for developing a better understanding of TB burden in the province (McBryde 2012).

2.8. Conclusion

The high burden of disease in PNG caused by TB makes national TB control critical. However, TB control across the country is made more challenging by geographic and access difficulties, limited resources, inadequate numbers of healthcare personnel, and the strong influence of sociocultural factors. Although there is a body of literature describing the epidemiology of TB in various regions of PNG, for other areas little is known. For these settings, understanding the burden of disease, the extent of drug resistance, diagnostic accuracy, and the challenges of treatment access, will be essential to the aim of locally-achievable TB management and care.

CHAPTER 3

THE EPIDEMIOLOGY OF TUBERCULOSIS IN PATIENTS DIAGNOSED AT BALIMO DISTRICT HOSPITAL

The majority of this chapter forms the basis of a manuscript published in *Tropical Medicine & International Health*, with publication details as follows:

> Diefenbach-Elstob T, Graves P, Dowi R, Gula B, Plummer D, McBryde E, Pelowa D, Siba P, Pomat W and Warner J (2018) The epidemiology of tuberculosis in the rural Balimo region of Papua New Guinea. *Tropical Medicine & International Health*. In Press. https://doi.org/10.1111/tmi.13118 © 2018 John Wiley & Sons Ltd

Some data that was not published in the manuscript has been included in the thesis chapter. In addition, the thesis chapter has been updated for clarity and currency, and to incorporate comparison data from the recently published article describing the countrywide TB burden in PNG (Aia et al. 2018), that had not been published at the time our manuscript was submitted.

3.1. Introduction

Tuberculosis (TB) is an urgent health concern in Papua New Guinea (PNG). In 2016, incidence was estimated at 432 cases per 100,000 people per year, which in a population of approximately 8.1 million, equated to almost 35,000 people with TB (World Health Organization 2017a). The burden of TB cases resistant to standard treatment is also substantial, with drug resistance estimated to occur in 3.4% of new cases, and 26% of retreatment cases (World Health Organization 2017a).

Western Province is the largest province in PNG by area. The province has three districts – North Fly, Middle Fly, and South Fly (Figure 3.1). Middle Fly District, the most populous of the three districts, includes five local level government (LLG) areas. These are Balimo Urban, Gogodala Rural, Bamu Rural, Lake Murray Rural, and Nomad Rural (Figure 3.1). Balimo District Hospital (BDH) primarily serves the Balimo Urban and Gogodala Rural LLGs, and to a lesser extent the Bamu Rural LLG. In this study, the area served by BDH is generally referred to as the 'Balimo region', while the term 'Gogodala region' refers to the combined Balimo Urban and Gogodala Rural LLGs only.

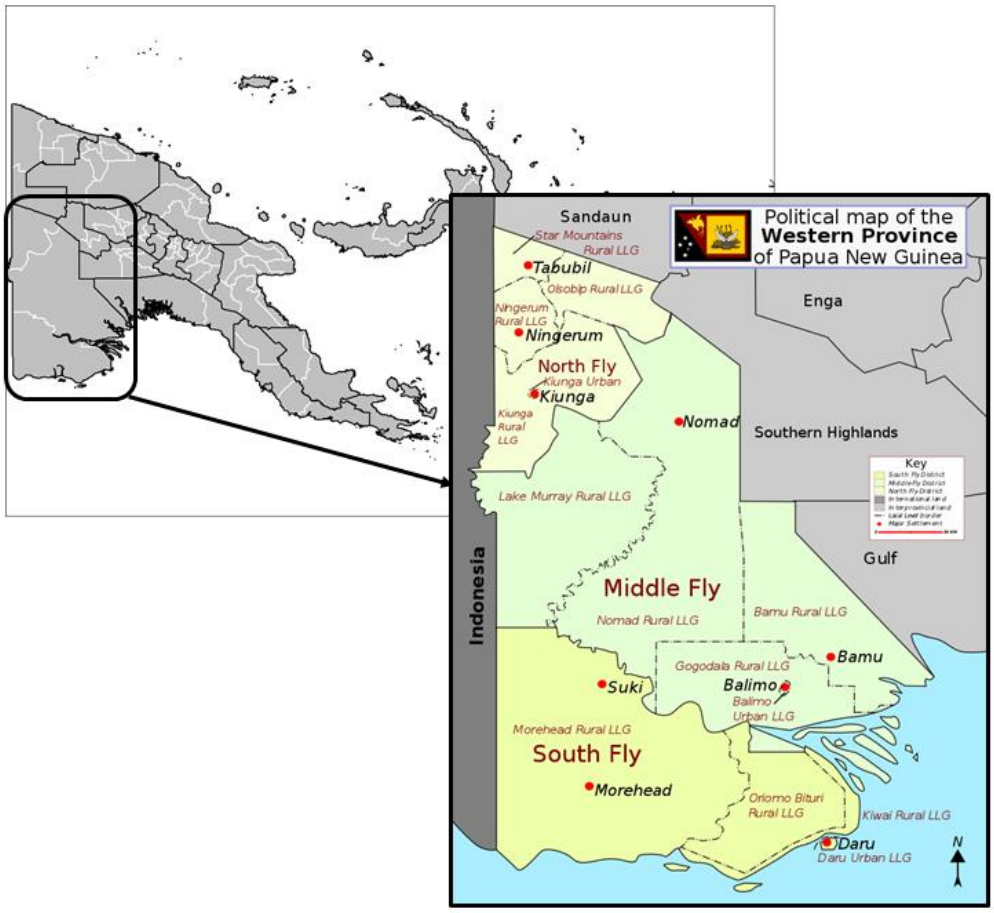


Figure 3.1: Western Province of Papua New Guinea (PNG), showing the North Fly, Middle Fly, and South Fly Districts. Balimo is located in the south-east of the Middle Fly District, and the provincial capital of Daru is on an island to the south-east of the provincial mainland. (Image attributions: (1) By Keith Edkins (Derived from File: Papua_New_Guinea_Districts.png) [Public domain], via Wikimedia Commons; (2) By Alaisd [Public domain], from Wikimedia Commons)

In Western Province, TB incidence has been estimated at 549 cases per 100,000 people per year (McBryde 2012). Drug resistance has been identified in 25% of Western Province-based TB patients presenting at Australian health clinics in the Torres Strait (Gilpin et al. 2008; Simpson et al. 2011), although patients presenting at these clinics may be more likely to have advanced disease. More recently, a multi-site study that included two sites in Western Province found multidrug-resistant TB (MDR-TB) in 34.1% of Daru Hospital TB cases (Aia et al. 2016). Cases with rifampicin (RIF) mono-resistance were also identified, including 7.3% of those from Daru, and 5.6% of those from Tabubil in the North Fly District (Aia et al. 2016) (Figure 3.1). However, data about the burden of TB in other areas of the province are scarce, particularly outside the South Fly District. Only one study examined TB in the province more broadly; this was an evaluation study which assessed the risks of TB across the province, and concluded that the provincial burdens of TB and MDR-TB were much higher than official estimates, and that improvements within the TB control programme were essential to limit the spread of drug resistance (McBryde 2012).

A small number of health facilities across Western Province are able to provide TB diagnosis and treatment. However, the lack of roads and cost of travel makes access difficult (Diefenbach-Elstob et al. 2017). Furthermore, TB patients with evidence of drug resistance, based on a lack of response to first-line treatment, or persistent positive smear microscopy results, must travel to the provincial capital on the island of Daru to access second-line treatment. Daru is therefore at risk of becoming a hotspot for complicated TB cases, with high numbers of drug-resistant cases concentrated on a small and densely populated island that has already suffered from other health crises, including cholera (Horwood et al. 2014; National 2010).

The known high rate of drug-resistant TB (DR-TB) cases in Daru, combined with the lack of research describing TB across the province more broadly, means there is an urgent need for epidemiological data describing TB patients from other areas of Western Province. This study describes the epidemiology of TB in the patient cohort of BDH located in the Middle Fly District of Western Province, PNG.

3.2. Materials and Methods

3.2.1. Study setting and patient characteristics

Balimo, located on the Aramia River floodplain in the Middle Fly District of Western Province, is a town of approximately 4400 people, and the urban centre of the Gogodala Rural LLG area (National Statistical Office 2014). Numerous small villages in the Gogodala Rural LLG area have a combined population of approximately 33,000 people (National Statistical Office 2014). There are very few roads outside of Balimo town, and travel is predominantly by foot, boat, or canoe (Figure 3.2).

Figure 3.2: Aerial view of the southern region of Western Province. Travel in the region is primarily by air, boat, or foot, and there are very few roads. (Image credit: Tanya Diefenbach-Elstob)

BDH is the primary health facility in the Middle Fly District capable of diagnosing TB and initiating treatment. As a district hospital, BDH is classified as a level four facility, based on the National Health Service Standards (Government of Papua New Guinea 2011a). This comprehensive guideline outlines minimum quality standards for patient care, leadership and management, human resources management, information systems, the environment, and improving performance (Government of Papua New Guinea 2011b). A number of

smaller health facilities in the Gogodala Rural LLG and Bamu Rural LLG areas are able to commence patients on TB treatment (see Appendix 2). However, these facilities can only diagnose TB clinically, and sputum or other clinical specimens should be sent to Balimo for laboratory confirmation. At BDH, TB diagnoses are primarily passive, based on symptomatic patients seeking care at the hospital through self-referral, or onwards referral from health workers at a peripheral health centre or aid post.

3.2.2. Diagnostic and treatment outcome definitions

BDH has not had the services of a resident medical officer since the 1990s. The only method available to confirm TB diagnoses is Ziehl-Neelsen (ZN)-stained smear microscopy, and X-ray was not available at the time of this study. As such, the majority of TB patients recorded in this study have received clinical diagnoses led by resident health extension officers (HEOs) trained in the management of drug-susceptible TB cases, and without laboratory confirmation of infection. In this region, the criteria for TB diagnoses and treatment outcomes are based on the PNG National TB Management Protocol and the WHO definitions (Department of Health 2011; World Health Organization 2013a). Based on these guidelines, all people for whom a health worker has decided to give a full course of TB treatment are recorded as a TB case (Department of Health 2011). This occurs regardless of laboratory confirmation of infection, or whether the diagnosis is pulmonary or extrapulmonary TB. A pulmonary TB diagnosis may be indicated by symptoms such as prolonged cough (more than 2 – 3 weeks), fever, weight loss, and night sweats; or in extrapulmonary TB by signs and symptoms including lymphadenitis, loss of function in the lower limbs, headache, or mental confusion, according to the PNG National TB Management Protocol (Department of Health 2011).

Standardised TB treatment in the region is available only for drug-susceptible TB cases. Six months of treatment is provided using fixed dose combination (FDC) drugs (Figure 3.3). Category 1 treatment is used for new TB patients, and is comprised of a two-month intensive phase with RIF, isoniazid (INH), ethambutol (EMB), and pyrazinamide (PZA); followed by a four-month continuation phase with RIF and INH only (World Health Organization 2010).

Category 2 treatment is indicated for retreatment patients (those classified as relapse, treatment after default, treatment after failure, or other), and includes a three-month intensive phase followed by a five-month continuation phase (Department of Health 2011). This regimen includes daily streptomycin (STR) injections for the first 56 days, and EMB is included in the continuation phase (Department of Health 2011). In Balimo medications for DR-TB are not available, and patients who may have drug resistance must travel to Daru for diagnosis and treatment.

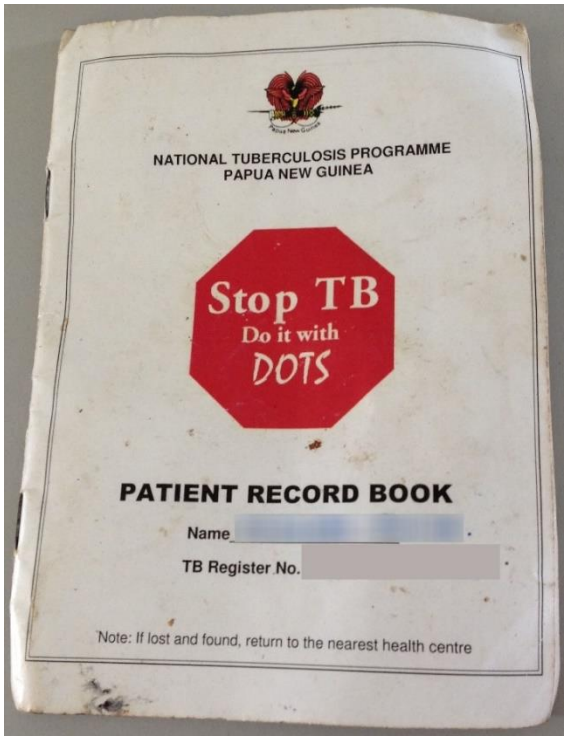


Figure 3.3: A patient record book used for details of a patient's TB diagnosis and treatment in PNG. (Image credit: Tanya Diefenbach-Elstob)

Treatment outcomes are defined in Table 3.1, based on the PNG National TB Management Protocol (Department of Health 2011). In addition, binary categorisation of treatment success and treatment failure has been used to define treatment outcomes in this study (Table 3.1).

Table 3.1: Definitions used for treatment outcomes, based on the PNG National TB Management Protocol (Department of Health 2011).

Group (this study)	Treatment outcome	Definition
Treatment success	Cured	Initially sputum smear-positive, but smear-negative in last month of treatment, and on at least one previous follow-up occasion
	Treatment complete	Completed treatment, but not classified as cure or treatment failure
Treatment failure	Treatment failure	Smear positive at five months or later, or at two months if initially smear negative
	Died	Death for any reason during treatment
	Default	Treatment interruption for two or more consecutive months

3.2.3. Data collection, transcription, and editing

Patient data were primarily obtained from the TB patient register (hereafter called the TB register) held at BDH, including demographic, clinical, and laboratory details of all patients diagnosed with TB and commenced on treatment at the hospital during the period 26th April 2013 to 25th February 2017. All pages of the register books available at the time of two study visits (April 2016 and February 2017) were transcribed into the TB study spreadsheet in Microsoft Excel. At the second study visit, the TB register was studied again, to update treatment outcomes added since the first visit.

In addition to the TB register, patient data were obtained from a secondary source – the BDH TB laboratory database. This database is a register of all clinical samples investigated for TB at BDH using ZN-stained smear microscopy, and the results of those investigations.

The TB register records were matched with patients recorded in the BDH TB laboratory database, based on name and demographic data, and any additional demographic or laboratory data were added to the TB study spreadsheet. For minor discrepancies (e.g. similar but not exact age), the TB register was assumed to be correct for demographic information. For any discrepancies in reported microscopy results, the laboratory database was assumed to be correct, as microscopy results are obtained directly from the laboratory database maintained by the BDH laboratory technician, Mr Daniel Pelowa (Figure 3.4).

Additional microscopy results included in the laboratory database, but not in the patient register, were added to the register data. This occurred due to some information in the patient register not always being up-to-date, especially in the case of follow-up microscopy results. However, the majority of patients (1294/1614, 80.2%) could not be matched to the laboratory database due to both a high proportion of clinical diagnoses and limited overlap of the data sources.

Figure 3.4: Laboratory technician Mr Daniel Pelowa at the BDH laboratory. (Image credit: Jeffrey Warner)

3.2.4. Data cleaning

In this region, people are often uncertain of their age, and there was evident bias towards reporting of even-numbered ages (see Appendix 3). For this reason, age range categories were used, and where multiple different ages were recorded (e.g. in the TB register and the

laboratory database) people were placed in the age category that matched the average of the reported ages.

Patient records frequently listed more than one town or village as the residential address. In these cases, the first location listed in the TB register was assumed to be the current residential address. The rationale for this assumption was that the recorded residential address is used to locate the patient if required. Subsequent towns or villages listed were assumed to be secondary residential addresses, such as the location of a person's garden, or their home village.

Some adjustments were made for the recording of primary residential addresses, as follows:

- Localities within a town or village were adjusted to show the main village as the primary residential address. For example, Adiba Sub-Health Centre would be recorded as Adiba; while Page, which is a ward in Balimo town, would be recorded as Balimo.
- There are two villages named Saweta. Based on advice from Sister Bisato Gula (a senior nurse at BDH, and the acting district TB coordinator), patients who listed both Waligi and Saweta were recorded as Waligi (a village close to Balimo), while patients who listed only Saweta were recorded as Saweta (a village near Awaba).

Finally, where treatment commencement dates were missing, the treatment year and month were assumed to be the same as the patient registration date.

3.2.5. Formulation of additional categories

An additional category for language group was added to the data. This was based on the first village given in the address category. If Balimo was listed first, then the language group was instead based on the second address if one was given, as Balimo is an urban centre with a greater mix of people from the surrounding regions. For patients who listed a logging camp (Kamusie, Panakawa, or Sasareme) as their address, the language group was based on the second address if one was given. The justification for this decision was that logging camp

workers originate from a wide range of villages, and people from certain villages are more likely to work at one particular logging camp but not another. If no second address was given, the language group was recorded as 'unknown'. Boundaries and included villages for the language groups were determined based on information available from the Summer Institute of Linguistics (2004) and Lewis (2009). It should be noted that the language group categorisations in this study do not necessarily reflect the language spoken by each patient with certainty, but rather are intended to indicate the diversity of language groups in the region from which patients in this study originate.

3.2.6. Data analysis

Statistical analyses were undertaken for 1518 of the 1614 patients recorded in the TB register. The 96 excluded patients were diagnosed through non-routine active case detection activities undertaken in the Bamu region, and were thus not considered to be representative of the usual patient cohort seen at BDH. These 96 patients are described separately in the results (see Section 3.3.6). Note that unless stated otherwise, any references to 'all' patients in the subsequent text of this chapter refer to all patients except the 96 excluded patients.

Incidence of reported cases was estimated for the Gogodala region only, which consists of Balimo Urban LLG and Gogodala Rural LLG. Incidence was based on case numbers for new patients commenced on treatment in all full months and years of the study period. The total population for the two Gogodala LLG regions was used as the denominator, based on the 2011 census data (National Statistical Office 2014). The most recent estimate of population growth in the province (2.5% per annum for the 2000 – 2011 period) was used to estimate population for all years subsequent to 2011 (National Statistical Office 2013), as shown in Table 3.2. The monthly trend of reported TB cases was based on the month of treatment commencement.

Table 3.2: Actual and calculated population figures for the combined Balimo Urban and Gogodala Rural LLGs in 2011 – 2017.

Year	Population	Notes
2011	37,427	Actual census figure
2012	38,363	Calculated (2.5% population growth)
2013	39,322	Calculated (2.5% population growth)
2014	40,305	Calculated (2.5% population growth)
2015	41,313	Calculated (2.5% population growth)
2016	42,346	Calculated (2.5% population growth)
2017	43,405	Calculated (2.5% population growth)

Demographic and clinical categories were tabulated. Chi-square analyses were used to assess differences between patient groups based on TB presentation (pulmonary or extrapulmonary TB, with concurrent or unknown presentations excluded), and LLG area (combined Balimo Urban LLG and Gogodala Rural LLG, compared with Bamu Rural LLG, with any patients not from these regions excluded). In the analysis of LLG groups, the 96 previously excluded patients were included in the Bamu Rural LLG group, as this analysis was intended to directly compare the demographic and infection characteristics of these patients.

The monthly trend of TB patient diagnoses was assessed based on the month treatment was commenced. A bar chart was used to show the total number of cases per month, and the Chi-square goodness-of-fit test was used to analyse case numbers per month. Line graphs were used to show changes in the proportion of patients in the sex, incoming patient status, TB type, LLG area, and treatment outcome categories over time.

Predictors of treatment failure were analysed using univariate and multivariate logistic regression, based on binary categorisation of treatment success (cured and treatment complete outcomes) or treatment failure (treatment default, death, and treatment failure outcomes). Excluded patients were those with a ‘transfer out’ status (n = 127) or unknown outcome (n = 293); as well as those patients commenced on treatment in the last six full months of the study period (August 2016 to February 2017) (n = 368), as insufficient time

had elapsed for these patients to have a treatment outcome recorded. All variables included in the univariate model were also included in the multivariate model.

Tabulations, charts, and statistical analyses were performed using Stata/IC, version 14. Confidence intervals were calculated using the AusVet 'Confidence limits for a proportion' calculator (Sergeant 2017). Specific and direct standardised incidence rates were calculated using the Epi_Tools spreadsheet (LaMorte 2006). The pie chart and line graphs were created using GraphPad Prism 7.

3.3. Results

3.3.1. Incidence and distribution of the reported tuberculosis patients

The TB patient numbers and the incidence of reported cases for patients commenced on treatment across the study period are shown in Figure 3.5. The average yearly reported incidence for the three complete years of the study period was 727 TB cases per 100,000 people per year (Table 3.3).

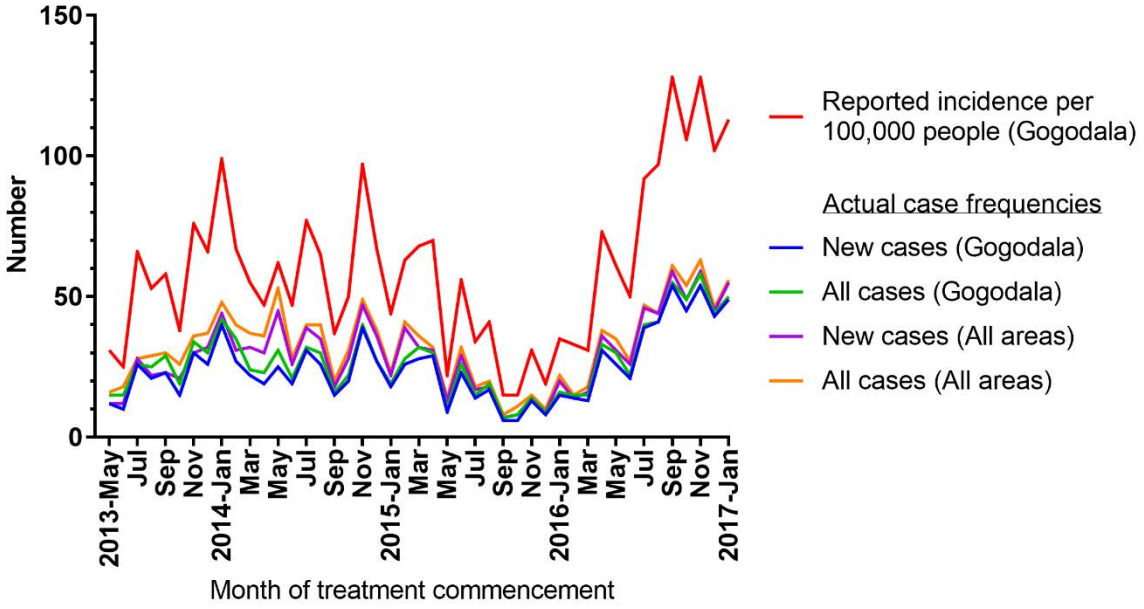


Figure 3.5: Actual case frequencies, and incidence of reported new cases (per 100,000 people) per month for TB across the study period. Cases in the Gogodala region include the Balimo Urban and Gogodala Rural LLG areas only.

Table 3.3: Case numbers and incidence of reported cases per 100,000 people per year for new TB patients in the Gogodala region.

Year	n	Calculated population	Reported incidence (per 100,000)
2014	310	40,305	769
2015	197	41,313	477
2016	396	42,346	935
2014 – 2016	903		727 (average)

n: number

The distributions of patient demographic and clinical data are shown in Table 3.4. Of note are the large proportion of cases in children (25.0%, 95% CI 22.9 – 27.2), and that 77.1% (95% CI 75.0 – 79.2) of all TB patients had a diagnosis of extrapulmonary TB. The site of extrapulmonary infection was reported for 275 TB patients. The distribution of sites is shown

in Figure 3.6, with the majority localised to the glands or lymph nodes (n = 108, 37.6%, 95% CI 32.2 – 43.4); or the spine (n = 88, 30.7%, 95% CI 25.6 – 36.2).

The 2014 – 2016 average specific and direct standardised rates per 100,000 people per year for sex are shown in Table 3.4, according to the national population proportion for PNG from the 2011 census (National Statistical Office 2015). Based on the standard population proportion, the number of cases in females was smaller than the reported incidence based on their proportion of the population, while the number of cases in males was higher than the reported incidence based on their proportion of the population.

Table 3.4: Patient demographic and clinical data for sex, age category, LLG area, TB type, and patient status.

	Variable	n	% (95% CI)	Specific and direct standardised incidence rates per 100,000 people (2014 - 2016 avg)
Sex	Female	722	47.6 (45.1 – 50.1)	709 (specific)
	Male	793	52.2 (49.7 – 54.7)	742 (specific)
	Unknown	3	0.2 (0.1 – 0.6)	726 (standardised)
Age category	0 – 14 years	379	25.0 (22.9 – 27.2)	
	15 – 24 years	254	16.7 (14.9 – 18.7)	
	25 – 34 years	259	17.1 (15.3 – 19.0)	
	35 – 44 years	211	13.9 (12.3 – 15.7)	
	45 – 54 years	192	12.6 (11.1 – 14.4)	
	55 – 64 years	138	9.1 (7.7 – 10.6)	
	65+ years	31	2.0 (1.4 – 2.9)	
	Unknown	54	3.6 (2.7 – 4.6)	
LLG area	Balimo Urban	252	16.6 (14.8 – 18.6)	
	Gogodala Rural	1010	66.5 (64.1 – 68.9)	
	Bamu Rural	199	13.1 (11.5 – 14.9)	
	Kiwai Rural	6	0.4 (0.2 – 0.9)	
	Morehead Rural	4	0.3 (0.1 – 0.7)	
	Nomad Rural	1	0.1 (0.1 – 0.4)	
	Unknown	46	3.0 (2.3 – 4.0)	
TB type	Pulmonary	321	21.1 (19.2 – 23.3)	
	Extrapulmonary	1171	77.1 (75.0 – 79.2)	
	Both	6	0.4 (0.2 – 0.9)	
	Unknown	20	1.3 (0.9 – 2.0)	
Patient status	New	1375	90.6 (89.0 – 91.9)	
	Treatment after relapse	26	1.7 (1.2 – 2.5)	
	Treatment after failure	11	0.7 (0.4 – 1.3)	
	Treatment after default	77	5.1 (4.1 – 6.3)	
	Transfer in	6	0.4 (0.2 – 0.9)	
	Other	2	0.1 (0.0 – 0.5)	
	Unknown	21	1.4 (0.9 – 2.1)	
Treatment category	Category 1	1376	90.7 (89.1 – 92.0)	
	Category 2	122	8.0 (6.8 – 9.5)	
	Unknown	20	1.3 (0.9 – 2.0)	

avg: average; CI: confidence interval; LLG: local level government; n: number; TB: tuberculosis

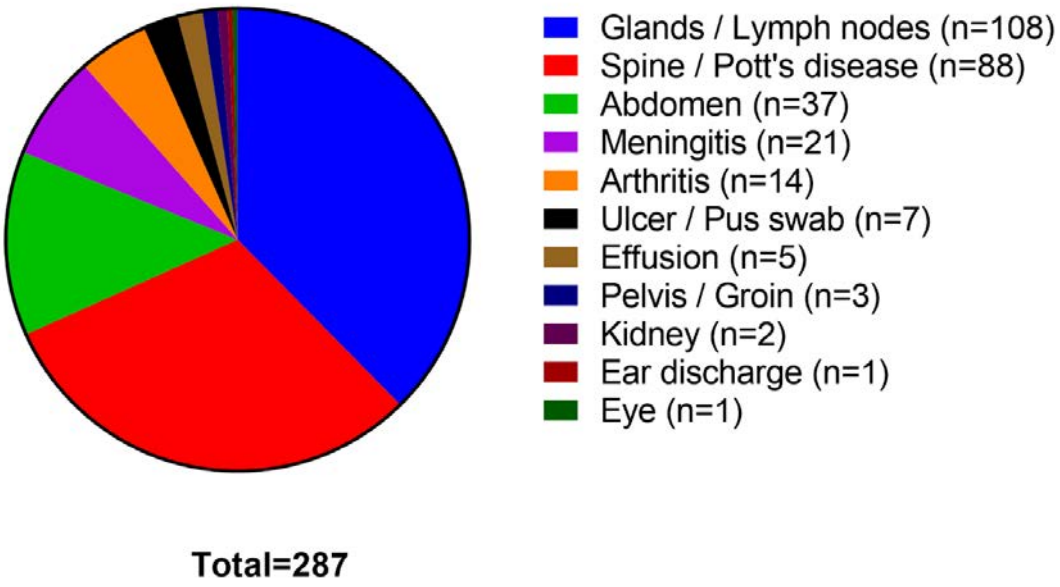


Figure 3.6: Distribution of extrapulmonary TB site of infection. The site was known for 275 TB patients, with a total of 287 sites recorded as some patients had more than one site recorded.

The distribution of TB cases by age and sex is shown in Figure 3.7. In the age categories for 25 years and over, the categories by sex contained similar numbers of cases. However, for cases aged up to 24, there were substantially more male cases in both the 0 – 14 and 15 – 24 years age categories.

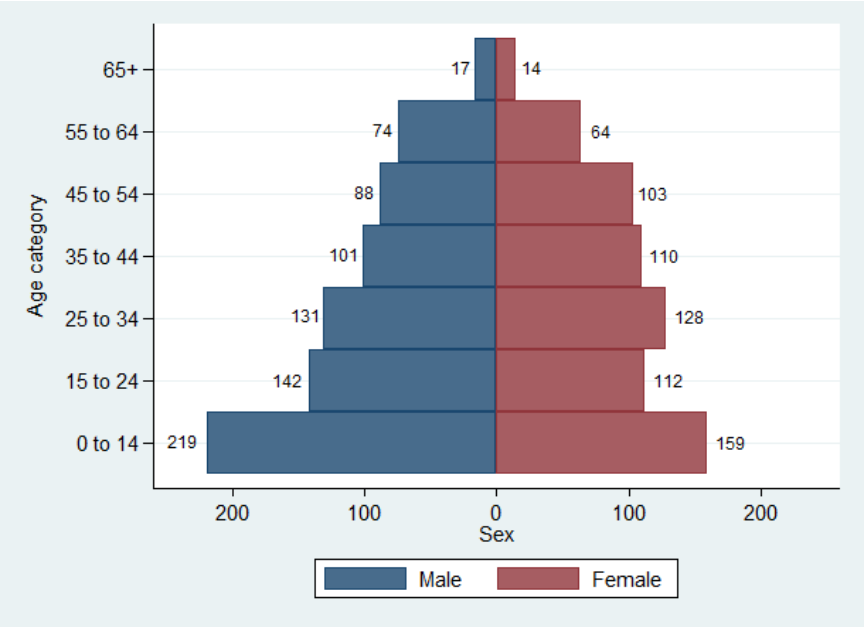


Figure 3.7: Distribution of TB cases by age and sex. Substantially more male cases were evident in the 0 – 14 and 15 – 24 years age groups.

The residential address reported by patients included 103 towns and villages across five PNG provinces. The largest proportion of cases came from Balimo (n = 244), however other locations with substantial numbers of cases included Adiba (n = 59), Aketa (n = 47), Kimama (n = 162), Pisi (n = 73), and Widama (n = 48). The residential address was unknown for 33 patients, due to insufficient information or no address being recorded. Patients came from 19 different language group regions, with the majority from Gogodala region villages. However, substantial numbers of patients also came from villages in the Bamu (n = 213), Dibiyaso (n = 95), and Mubami (n = 35) language group regions. The association between geography and TB distribution will be explored further in Chapter 5.

3.3.2. Smear microscopy results for tuberculosis patients

Pre-treatment ZN smear microscopy results were recorded for 359 of the 1518 TB patients (23.6%, 95% CI 21.6 – 25.9), including 101/1171 (8.6%, 95% CI 7.2 – 10.4) patients diagnosed with extrapulmonary TB, 250/321 (77.9%, 95% CI 73.0 – 82.1) with pulmonary TB, 4/6

(66.7%, 95% CI 30.0 – 90.3) with concurrent infection, and 4/20 (20.0%, 95% CI 8.1 – 41.6) with unknown infection. Although the majority of smear results were obtained from sputum samples, a small proportion of extrapulmonary TB smear results were from other clinical specimens (e.g. discharge). For pulmonary TB patients, 65.7% (n = 211) were smear-positive, and 12.1% (n = 39) were smear-negative; no smear result was recorded for the remaining pulmonary TB patients. The microscopy results for each of the patient groups are shown in Table 3.5. Where more than one smear result was recorded, only the highest smear grade is shown here.

Table 3.5: Pre-treatment ZN smear microscopy results for patients diagnosed with TB at BDH, based on the highest smear grade recorded for each patient.

	Extrapulmonary, n (%)	**Pulmonary, n (%)**	**Concurrent, n (%)**	**Unknown, n (%)**
3+	4 (0.3)	100 (31.2)	0 (0)	1 (5.0)
2+	2 (0.2)	58 (18.1)	1 (16.7)	0 (0)
1+	4 (0.3)	32 (10.0)	0 (0)	1 (5.0)
Scanty	0 (0)	19 (5.9)	0 (0)	0 (0)
Positive[1]	0 (0)	2 (0.6)	0 (0)	0 (0)
NAFB	91 (7.8)	39 (12.1)	3 (50.0)	2 (10.0)
Unsatisfactory	1 (0.1)	0 (0)	0 (0)	0 (0)
Unknown	1 (0.1)	0 (0)	0 (0)	0 (0)
No smear	1068 (91.2)	71 (22.1)	2 (33.3)	16 (80.0)
Total	**1171 (100.0)**	**321 (100.0)**	**6 (100.0)**	**20 (100.0)**

[1]Grade unknown; n: number; NAFB: no acid-fast bacilli (negative)

3.3.3. Distribution of tuberculosis cases over time

TB patient data for complete years were available for 2014, 2015, and 2016. Case numbers for all TB patients commenced on treatment in these years are shown in Table 3.6. Case numbers were similar in 2014 and 2016, but substantially lower in 2015.

Table 3.6: TB case numbers recorded in 2014, 2015, and 2016 for patients from all regions.

Treatment year	n	%
2014	460	38.7
2015	260	21.9
2016	470	39.5
Total	**1190**	**100.00**

n: number

Variability in the TB patient numbers was evident over time (Figure 3.8). However, there was no pattern evident in the distribution of TB patients by month across the three complete years of the study. When grouped by month, November had the largest number of cases (Figure 3.9), although no significant difference was found in TB patient numbers among the calendar months ($X^2 = 13.01$, $p = 0.293$).

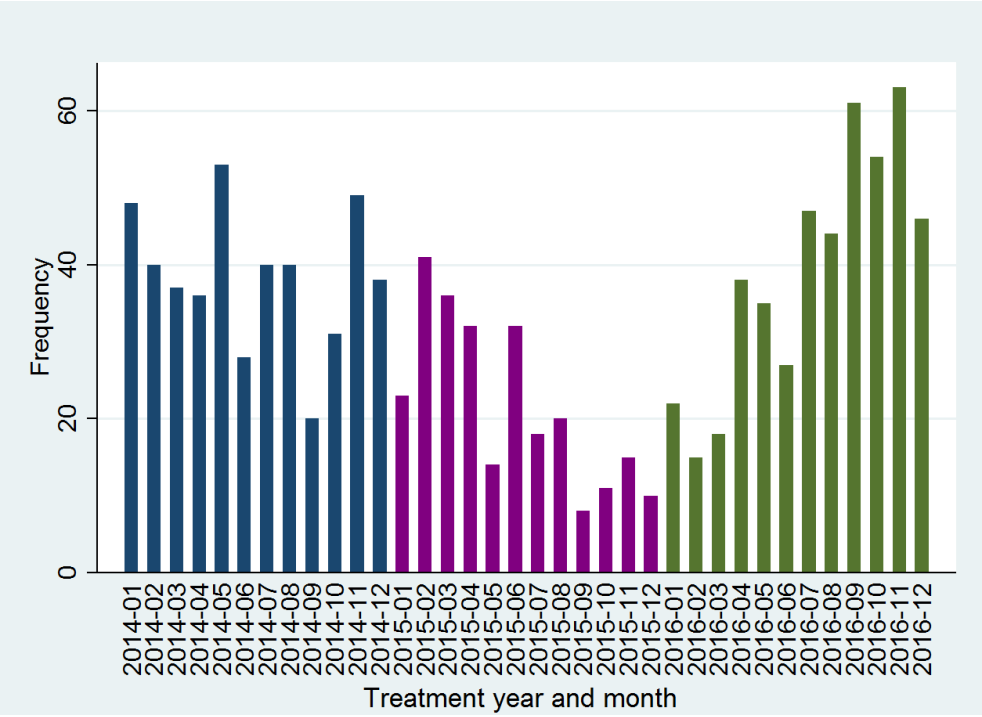


Figure 3.8: Distribution of all TB cases by month and year in the three complete years of the study period (2014 – 2016).

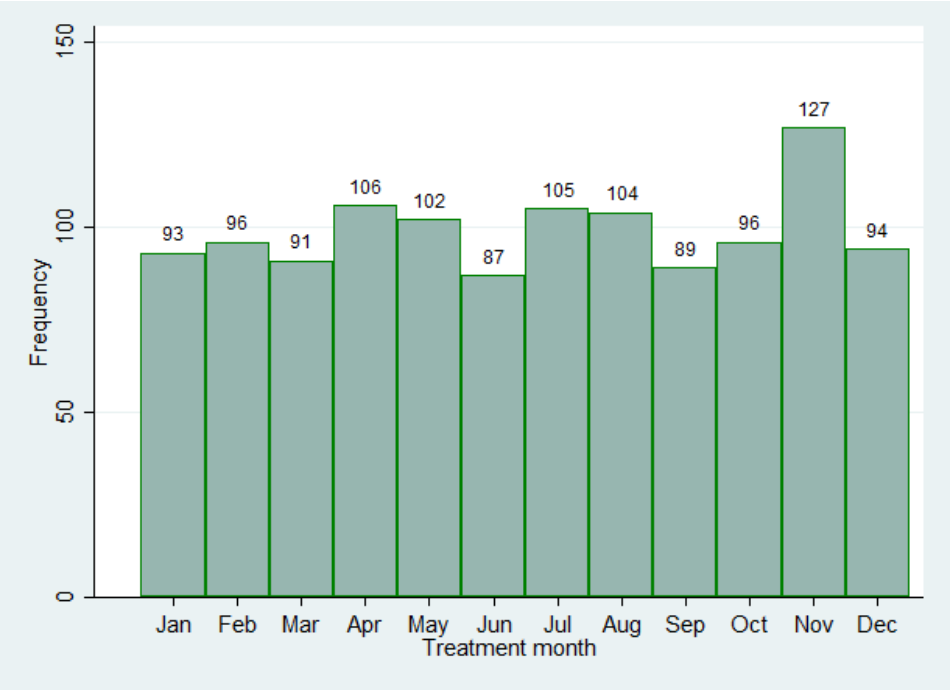


Figure 3.9: TB case numbers by month for all patients in the years 2014 – 2016.

The proportions of TB patients by sex are shown in Figure 3.10. Although distributions remained approximately equal over the full study period, substantially more male cases and fewer female cases were seen in the March 2015 – August 2015 period.

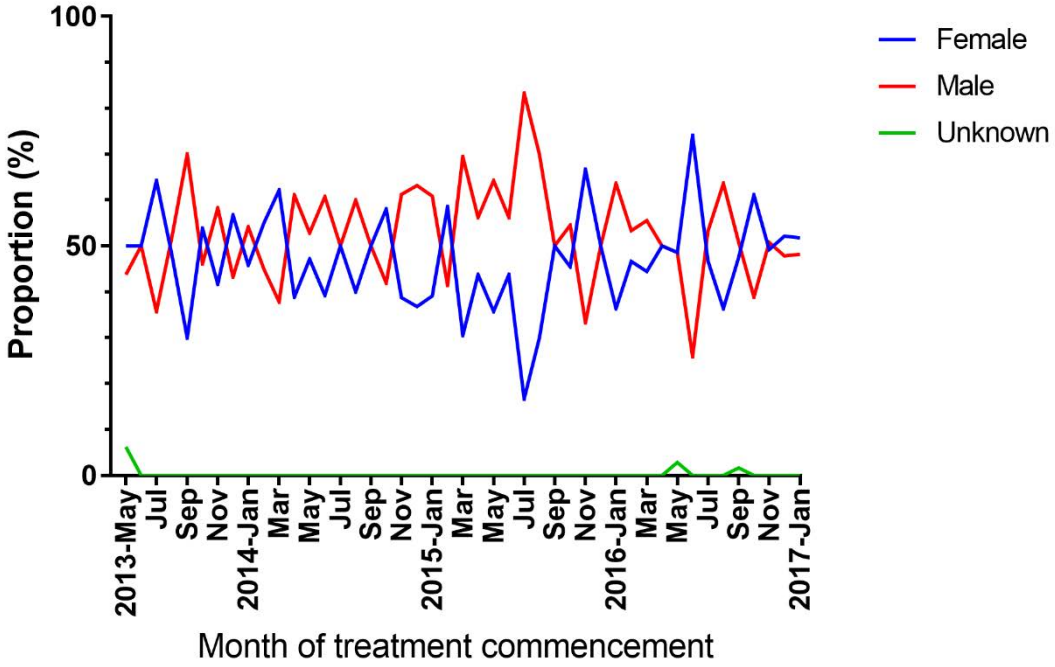


Figure 3.10: Distribution of TB cases by sex over the May 2013 – January 2017 period.

The incoming patient status distribution is shown in Figure 3.11. In general, new cases gradually increased while retreatment cases gradually decreased, with the exception of in October 2015.

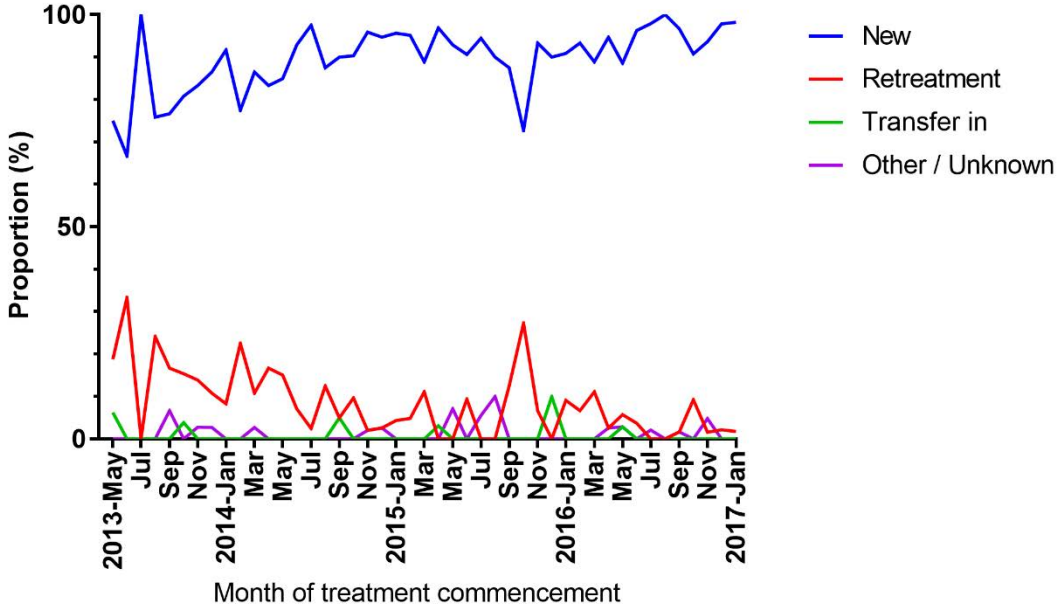


Figure 3.11: Distribution of TB cases by incoming patient status over the May 2013 – January 2017 period.

The distribution of TB patients by infection type is shown in Figure 3.12. The higher proportion of extrapulmonary TB patients seen in this study has been consistent across the full study period. However, it appears that pulmonary TB cases may have been higher earlier in the study period, while extrapulmonary cases appear higher more recently, although this observation requires further investigation.

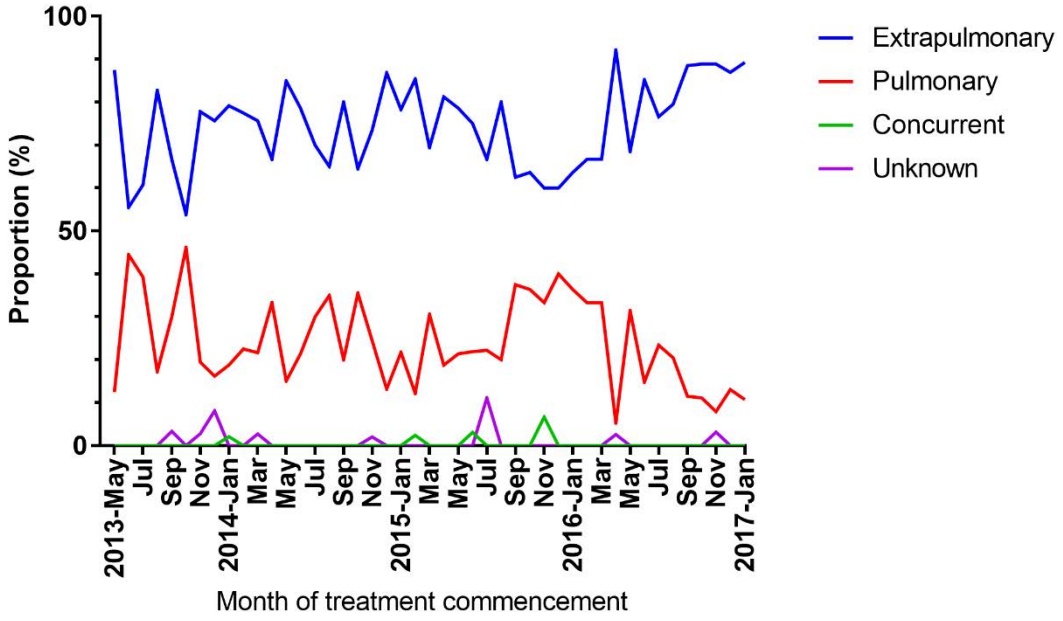


Figure 3.12: Distribution by TB patients by infection type over the May 2013 – January 2017 period.

The proportions of TB patients by LLG area are shown in Figure 3.13. Increased proportions of patients from the Bamu region were seen in March – May 2014, and February 2015. Although fluctuating, there appears to be an upward trend in Balimo Urban LLG patients in the final year of the study period.

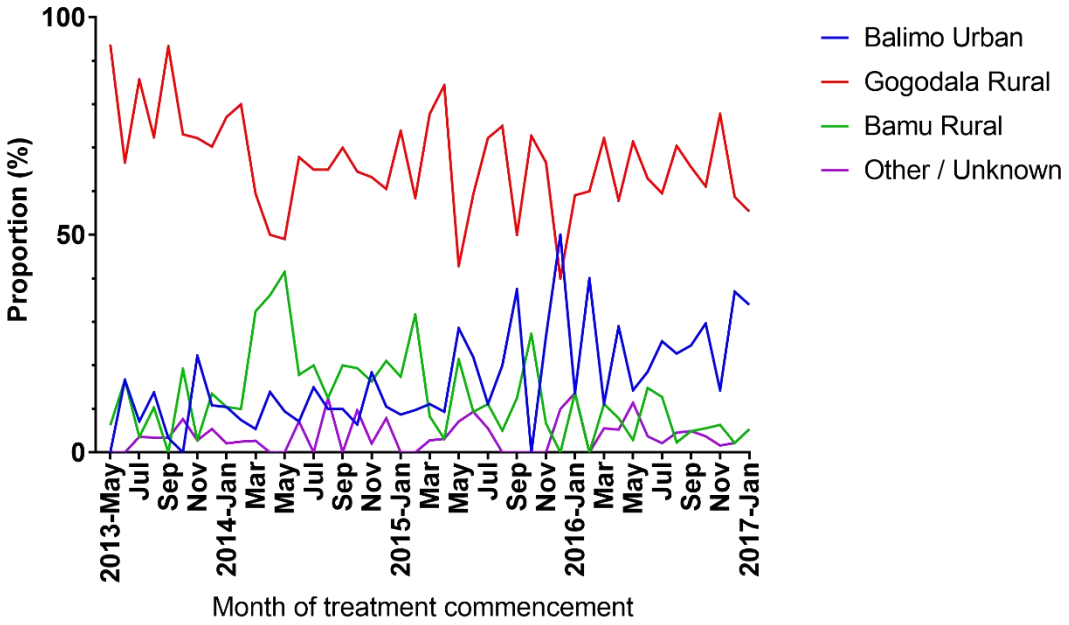


Figure 3.13: Distribution of TB cases by LLG over the May 2013 – January 2017 study period.

Proportions for treatment outcomes are shown in Figure 3.14. The reason for the large number of unknown outcomes early in the study period is unknown. However, the increasing number of unknown outcomes in the final six months likely reflects the access challenges generally associated with TB management in this region, which may delay patient presentation at BDH for post-treatment evaluation.

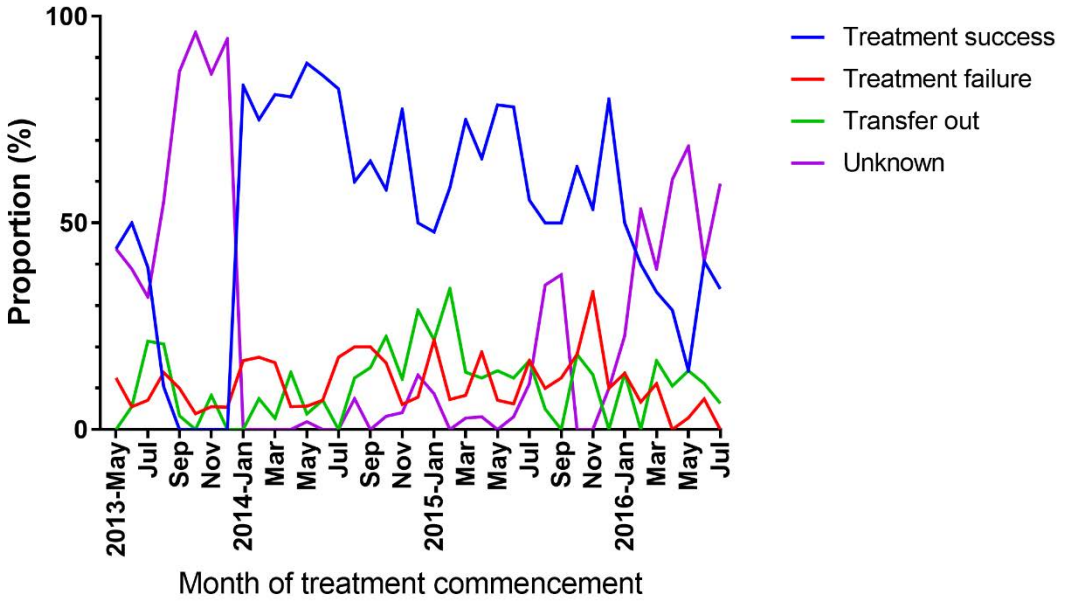


Figure 3.14: Distribution of treatment outcomes over the May 2013 – July 2016 study period. Note that this graph excludes the final six months of data shown in the other graphs, as insufficient time had elapsed for a treatment outcome to be known for these patients.

3.3.4. Analysis of pulmonary and extrapulmonary tuberculosis patients

The analysis comparing pulmonary and extrapulmonary patient groups (concurrent and unknown infection excluded) is shown in Table 3.7. There was a greater proportion of extrapulmonary TB patients in the 0 – 14 years age group ($X^2 = 117.45$, $p < 0.01$) and ages 55 years and over ($X^2 = 11.45$, $p < 0.01$); and a greater proportion of pulmonary patients in the 15 – 54 years age groups compared to both the 0 – 14 years and 55+ years age groups (Table

3.7). New patients were more likely to be diagnosed with extrapulmonary than pulmonary TB when compared to previously treated patients ($X^2 = 6.15$, $p = 0.01$) (Table 3.7).

Table 3.7: Chi-square analysis comparing pulmonary and extrapulmonary TB patient groups by sex, age, incoming patient status, LLG area, and treatment outcome.

Variable	Number	TB type	
		Pulmonary	Extrapulmonary
Sex	**n**	**n (%)**	**n (%)**
Female	706	141 (20.0)	565 (80.0)
Male	783	180 (23.0)	603 (77.0)
Total	**1489**	**321**	**1168**
			Pearson X^2 = 2.00, df = 1, p = 0.16
Age category	**n**	**n (%)**	**n (%)**
0 – 14 years	376	11 (2.9)	365 (97.1)
15 – 54 years	905	278 (30.7)	627 (69.3)
Total	**1281**	**289**	**992**
			Pearson X^2 = 117.45, df = 1, p < 0.01
15 – 54 years	905	278 (30.7)	627 (69.3)
55+ years	168	30 (17.9)	138 (82.1)
Total	**1073**	**308**	**765**
			Pearson X^2 = 11.45, df = 1, p < 0.01
Patient status	**n**	**n (%)**	**n (%)**
New	1363	283 (20.8)	1080 (79.2)
Previously treated	114	35 (30.7)	79 (69.3)
Total	**1477**	**318**	**1159**
			Pearson X^2 = 6.15, df = 1, p = 0.01
Treatment outcome	**n**	**n (%)**	**n (%)**
Treatment success	605	169 (27.9)	436 (72.1)
Treatment failure	119	25 (21.0)	94 (79.0)
Total	**724**	**194**	**530**
			Pearson X^2 = 2.43, df = 1, p = 0.12
LLG area	**n**	**n (%)**	**n (%)**
Balimo Urban	249	43 (17.3)	206 (82.7)
Gogodala Rural	995	223 (22.4)	772 (77.6)
Bamu Rural	192	45 (23.4)	147 (76.6)
Total	**1436**	**311**	**1125**
			Pearson X^2 = 3.52, df = 2, p = 0.17

df: degrees of freedom; LLG: local level government; n: number; TB: tuberculosis

3.3.5. Analysis of treatment outcomes

Of the 1518 recorded TB patients, 368 who commenced treatment during the final six months of the study period were not under treatment long enough to have a known treatment outcome. Of the remaining 1150 patients registered early enough to have completed the full six-month treatment period, the treatment outcome was unknown for 293 (25.5%, 95% CI 23.0 – 28.1). Treatment outcomes are shown in Table 3.8, for patient groups both including and excluding unknown treatment outcomes. Based on the overall treatment outcome categories for patients where the outcome was known, 611 patients (71.3%, 95% CI 68.2 – 74.2) had a treatment success outcome (cured or treatment complete), and 119 patients (13.9%, 95% CI 11.7 – 16.4) had a treatment failure outcome (default, failure, or died). A complete summary of all registered patients and treatment outcomes is shown in Figure 3.15.

Table 3.8: TB treatment outcomes for all patients who had completed the six-month treatment period. The data on the left detail distributions where the treatment outcome was known, and the data on the right detail distributions with the inclusion of unknown treatment outcomes.

Treatment outcome	**n**	**% (95% CI)**	**n**	**% (95% CI)**
Cured	90	10.5 (8.6 – 12.7)	90	7.8 (6.4 – 9.5)
Treatment complete	521	60.8 (57.5 – 64.0)	521	45.3 (42.5 – 48.2)
Default	47	5.5 (4.1 – 7.2)	47	4.1 (3.1 – 5.4)
Treatment failure	5	0.6 (0.3 – 1.4)	5	0.4 (0.2 – 1.0)
Died	67	7.8 (6.2 – 9.8)	67	5.8 (4.6 – 7.3)
Transfer out	127	14.8 (12.6 – 17.4)	127	11.0 (9.4 – 13.0)
Unknown	-	-	293	25.5 (23.0 – 28.1)
Total	**857**	**100.0**	**1150**	**100.0**

CI: confidence interval; n: number

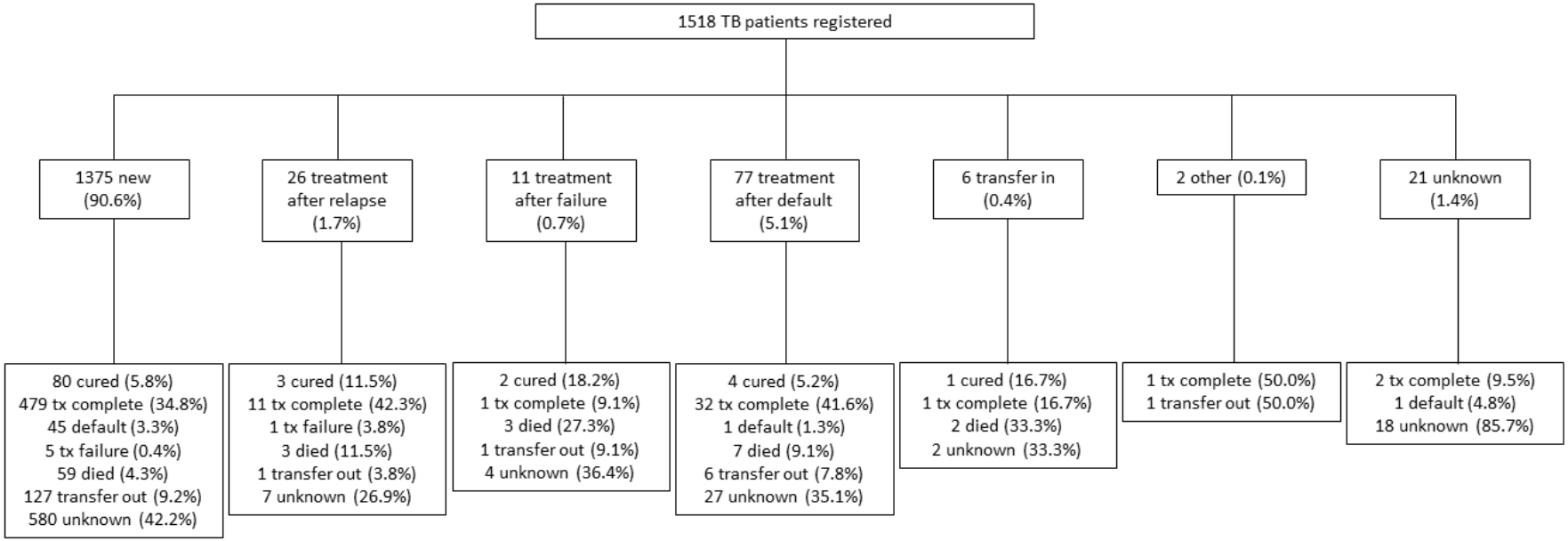


Figure 3.15: Incoming patient status and treatment outcomes for all TB patients registered at BDH during the study period. All unknown treatment outcomes are included, regardless of whether the patient had been under treatment long enough to have completed their course of medication.

The logistic regression results for the analysis of treatment outcomes are shown in Table 3.9. In the univariate analysis, residence in the Bamu Rural LLG was a predictor of treatment failure (OR = 3.00, p = 0.01), while in the multivariate analysis, residence in either the Gogodala Rural LLG or Bamu Rural LLG was a predictor of treatment failure (Gogodala Rural, OR = 2.49, p = 0.02; Bamu Rural, OR = 4.11, p < 0.01).

Table 3.9: Univariate and multivariate logistic regression examining predictors of treatment failure in all TB patients. A total of 680 complete observations were included in the multivariate model.

		Univariate			Multivariate	
Predictor variables		**n**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
Sex	Female	343	1.0		1.0	
	Male	385	1.45 (0.97-2.16)	0.07	1.45 (0.94-2.22)	0.09
Age	0 – 14 years	188	1.0		1.0	
	15 – 24 years	140	0.62 (0.34-1.15)	0.13	0.81 (0.42-1.56)	0.53
	25 – 34 years	132	0.75 (0.41-1.37)	0.36	0.90 (0.46-1.73)	0.75
	35 – 44 years	102	0.62 (0.31-1.23)	0.17	0.67 (0.31-1.43)	0.30
	45 – 54 years	78	0.84 (0.42-1.70)	0.64	1.10 (0.52-2.37)	0.80
	55 – 64 years	64	1.18 (0.59-2.37)	0.64	1.63 (0.78-3.41)	0.20
	65+ years	12	1.41 (0.36-5.46)	0.62	1.65 (0.42-6.52)	0.48
Patient status	New	655	1.0		1.0	
	Prev. treated	67	1.43 (0.77-2.68)	0.26	1.47 (0.77-2.79)	0.24
TB type	Pulmonary	194	1.0		1.0	
	EP	530	1.46 (0.91-2.35)	0.12	1.47 (0.86-2.50)	0.16
LLG area	Balimo	110	1.0		1.0	
	Gogodala	492	1.97 (0.99-3.93)	0.06	2.49 (1.15-5.40)	0.02
	Bamu	104	3.00 (1.36-6.64)	0.01	4.11 (1.70-9.96)	<0.01

CI: confidence interval; EP: extrapulmonary; LLG: local level government; n: number; OR: odds ratio; Prev: previously; TB: tuberculosis

3.3.6. Description of tuberculosis patients diagnosed through non-routine case-finding activities

A total of 96 TB patients from eight Bamu-region villages were diagnosed through collaborative outreach activities between BDH and Youth with a Mission (YWAM). All of these diagnoses were made in the period 11th to 18th March 2016. The majority of patients

were female (n = 56, 58.3%, 95% CI 48.3 – 67.7). More than half of patients were aged 0 – 14 years (n = 57, 59.4%, 95% CI 49.4 – 68.7) (Table 3.10). All patients were new TB diagnoses. Only two patients were diagnosed with pulmonary TB (2.1%, 95% CI 0.6 – 7.3). A treatment outcome was available for one patient, recorded as treatment complete.

Table 3.10: Age distribution of Bamu Rural LLG area TB patients diagnosed through BDH and YWAM collaborative outreach activities.

Age category	n	Percent
0 – 14 years	57	59.4
15 – 24 years	10	10.4
25 – 34 years	12	12.5
35 – 44 years	6	6.3
45 – 54 years	7	7.3
55 – 64 years	3	3.1
65+ years	1	1.0
Total	**96**	**100.0**

n: number

3.3.7. Comparison of demographic and diagnostic categories between Bamu- and Gogodala-region patients

Of the 1557 TB patients who came from the Bamu or Gogodala regions only, 1262 (81.1%, 95% CI 79.0 – 82.9) were from the Gogodala region (Balimo Urban LLG or Gogodala Rural LLG), while 295 (19.0%, 95% CI 17.1 – 21.0) were from the Bamu region (Bamu Rural LLG).

For the Gogodala region, a slight majority of patients were male (n = 659, 52.3%, 95% CI 49.6 – 55.1), while for the Bamu region, a slight majority were female (n = 151, 51.2%, 95% CI 45.5 – 56.8). However, there was not a significant difference in sex between the two regions ($X^2 = 1.19$, $p = 0.28$) (Table 3.11). Based on age categories, there was a significant difference between patients from the Bamu and Gogodala regions. In the Bamu region, patients were more likely to be aged 0 – 14 years, and less likely to be aged 55 years or older, when compared to the Gogodala region patients (Table 3.11).

When analysing the incoming patient status, there was no significant difference between the Gogodala and Bamu patient groups based on new or previously treated patient status (X^2 = 0.21, p = 0.65) (Table 3.11). There was a significant difference based on success or failure treatment outcomes between these two patient groups, with those from the Bamu region more likely to have a treatment failure outcome, and those from the Gogodala region more likely to have a treatment success outcome (X^2 = 3.74, p = 0.05) (Table 3.11). The analysis of TB type between the two regions was suggestive of patients from the Gogodala region being more likely to be diagnosed with pulmonary TB, and patients from the Bamu region being more likely to be diagnosed with extrapulmonary TB (X^2 = 3.69, p = 0.06) (Table 3.11).

Table 3.11: Chi-square analysis comparing TB patients from the Gogodala and Bamu regions by sex, age category, incoming patient status, TB type, and treatment outcome.

		Residential region	
		Gogodala region	**Bamu region**
Sex	**n**	**n (%)**	**n (%)**
Female	751	600 (79.9)	151 (20.1)
Male	803	659 (82.1)	144 (17.9)
Total	**1554**	**1259**	**295**
			Pearson X^2 = 1.19, df = 1, p = 0.28
Age category	**n**	**n (%)**	**n (%)**
0 – 14 years	420	283 (67.4)	137 (32.6)
15 – 54 years	914	774 (84.7)	140 (15.3)
Total	**1334**	**1057**	**277**
			Pearson X^2 = 52.36, df = 1, p < 0.01
15 – 54 years	914	774 (84.7)	140 (15.3)
55+ years	171	156 (91.2)	15 (8.8)
Total	**1085**	**930**	**155**
			Pearson X^2 = 5.04, df = 1, p = 0.03
Patient status	**n**	**n (%)**	**n (%)**
New	1419	1150 (81.0)	269 (19.0)
Previously treated	111	88 (79.3)	23 (20.7)
Total	**1530**	**1238**	**292**
			Pearson X^2 = 0.21, df = 1, p = 0.65
TB type	**n**	**n (%)**	**n (%)**
Pulmonary	313	266 (85.0)	47 (15.0)
Extrapulmonary	1219	978 (80.2)	241 (19.8)
Total	**1532**	**1244**	**288**
			Pearson X^2 = 3.69, df = 1, p = 0.06
Treatment outcome	**n**	**n (%)**	**n (%)**
Treatment success	598	517 (86.5)	81 (13.6)
Treatment failure	123	98 (79.7)	25 (20.3)
Total	**721**	**615**	**106**
			Pearson X^2 = 3.74, df = 1, p = 0.05

df: degrees of freedom; n: number; TB: tuberculosis

3.3.8. Co-infection of HIV and tuberculosis

Provider-initiated counselling and testing (PICT) for HIV is recommended for all TB patients and people being investigated for TB in PNG (Department of Health 2011). However, HIV testing was not routinely performed for TB patients at BDH at the time of this study. Of the 1614 patients recorded in the TB register, 74 (4.6%, 95% CI 3.7 – 5.7) had a HIV test outcome recorded. Of these, four were positive (5.4%, 95% CI 2.1 – 13.1), one had no result recorded, and the remainder were negative.

3.4. Discussion

This study has described the high burden of TB in the Balimo region of PNG, and has particularly highlighted a very high proportion of clinically-diagnosed extrapulmonary TB patients, a high burden of childhood TB, and the increased likelihood of poor treatment outcomes for people from rural areas.

3.4.1. The Gogodala region has a high burden of tuberculosis

The overall incidence of reported new cases for TB in the Balimo Urban and Gogodala Rural LLG areas, estimated here to range from 477 – 935 cases per 100,000 people per year, is substantially higher than the PNG-wide WHO estimates. In 2016, the WHO reported that 27,294 new TB cases were notified in an estimated population of 8.1 million people, equating to 337 case notifications per 100,000 people per year (World Health Organization 2018a). By further comparison, the Western Pacific Region reported 1,305,408 new cases in an estimated population of 1.9 billion people, equating to 69 case notifications per 100,000 people per year (World Health Organization 2018a).

The extremely high number of TB diagnoses emphasises the heavy burden of disease that TB causes in this region. Our estimate for the Balimo region is higher than the previous Western Province region incidence estimate of 549 cases per 100,000 people per year (McBryde 2012), as well as the more recent case notification rate of 674 cases per 100,000 people per

year (Aia et al. 2018). The South Fly District and the provincial capital of Daru have previously been noted for having a high burden of TB, and particularly DR-TB (Aia et al. 2016; Chandler 2016; Furin & Cox 2016; Gilpin et al. 2008; Kase et al. 2016; McBryde 2012; Simpson et al. 2011). A heavy burden of TB cases in the North Fly District has previously been observed, while more recent research described 18 cases of TB from Tabubil, with one of these identified as RIF-resistant (Aia et al. 2016; McBryde 2012). The data from the North Fly District, in combination with the Middle Fly District data described in our study, highlight the broad reach of TB across Western Province. Well-resourced health services across all three districts are essential to respond to TB across the province, and until that time, the burden on the urban capital Daru will continue.

In this study of the Balimo region, extrapulmonary TB accounted for more than 75% of TB diagnoses. Globally, extrapulmonary TB usually accounts for about 15% of TB cases, although in the WHO Western Pacific Region, extrapulmonary TB cases are reported to be as low as 8% (World Health Organization 2017a). The most recent nation-wide data for TB in PNG reported that 42.4% of cases were extrapulmonary, while 62.1% of notified cases in Western Province were extrapulmonary TB (Aia et al. 2018). The authors of one study from PNG have commented on the prevalence of extrapulmonary TB in the country, noting that both under-diagnosis and over-diagnosis are possible outcomes in settings where diagnoses are predominantly symptom-based (Karki et al. 2017). The large proportion of extrapulmonary TB diagnoses in the Balimo region requires further investigation. Greater understanding of the epidemiology of extrapulmonary TB in this setting is particularly important given the presence of severe forms such as spinal TB and meningitis.

The 1:1.1 ratio of female to male TB cases identified in this study differs from the PNG national, South-East Asia, and Western Pacific Region ratios, which in 2016 had estimated female:male incidence ratios of 1:1.7, 1:1.9, and 1:2.1 respectively (World Health Organization 2017a). Various factors have been suggested to contribute to the higher proportion of TB cases in men, including TB contacts, health-seeking behaviour, and smoking (Grandjean et al. 2011; Mason et al. 2016; Mason et al. 2017; Watkins & Plant 2006). In PNG, the prevalence of tobacco smoking in males is 37.3%, compared to only 14.5% in females (World Health Organization 2017c). As a result, it appears that smoking may not be a major

risk factor affecting the female:male ratio of TB patients in the Balimo setting, given the more equal proportion of TB cases. However, it should be noted that cooking fires are used extensively in this setting, which may affect more females and children. This observation is notable as the use of biomass stoves has been associated with TB in other settings, including India, Nepal, and Mexico (Kolappan & Subramani 2009; Pérez-Padilla et al. 2001; Pokhrel et al. 2010; Sehgal et al. 2014).

The age distribution of TB cases in the Balimo region showed patients aged 0 – 14 years to be the largest group. In the analysis of pulmonary and extrapulmonary TB cases, the very low number of pulmonary cases in children aged up to 14 years is also of note. These results correspond with the general presentation of TB in children, which tends to be paucibacillary and have lymph node involvement (Marais 2014). However, from an epidemiological perspective high numbers of TB cases in children are concerning, as they tend to indicate recent MTB transmission, as well as ongoing transmission within the community (Marais et al. 2005). Furthermore, the distribution of childhood TB in Balimo suggests possible over-diagnosis of extrapulmonary TB and under-diagnosis of pulmonary TB (Seddon et al. 2015). Further research would be necessary to understand TB transmission dynamics in this setting, particularly the risk of children acquiring TB and the disease presentation leading to TB childhood diagnoses. Understanding factors such as Bacille Calmette-Guérin (BCG) vaccination coverage would also be important, as this will likely play a role in the local epidemiology of childhood TB, and the forms of childhood extrapulmonary TB that may develop (Roy et al. 2014; Trunz et al. 2006).

Figure 3.16: Children in a rural village in the Gogodala region of PNG. (Image credit: David Plummer)

Given that many of the TB cases reported in this study are clinically diagnosed without bacteriological confirmation, according to the PNG National TB Management Protocol (Department of Health 2011), there may be TB misdiagnosis among presenting patients. Factors such as case detection bias should also be considered, particularly in relation to high case numbers in children, and of extrapulmonary TB. However, treatment outcomes suggest that over-diagnosis is not a major concern. In this study, more than 70% of TB patients with a known treatment outcome were classified as 'treatment success', meaning that they were either cured or successfully completed treatment, in the absence of classification as treatment failure. This proportion is higher than the previously published data for Balimo, which showed treatment success of approximately 50% in 2011 TB cases at BDH (McBryde 2012); and is moderately higher than the data for the southern region of PNG, which recorded a treatment success (combined treatment completion and cure) proportion of about 62% (Aia et al. 2018). Also of note is the substantially lower treatment default rate in this study, being approximately 5% compared to about 18% in the 2011 evaluation study (McBryde 2012). In a region where successful treatment is often the best supporting evidence for a TB diagnosis, this suggests that at BDH the recognition of non-laboratory confirmed cases of TB by clinicians is reasonable. Clinical diagnostic accuracy, particularly in

the context of extrapulmonary TB, will be investigated further in Chapter 7. It should also be noted that the use standard treatment approaches such as amoxicillin and aspirin or paracetamol have previously been described in the Balimo region (Diefenbach-Elstob et al. 2017). This is a standard treatment approach to febrile illness throughout PNG (Saweri et al. 2017), and there are a number of standard guidelines for the management of various disease presentations in PNG (National Department of Health 2012; Paediatric Society of Papua New Guinea 2016; Shann et al. 2003). However, whether such treatments are used concomitantly with TB treatment is unknown. The possibility of symptom resolution even if extrapulmonary TB has been incorrectly diagnosed should therefore be considered. However, regardless of the accuracy of TB diagnoses in this setting, misdiagnoses of TB may in fact place greater burdens on the health system, particularly if symptoms are not resolved.

It is also of note that in this study a relatively high proportion of pulmonary TB cases had a positive smear result, being 65.8% compared to 31% of pulmonary TB cases with bacteriological confirmation across PNG (World Health Organization 2017a). The low proportion of smear-negative pulmonary TB cases may reflect additional challenges in pulmonary TB diagnosis, particularly in this setting where chest X-ray was not available at the time of the study. In addition, the low proportion of smear-negative pulmonary TB patients suggests under-diagnosis of this form of TB. This result supports earlier research undertaken by our group, where patient data was compared with molecular analysis of DNA extracted from TB sputum smear slides, and identified a substantial proportion of missed smear-negative pulmonary TB diagnoses (Guernier et al. 2018).

The increased likelihood of new patients being diagnosed with extrapulmonary TB, while previously treated patients were more likely to be diagnosed with pulmonary TB, may have several possible explanations. The higher proportion of pulmonary TB diagnoses in previously treated cases may be influenced by emerging drug resistance in the region. DR-TB is known to occur in Balimo, and is likely to be more prevalent among pulmonary cases if these cases are more likely to truly be TB. An additional possibility for the increased likelihood of previous treatment among pulmonary cases is the status of the Balimo region as endemic for melioidosis (Diefenbach-Elstob et al. 2015; Warner et al. 2007; Warner et al.

2008). Melioidosis frequently presents with pulmonary symptoms, and may initially be misdiagnosed as TB (Bala Raghu Raji et al. 2018; Warner et al. 2010), thus increasing the possibility of a patient returning with ongoing symptoms, and potentially being retreated. Furthermore, limited diagnostic facilities in general result in challenges to infectious disease investigation and management in this region.

In this study, approximately 90% of all TB cases were placed on Category 1 treatment, while 8.0% were commenced on Category 2 treatment (Figure 3.17). However, the use of Category 2 treatment in PNG is problematic due to high levels of generalised STR resistance, possibly as a result of extensive use of STR to treat TB, urinary tract infections, and *Klebsiella* infections in PNG in the past (Ley et al. 2014a; Maddocks et al. 1976; McBryde 2012; Simpson et al. 2011).

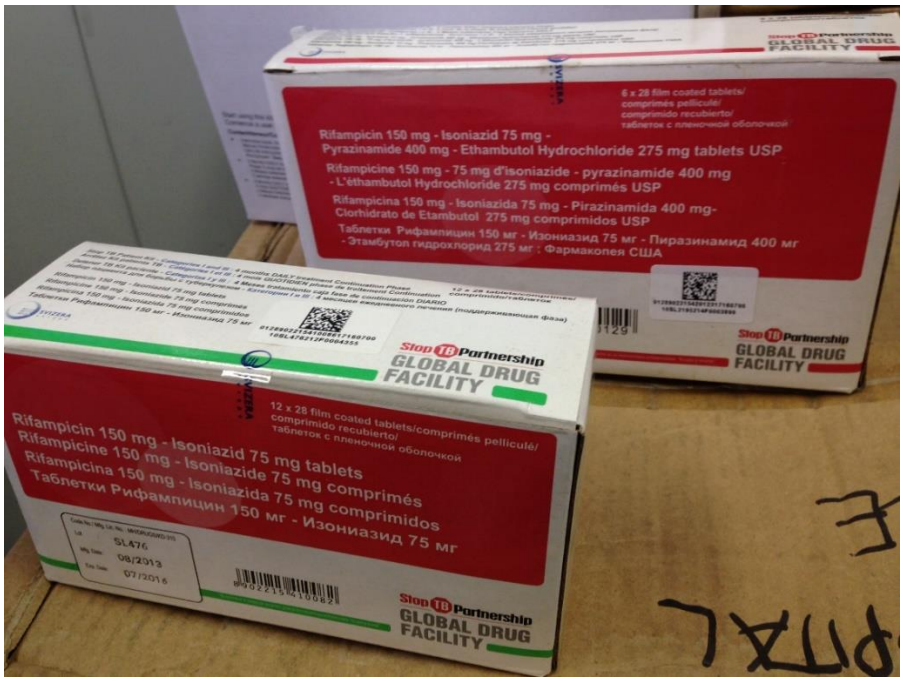


Figure 3.17: The Category 1 standardised DOTS treatment packs used for TB in the Balimo region. The rear box shows the four-drug intensive phase treatment used for the first two months, while the front box shows the two-drug continuation phase treatment used in the last four months. (Image credit: Tanya Diefenbach-Elstob)

Treatment outcomes were unknown for just over 25% of TB patients who completed treatment at least six months before the end of the study period. This proportion may reflect the flexibility with which DOTS is often administered in the region, where patients may take their treatment packs back to their home village for the duration of treatment (Diefenbach-Elstob et al. 2017). In this study, the only factor found to be significantly associated with poorer treatment outcomes was the LLG area in which a patient resided, with poorer outcomes more likely in the rural LLGs. This finding may be linked to the challenges associated with obtaining healthcare for those patients who have a rural residence (Adamu et al. 2018; Gebrezgabiher et al. 2016). TB patients living in or around the Balimo Urban LLG area will face substantially lower travel and economic burdens in obtaining care initially, and continuing treatment once diagnosed. Furthermore, patients who have resolution of their symptoms may not return for post-treatment evaluation at the conclusion of treatment. Overall, unknown treatment outcomes are likely to result from a number of factors, including loss to follow-up, geographic and access challenges, and late updating or non-recording of outcomes in the TB register.

3.4.2. Time trends of tuberculosis cases

Although the reason for the fluctuation in TB cases over the three complete years of the study is uncertain, there are a number of potential influencing factors. These include staffing levels and practices; as well as the timing of TB awareness, education or outreach activities undertaken in Balimo and surrounds. It would be useful to evaluate community-level TB activities, and to investigate their impact on TB case detection. Although the difference in case numbers across the calendar months was not significant, there were higher case numbers overall in November. There are two factors that may influence patient numbers at this time. Ease of travel may be a factor, as November is the first month of the wet season. Travel in the region is easier when water levels are higher, which may result in a small influx of patients to the hospital in this month (Diefenbach-Elstob et al. 2017). Additionally, school holidays across the Christmas period will result in increased travel around the region.

Figure 3.18: Village houses in the Balimo region, with dugout canoes in the foreground. (Image credit: David Plummer)

3.4.3. Comparison of Bamu- and Gogodala-region patients

The majority of patients in this study originated from the Balimo Urban and Gogodala Rural LLG areas. However, with almost 20% of patients based in Bamu Rural LLG villages, people from the Bamu region also contribute substantially to TB patient presentations at BDH.

In the analyses, Bamu-region patients were more likely to be children, and more likely to have a treatment failure outcome when compared with Gogodala-region patients. As already noted, the overall study found higher proportions of extrapulmonary cases in children, with this trend continuing in the comparison of the two patient groups.

It is difficult to speculate on the reasons why these factors are more common in the Bamu region patients. However, the region is remote, and has been described as one of the 'poorest and least developed regions' of the province (Jennifery 2012). Thus the extreme living conditions, combined with a lack of healthcare facilities capable of dealing with TB, may be contributors.

3.4.4. Limitations of the tuberculosis patient register analysis

There are a number of limitations that may have influenced patient data analysis. Patient data is recorded in hand-written registers by a number of different health workers. As such, data entry mistakes may occur, and some patient data may go unrecorded. Record-keeping in this resource-limited region is undoubtedly difficult, especially given the lack of digital records. There are also inherent challenges resulting from the use of records in separate patient and laboratory registers, particularly in locating and updating historical records. Despite this, the TB register was assumed to be the most accurate record in the classification of patients, even if it was unclear how decisions were reached, given that the TB register represents the official TB record. For example, if a patient was classified as cured, but only one negative smear was recorded in the register, the cured classification was still used in this study. Furthermore, any missing treatment commencement dates were assumed to be the same as the patient registration date. As already noted in the methods (see Section 3.2.4), there are inherent problems with some data, including age inaccuracies and assumptions regarding patient residential locations. These problems are unavoidable in a data set such as that used in this study, but are mentioned here due to the need to interpret the results with caution.

There was a substantial proportion (25.5%, 95% CI 23.1 – 28.1) of patients who had completed the six-month treatment period, but who did not have a treatment outcome recorded. In this setting where it is not unusual for patients to undertake TB treatment at home (Diefenbach-Elstob et al. 2017), it is possible that not returning for post-treatment evaluation could be linked with either successful or unsuccessful treatment. As a result, the potential influence of unknown treatment outcomes should be considered in the context of these results.

Given the challenges of travel throughout the South Fly and Middle Fly Districts of the province, it is possible that some Gogodala- and Bamu-LLG area patients present directly at Daru or other peripheral health facilities instead of at Balimo. This may be particularly so for people located close to the Fly River, for whom water-based transport will be faster and easier than walking. Further research into travel patterns and seasonal factors would be required to quantify the number of Balimo-region TB patients who are not seen at BDH. Thus

the TB data presented here are not intended to be an exhaustive record of TB patients for the Balimo region, but a description of the TB cases presenting at BDH, the only hospital in this region of the Middle Fly District.

Figure 3.19: Passengers preparing to travel by boat from Kawito. (Image credit: David Plummer)

3.5. Conclusion

The extremely high reported incidence of TB, and particularly the high proportion of extrapulmonary TB, demonstrates a heavy burden of TB disease in the Balimo region. Increased understanding of the epidemiology of TB in this setting provides important information in the context of TB control and elimination in Western Province and PNG more broadly. This is through insight into the distribution of the disease burden, and indicators of areas of particular concern. Although improved resources and facilities are an urgent need at BDH, this study has also demonstrated the substantial success of health care workers (HEOs, clinical nurses, and laboratory technicians) in diagnosing, treating, and managing TB in this non-doctor-led model of care setting, and the dedication of these staff to this task. The

burden of disease in this newly described TB-endemic region emphasises the need for the role of BDH to be considered in the broader Western Province TB control program.

As described previously, TB in PNG may be diagnosed clinically based on the PNG National TB Management Protocol, and the WHO definitions (Department of Health 2011; World Health Organization 2013a). Misdiagnosis due to limited access to laboratory confirmation is possible, and indeed it is a feature of TB epidemics in these resource-limited settings. Therefore, this study also highlights the need to increase the capacity of laboratory and medical imaging-based diagnostics to aid in the accuracy of diagnosis, which will lead to more directed and evidence-based therapeutic interventions.

The setting-specific data given in this chapter provides a broad epidemiological description of TB in the Balimo region, and the potential influence that these TB cases have on the control of TB in Western Province and PNG more broadly. This research provides a baseline for informing future research questions, as well as information that may support and enhance the existing Balimo TB control program. In addition, the focused epidemiological data may be combined with setting-specific social science research to provide local solutions to TB control.

This thesis will continue to examine epidemiological aspects of TB in the Balimo region from a more focused perspective. The data presented in this chapter has described the incidence and distribution of TB, but noted that DR-TB should also be considered. Prior research from our group has identified genetic evidence of DR-TB in clinical samples from TB patients in the Balimo region (Diefenbach-Elstob and Moreau et al., unpublished data). Understanding the burden of DR-TB in the region is important as it will be linked to incidents of treatment failure in the context of either true DR-TB cases, or the possibility of misdiagnoses where other conditions such as melioidosis are also present.

CHAPTER 4

MOLECULAR CHARACTERISATION OF *MYCOBACTERIUM TUBERCULOSIS* DRUG RESISTANCE IN THE BALIMO REGION

4.1. Introduction

In Chapter 3, tuberculosis (TB) in the Gogodala region of Papua New Guinea (PNG) was described, with characteristics of TB in the region being the large proportion of extrapulmonary TB, a large number of childhood cases, and the equal distribution of male and female patients. In addition, smear microscopy is the only laboratory-based method of diagnosis available at Balimo District Hospital (BDH). In such a setting TB diagnosis is complicated by the inability to differentiate other conditions that may present similarly to TB, such as melioidosis (Bala Raghu Raji et al. 2018; Warner et al. 2010), which is also endemic in the Balimo region (Diefenbach-Elstob et al. 2015; Warner et al. 2007; Warner et al. 2008). These diagnostic challenges mean that sometimes the only method of confirming a TB diagnosis is a positive response to treatment. In Chapter 3, for those patients where the treatment outcome was known, 13.9% had a treatment failure outcome (death, default, or treatment failure). Many factors may contribute to treatment failure, and rural residence was noted for its association with treatment failure. Another reason for treatment failure is the presence of drug-resistant TB (DR-TB). In this chapter, the extent of DR-TB in the Balimo region will be investigated.

PNG is considered to have a high burden of both TB and DR-TB (World Health Organization 2017a). In general, drug resistance is usually first identified in the form of rifampicin (RIF) mono-resistant TB (RR-TB), or multidrug-resistant TB (MDR-TB), which is resistance to both RIF and isoniazid (INH) (World Health Organization 2013a). In 2016, PNG had an estimated proportion of RR-TB and MDR-TB in 3.4% of newly diagnosed TB cases, and 26% of previously treated TB cases (World Health Organization 2017a).

In PNG, Western Province has been particularly noted for a high burden of DR-TB, although as Daru Hospital is the referral site for Xpert MTB/RIF testing in Western Province (Department of Health 2011), the proportion of DR-TB would be expected to be higher. A multi-site study that included Western Province found that 34.1% of new and previously treated cases at Daru Hospital in the provincial capital were MDR-TB, while proportions of MDR-TB ranged from 1.8 to 13.3% at sites in Madang, Morobe, and the National Capital District (Aia et al. 2016). Extensively drug-resistant TB (XDR-TB), which is MDR-TB with additional fluoroquinolone (FLQ) and second-line injectable antibiotic resistance, has also been described in Daru (Chandler 2016; Furin & Cox 2016; Kase et al. 2016) (Figure 4.1). There are also a number of studies describing Western Province-based TB patients who presented at Australian health clinics in the Torres Strait, which found MDR-TB in 25% of TB patients (Gilpin et al. 2008; Simpson et al. 2011). These studies provide evidence of the substantial burden of DR-TB in PNG, and particularly Western Province.

Figure 4.1: A partial aerial view of Daru, the capital of Western Province, located on an island to the south of mainland PNG. (Image credit: David Plummer)

Phenotypic drug susceptibility testing (DST) of *Mycobacterium tuberculosis* (MTB) bacilli isolated and cultured from patient clinical samples (usually sputum) serves as a reference standard for the diagnosis of DR-TB (World Health Organization 2018d). However, this method is laborious and time-consuming, taking as long as 8 – 12 weeks to obtain a result (Migliori et al. 2008). Furthermore, culture and DST are high risk activities, requiring biosafety cabinets and appropriate physical containment facilities, thus limiting their use to centralised well-equipped laboratories (World Health Organization 2012, 2015b). The growing demand for rapid identification of drug resistance has resulted in molecular techniques being increasingly utilised, based on the identification of mutations in numerous genes in the MTB genome that have been associated with a drug-resistant phenotype (Ramaswamy & Musser 1998). These mutations occur as single base changes in the MTB genome, and are known as single nucleotide polymorphisms (SNP).

The molecular basis of RIF resistance has been well-characterised, with 96% of this resistance associated with mutations in an 81-base pair RIF-resistance determining region (RRDR) located in the centre of the *rpoB* gene (Ramaswamy & Musser 1998). Resistance to INH has been frequently associated with mutations in the *katG* and *inhA* genes (Ramaswamy & Musser 1998). Because a large proportion of RIF-resistant strains have concomitant INH resistance, the molecular detection of RIF resistance in MTB isolates is often used as an early indicator of MDR-TB before phenotypic susceptibilities are available, or in countries where DST is not routinely available (Traore et al. 2000). The Xpert MTB/RIF assay (Cepheid, USA) that operates on the GeneXpert platform has been recommended by the World Health Organization (WHO) for the detection of RIF resistance in all people suspected of having MDR-TB (World Health Organization 2013c). The test is based on the molecular detection of *rpoB* mutations (Helb et al. 2010; Ioannidis et al. 2011; World Health Organization 2013c). However, the Xpert MTB/RIF does not detect INH resistance. As such, in the absence of phenotypic DST the Xpert MTB/RIF and other molecular drug resistance detection techniques that focus on *rpoB* may overestimate MDR-TB, especially if the prevalence of RR-TB locally is unknown (Traore et al. 2000). An approach that can identify both *rpoB* and *katG* mutations is more likely to differentiate RR-TB, MDR-TB, and strains in which MDR-TB is likely to develop (Manson et al. 2017).

The presence of DR-TB in the Middle Fly District of Western Province, including the Balimo region, has already been noted (see Section 3.5). Given the challenges of diagnosis and the presence of DR-TB in this region of PNG, the aim of this study is to provide information about the molecular patterns and frequency of DR-TB in the Balimo region. The approach is based on genetic drug resistance characterisation of the *rpoB* and *katG* genes in MTB DNA extracted from sputum samples collected from TB patients and people undergoing TB investigations at BDH. This investigation will further understanding of the burden of DR-TB in the Balimo region of Western Province, as well as provide insights into the impact of DR-TB on TB diagnostics and treatment outcomes.

4.2. Materials and Methods

4.2.1. Study setting and patient characteristics

The Balimo setting and description of TB case-finding activities have been previously described in Chapter 3. However, the data presented in this chapter is focused on presumptive pulmonary TB patients only, and includes analysis of samples collected from people undergoing investigation for TB, newly diagnosed TB patients, and follow-up samples collected from people who had previously been commenced on TB treatment. Sputum samples were obtained through routine collection from presumptive TB patients who presented at BDH for either new or follow-up TB investigations.

4.2.2. Initial collection and preparation of samples

Sputum samples were collected during the period April 2016 to June 2017, with a total of 240 samples provided on a convenience sampling basis for this study. These samples represented approximately 37% of those collected and processed at the BDH laboratory during the corresponding time period. These included decontaminated and concentrated (n = 213) and fresh (n = 27) sputum samples. Processed sputum was prepared at the BDH laboratory, with decontamination and concentration procedures according to the modified Petroff's method (Kent & Kubica 1985; Petroff 1915). Following processing, sputum was

smeared onto microscope slides, and examined using Ziehl-Neelsen (ZN) staining for the presence of acid-fast bacilli. Fresh sputum samples were decontaminated and concentrated at James Cook University (JCU), Townsville, following the same procedure used at BDH.

4.2.3. Preparation of DNA template

DNA extraction procedures were undertaken in biosafety level three laboratories. Approximately 100 – 150 µL of each decontaminated sputum was transferred to a 2 mL tube and heat inactivated at 80^{o}C in a thermal block for 1 hour. The sputum was then centrifuged at 3,000 g for 15 minutes, and the supernatant removed. A volume of 1 mL of a 4M guanidine isothiocyanate (GIT) solution was added to each tube, and incubated overnight at 37^{o}C. The samples were then centrifuged at 13,000 rpm for 10 minutes, and the supernatant removed.

Following inactivation, DNA was extracted using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Germany). Initially, the spun pellets were resuspended in 200 µL of tissue lysis buffer and 40 µL of proteinase K solution, and incubated overnight at 55^{o}C, or until all sediment was dissolved. Subsequent steps were performed according to the manufacturer's instructions. Extracted DNA was stored at minus-80^{o}C until use.

4.2.4. Confirmation of *Mycobacterium tuberculosis* infection

Two TaqMan real-time PCR (qPCR) assays were used to confirm *M. tuberculosis* complex (MTBC) infection in the DNA extracts, according to protocols published by Broccolo et al. (2003). The first assay (IS*6110*) targeted a 122-base pair sequence located within the central region of the IS*6110* multicopy element, allowing detection of the *Mycobacterium* species. The second assay (*senX3-regX3*) targeted a 146-base pair sequence in the *senX3-regX3* intergenic region, which is present only in strains of the MTBC. Primer and probe sequences for the assays are shown in Table 4.1. The IS*6110* assay targets a multi-copy element, and as such has an increased ability to detect MTB DNA, even if the original DNA quantity is low. By

comparison, the *senX3-regX3* assay is more specific but less sensitive as it targets a single copy gene, and may thus have a reduced chance of reactivity when there is a low copy number of the DNA in the extraction product.

Table 4.1: Primer and probe sequences of the TaqMan qPCR assay targeting the IS*6110* multicopy element and the *senX3-regX3* intergenic region. (Source: Broccolo et al. 2003)

Name	**Primer direction**	**Sequence**
IS*6110* multicopy element (*Mycobacterium* species)		
TAQM3	Forward	5'-AGG CGA ACC CTG CCC AG-3'
TAQM4	Reverse	5'-GAT CGC TGA TCC GGC CA-3'
n/a	Probe	5'-TGT GGG TAG CAG ACC TCA CCT ATG TGT CGA-3'
***senX3-regX3* intergenic region (*M. tuberculosis* complex)**		
TAQregT2	Forward	5'-GTA GCG ATG AGG AGG AGT GG-3'
TAQreg2L	Reverse	5'-ACT CGG CGA GAG CTG CC-3'
	Probe	5'-ACG AGG AGT CGC TGG CCG ATC C-3'

Each qPCR reaction (20 µL) contained 1X GoTaq Probe qPCR Master Mix (Promega, Madison WI, USA), 0.8 µM each of forward and reverse primer, 0.1 µM of probe, and 2 µL of DNA template. The real-time PCR protocol consisted of an initial denaturation step of 95^{o}C for 2 minutes, followed by 45 cycles of denaturation at 95^{o}C for 5 seconds, and annealing/elongation at 60^{o}C for 15 seconds. The positive control used genomic DNA from the MTB H37Rv reference strain (NC_000962.3). The qPCR assays were carried out on a Rotor-Gene Q6000 (QIAGEN, Hilden, Germany).

The IS*6110* qPCR assay was undertaken in duplicate, with a triplicate run for samples with discordant results. The *senX3-regX3* qPCR assay was undertaken once on all samples. A DNA extract was considered to be reactive if the cycle threshold (Cq) was less than 40 cycles.

Samples that were reactive in both the IS*6110* and *senX3-regX3* assays were considered MTB-positive. In this setting, non-tuberculous mycobacteria (NTM) are considered to be rare, and the IS*6110* assay has been used previously for the detection of MTBC in PNG

(Guernier et al. 2018). Accordingly, for this study samples with duplicate reactivity of the IS*6110* assay but no reactivity of the *senX3-regX3* assay were classified as 'MTBC or NTM'.

4.2.5. Targeted PCR amplification and mutation analysis of the *rpoB* and *katG* genes

Of the 240 sputum samples that were initially assessed, 102 classified as either MTB (n = 62) or 'MTBC or NTM' (n = 25) were further tested using both the *rpoB* and *katG* targeted primer sets. A further 15 samples reactive for early runs of the IS*6110* assay, but ultimately classified as negative, were also included.

Targeted PCR was used to assess the DNA extracts for mutations in the resistance-associated regions of the *rpoB* and *katG* genes. The primer sets have been described elsewhere (Ballif et al. 2012b), and details are shown in Table 4.2.

Table 4.2: Targeted sequencing primers and PCR conditions used to identify drug resistance-associated markers in the *rpoB* and *katG* genes. The primer sets have been previously described in Ballif et al. 2010.

Gene	Primer direction	Sequence	Amplicon size (bp)
***rpoB* (Rv0667)**	Forward	5'- TCG GCG AGC TGA TCC AAA ACC A -3'	601
	Reverse	5'- ACG TCC ATG TAG TCC ACC TCA G -3'	
***katG* (Rv1908c)**	Forward	5'- CCA GCG GCC CAA GGT ATC -3'	850
	Reverse	5'- GCT GTG GCC GGT CAA GAA GAA GTA -3'	

bp: base pairs; PCR: polymerase chain reaction

Each PCR reaction (25 µL) contained 1X GoTaq G2 Green Master Mix (Promega, Madison WI, USA), 0.5 (*rpoB*) or 0.8 (*katG*) µM each of forward and reverse primer, and 2 µL of DNA template. The positive control consisted of genomic DNA from the MTB H37Rv reference strain (NC_000962.3).

The optimised *rpoB* PCR protocol consisted of an activation step of 94°C for 2 minutes; followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 40 seconds; and a final extension step of 72°C for 5 minutes. The optimised *katG* protocol consisted of an activation step of 94°C for 2 minutes; followed by 40 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 60 seconds; and a final extension step of 72°C for 5 minutes. The PCR assays were undertaken on the BIOER GeneTouch and Kyratec SuperCycler Trinity conventional PCR machines. Amplicons were analysed by agarose gel electrophoresis using a 1% agarose gel. Where clear bands of the correct size were visible, the remaining aliquot was dispatched to Macrogen Korea for sequencing of the amplicons in both directions to ensure accurate results. Chromatograms were analysed using Geneious R10 (Biomatters Limited, Auckland, New Zealand). The consensus sequences of each sample were compared to the wild-type (WT) MTB *rpoB* (GeneID 888164) and *katG* (GeneID 885638) gene sequences of the H37Rv reference strain (NC_000962), downloaded from the National Center for Biotechnology Information (NCBI) in order to identify the mutations.

Throughout the results and discussion, the nomenclature used to describe identified SNPs is as follows:

- Nucleotide mutations: #N→N (where # refers to the nucleotide number, and N refers to the WT and mutated nucleotides respectively)
- Codon mutations: A#A (where # refers to the codon number, and A refers to the WT and mutated amino acids respectively)

4.2.6. Samples excluded from the analyses

Three samples were excluded from the analyses, being duplicates from three patients. One patient returned identical lab results for the two duplicate samples, so the second sample was excluded. The two other patients returned identical microscopy results, but discrepant qPCR results, i.e. negative / 'MTBC or NTM' for one patient, and MTB / 'MTBC or NTM' for the other. In the first case, only the 'MTBC or NTM' sample was retained, while in the second case, only the MTB sample was retained.

4.3. Results

4.3.1. Demographic information and detection of *Mycobacterium tuberculosis* infection

Of the 240 sputum samples tested in this study, the analysis included samples collected from 237 people based on the exclusion criteria described previously (see Section 4.2.6). The analysis included 125 (52.7%) females, 111 (46.8%) males, and one person for whom sex was not recorded. Age at the time of collection was recorded for 133 participants. These people ranged in age from 3 – 75 years, including 16 children (aged up to 17 years), and 117 adults. The remainder of participants included 103 adults (age unknown), and one unknown. Of the 237 sputum samples that were analysed, 212 (89.5%) were new samples collected for TB investigation, 21 (8.9%) were follow-up samples collected after the patient had been commenced on TB treatment, and four (1.7%) had an unknown sample status. Of the 212 new sputum samples, 32 (15.1%) had a positive smear status, 177 (83.5%) were smear-negative, and three (1.4%) were unknown. The complete sputum smear microscopy results, stratified by sample status, are shown in Table 4.3. Where more than one smear result was recorded for a single sample, only the highest smear grade is shown here.

Table 4.3: Balimo sputum smear microscopy results stratified by sample status.

Sample status	ZN microscopy	n (%)
New	NAFB	177 (74.7)
	Scanty	1 (0.4)
	1+	11 (4.6)
	2+	6 (2.5)
	3+	14 (5.9)
	Unknown	3 (1.3)
Follow-up	NAFB	16 (6.8)
	1+	1 (0.4)
	2+	2 (0.8)
	3+	2 (0.8)
Unknown	NAFB	1 (0.4)
	Unknown	3 (1.1)
Total		**237**

n: number; NAFB: no acid-fast bacilli; ZN: Ziehl-Neelsen

Based on the classification criteria for molecular detection of MTB, 62/237 (26.2%, 95% CI 21.0 – 32.1) samples were classified as MTB, and 24/237 (10.1%, 95% CI 6.9 – 14.6) were classified as 'MTBC or NTM'. The remainder (151/237, 63.7%, 95% CI 57.4 – 69.6) of the samples were classified as negative for MTB.

4.3.2. Prevalence and characteristics of single nucleotide polymorphisms in Balimo-region tuberculosis patients

Adequate amplicons for sequencing were obtained from 50 samples using the *rpoB* primer set, and 48 samples using the *katG* primer set (Figure 4.2). Five of the samples that returned *rpoB* amplicons did not return adequate *katG* amplicons, while three samples that returned *katG* amplicons did not return adequate *rpoB* amplicons, resulting in a total of 53 amplicons that were suitable for sequencing. One 'MTBC or NTM' sample returned good *rpoB* sequences, while all other sequences were obtained from samples classified as MTB. One sample found to be MTB by qPCR returned a clean sequence in both directions for the *rpoB*

target region, but the BLAST results revealed that the closest matches were the *Corynebacterium* species (100% query cover out of 589 nucleotides and 92% identity). Sequencing results are shown in Table 4.4. A total of 34 of the 53 sequenced samples (64.2%) had no mutations identified and were classified as WT. A further six samples were WT for either *rpoB* or *katG* only, as the other gene sequencing was unsuccessful.

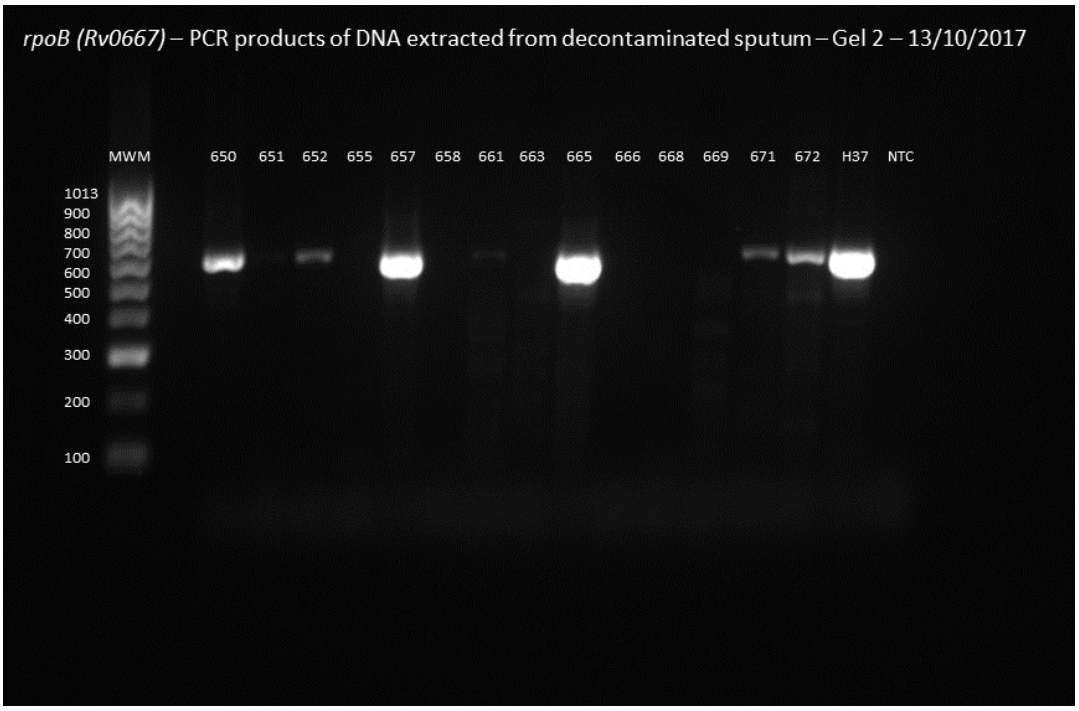


Figure 4.2: A gel image showing PCR products obtained from amplification of DNA extracted from decontaminated sputum samples using the *rpoB* primer set. PCR products from extract numbers 650, 652, 657, 665, 671, and 672 were considered adequate for sequencing.

Table 4.4: Summary of sequencing results and codon mutations for amplicons that were successfully sequenced.

***rpoB* conclusion**	***katG* conclusion**	**Combined**	**n**
WT	WT	WT	34
WT	n/a	*rpoB* WT *katG* unknown	5
S450L*	WT	RIF DR *katG* WT	4
S450L* and I480V	WT	RIF DR *katG* WT	1
WT or F548L	WT	*rpoB* uncertain *katG* WT	1
n/a	WT	*rpoB* unknown *katG* WT	1
n/a	P219L^	*rpoB* unknown *katG* mutated	1
n/a	A361V^ P365S^ S383L^ R396C^ D419D^	*rpoB* unknown *katG* mutated	1
WT	WT or E261K^	*rpoB* WT *katG* uncertain	1
WT	WT or G279R^	*rpoB* WT *katG* uncertain	3
Other species	n/a	Other species	1
Total			**53**

n: number; n/a: not available (amplification or sequencing failed); RIF DR: rifampicin drug resistance; WT: wild-type

*Codon mutation located within the RRDR of the *rpoB* gene

^*katG* mutations with uncertain association with drug resistance

The S450L (1349C→T) mutation in the *rpoB* gene was identified in five of the sequenced extracts. One of these extracts also carried an additional I480V (1438A→G) mutation in the same gene. When considering all samples that were classified as either MTB or 'MTBC or NTM' (n = 86), but excluding the follow-up samples for those participants who also had a new sample included (n = 3), 6.0% (5/83, 95% CI 2.6 – 13.3) of tested samples were

considered to be RIF-resistant. When those samples with a 'MTBC or NTM' classification were excluded, the proportion considered to be RIF-resistant was 8.1% (5/62, 95% CI 3.5 – 17.5).

A number of *katG* mutations were identified in two extracts that were WT for the *rpoB* gene target. The *katG* P219L (656C→T) mutation was identified in one sample, while five *katG* mutations, namely A361V (1082C→T), P365S (1093C→T), S383L (1148C→T), R396C (1186C→T), and the silent mutation D419D (1257C→T) were identified in a second sample.

In five samples, there were unconfirmed mutations based on the identification of a mutation in one sequence direction, but a WT nucleotide in the other sequence direction (Figure 4.3). These were *rpoB* WT or F548L (1644C→A) in one extract, *katG* WT or E261K (781G→A) in one extract, and *katG* WT or G279R (835G→C) in three extracts. These mutations were not investigated further as they are not known to be associated with any drug resistance.

Figure 4.3: Sequencing chromatogram showing an uncertain *katG* mutation, due to a SNP in one direction, but a WT genotype in the other direction.

4.3.3. Repeat testing of new and follow-up samples

Both new and follow-up samples were available for three patients. All three patients were sputum smear-positive on their initial sample collection, with smear grades of 2+ or 3+; and classified as MTB and WT based on the molecular and sequencing assays. The follow-up samples collected from these patients were obtained at different time-points, ranging from two to four months following treatment commencement. The follow-up samples were all sputum smear-negative, although interestingly, two samples were classified as MTB based on the molecular assays, while the third sample was classified as 'MTBC or NTM'. The MTB samples were WT for *rpoB* and *katG* based on the sequencing results. The 'MTBC or NTM' sample was WT for *rpoB*, although a suitable amplicon was not available for *katG* sequencing. This later sample reacted twice with the IS*6110* assay but had very late reactivity (40 < Cq < 45) with the *senX3-regX3* assay. Comparing each new sample with its paired follow-up, the average Cq values of the IS*6110* assays had increased for all three samples; very slightly for one sample (Cq 18.5 to 19.5), and substantially for the other two samples (Cq 14.5 to 23, and Cq 20 to 33.5).

4.4. Discussion

This study was undertaken on sputum samples collected in the rural Balimo region of PNG from patients suspected or confirmed to have TB. Molecular diagnostic techniques were able to identify MTB in 26 – 36% of the tested presumptive TB samples (depending on chosen positivity criteria), and RIF resistance-associated SNPs were identified in 6.0% (n = 5) of the tested samples with MTB and 'MTBC or NTM' classifications.

4.4.1. Drug resistance-associated single nucleotide polymorphisms identified in the Balimo region

The *rpoB* S450L (1349C→T) codon mutation identified in five extracts in this study is arguably the most frequently identified RIF resistance-associated mutation in the *rpoB* gene

(Brandis & Hughes 2013; Heep et al. 2001). This codon mutation has been described previously in three studies examining genetic drug resistance in samples from PNG, including in Western Province (Bainomugisa et al. 2018; Ballif et al. 2012b; Ley et al. 2014a). The I480V (1438A→G) codon mutation, identified in one extract in this study, is much less common, and was first described in a study from Mexico where it appeared as a double mutation alongside S450L as in our sample (Ramaswamy et al. 2004). Interestingly, the same double mutation has recently been identified in a single clinical sample investigated at Daru General Hospital in Western Province, PNG (Bainomugisa et al. 2018). However, the origin of the patient sample was not stated. Given that Daru is the provincial hospital, as well as the referral hospital for presumptive DR-TB patients from Balimo and across Western Province, clinical samples collected there cannot be assumed to be from people who are also Daru residents.

Figure 4.4: Researchers and health workers at the hospital in Newtown (Balimo), including from left (1) Sister Keyanato Siwaeya, (3) Prof David Plummer, (4) Sister Bisato Gula, (5) myself, and (9) Mr Daniel Pelowa. (Image credit: Jeffrey Warner)

There is less certainty regarding the drug resistance association of the mutations identified in the *katG* gene. In this study S315T, the most common INH resistance-associated *katG*

mutation, was not identified. As a result, neither genotypic INH mono-resistance nor MDR-TB were identified. Despite this finding, genotypic INH resistance should continue to be monitored, especially as the *katG* S315T mutation has been identified in other studies from PNG, including in Western Province (Bainomugisa et al. 2018; Ballif et al. 2012b; Ley et al. 2014a). Furthermore, several hundred different *katG* mutations have been documented in INH-resistant TB samples (Sandgren et al. 2009; Seifert et al. 2015; Vilchèze & Jacobs Jr 2014), and descriptions of new mutations conferring INH resistance are likely to occur in the future.

Only one of the *katG* mutations identified in the Balimo collection has been previously described. This was the P365S codon mutation, which has been identified in a single INH-susceptible sample from Korea (Yoon et al. 2012). One other similar mutation has been identified, although the nucleotide substitution was different and coded for another amino acid, i.e. S383L in Balimo versus S383P in Japan (Ando et al. 2010). In the Balimo study, the mutation at codon 419 was silent. However, this codon is a site where a number of INH-resistant mutations have been described, including D419A, D419Y, D419E, and D419H (Brossier et al. 2006; Cardoso et al. 2004; Huang et al. 2009). Based on currently available data (Sandgren et al. 2009; Vilchèze & Jacobs Jr 2014), the other *katG* mutations identified in Balimo (P219L, A361V, and R396C) appear to be not previously reported. However, given the uncertain drug resistance association of the *katG* mutations described in this study, the related samples would need to be confirmed as phenotypically resistant using DST.

4.4.2. Comparison of *Mycobacterium tuberculosis* drug resistance to global and Papua New Guinea data

This study found that 6.0% (95% CI 2.6 – 13.3) of the 83 MTB and 'MTBC or NTM' samples collected at BDH are RIF-resistant, based on the presence of drug resistance-associated mutations in the *rpoB* gene. These results demonstrate that DR-TB is already an established concern in the Middle Fly District. The proportion of drug resistance is comparable to the global, regional (Western Pacific) and PNG national estimates for RR-TB and MDR-TB, which

are 4.1% (95% CI 2.8 – 5.3), 5.3% (95% CI 2.9 – 7.8), and 3.4% (95% CI 1.7 – 5.0) respectively (World Health Organization 2017a).

However, regional estimates from site-specific studies undertaken in PNG have been variable. In Gulf Province (Figure 4.5), 3/48 (6.3%) sputum samples from TB patients tested with Xpert MTB/RIF were found to be positive for RIF resistance (Cross et al. 2014). In Madang Province, drug resistance was identified based on DST results in 8.7% of a collection of 69 isolates from new and previously treated TB patients (Luke et al. 2008; Phuanukoonnon et al. 2008). Another study from Madang Province identified RR-TB and/or MDR-TB in 5.8% (10/172) of samples that underwent DST (Ballif et al. 2012a). Two multi-site studies have been undertaken, with results based on DST. A study of patient samples from Eastern Highlands, Milne Bay, and Madang Provinces found 4.2% (9/212) of samples to have RR-TB and/or MDR-TB (Ley et al. 2014a). A larger study undertaken in Madang, Morobe, National Capital District (NCD), and Western Provinces found MDR-TB in 44/1145 (3.8%) new and previously treated TB patients, with site-specific distributions for MDR-TB ranging from 1.8% at Kilakila and Port Moresby General Hospital in the NCD, to 34.1% at Daru Hospital in Western Province (Aia et al. 2016).

Figure 4.5: A populated area near Kerema, the capital of Gulf Province, PNG. (Image credit: Tanya Diefenbach-Elstob)

There are also a number of studies focused on TB patients in Australia that have described drug resistance in patients from PNG. During the 1998 – 2012 period, 244 MDR-TB cases were notified in Australia, and 72 (29.5%) of these were from PNG (Francis et al. 2018). In Queensland, 96 MDR-TB patients were diagnosed during the 2000 – 2014 period, with 73 (76.0%) of these patients from PNG (Baird et al. 2018). Two studies focused on Western Province-based TB patients who presented at Australian health clinics in the Torres Strait found MDR-TB in 25% of these patients, based on culture and DST results (Gilpin et al. 2008; Simpson et al. 2011). This evidence confirms the substantial burden of DR-TB in PNG, and particularly in Western Province.

As described earlier, the proportion of DR-TB cases identified in Western Province is higher than the PNG national proportion, based on research undertaken in Daru and from

Australian studies reporting data from Western Province-based TB patients (Aia et al. 2016; Gilpin et al. 2008; Simpson et al. 2011; World Health Organization 2017a). However, clinical samples tested at the hospital in Daru will be collected from local residents as well as patients from across Western Province, as Daru is the referral hospital for presumptive DR-TB patients from Balimo, the Middle Fly District, and across the province (Department of Health 2011). In order to investigate possible heterogeneity between different areas of the province, it would be important to investigate the geographic origins of samples collected and analysed at Daru. This knowledge is essential to the implementation of strategic efforts aimed at control of DR-TB across Western Province.

Currently in Balimo, TB patients with presumptive DR-TB must provide a sputum sample which is sent to the provincial capital of Daru for Xpert MTB/RIF testing (Department of Health 2011). If positive, the patient must then travel to Daru to be commenced on DR-TB treatment, which is to be completed under the supervision of an appropriately trained health worker (Department of Health 2011). There are a number of potential patient and provider challenges that are associated with the absence of a rapid diagnostic for DR-TB in Balimo. These include (i) delays in initiation of adequate treatment, (ii) active transmission of DR-TB to others, (iii) loss-to-follow-up while waiting for diagnostic results, and (iv) access challenges due to the long travel time to reach Daru (approximately eight hours in a motorised dinghy). Our earlier work from the Balimo region has described the difficulties associated with treatment adherence if people do not have a support network (Diefenbach-Elstob et al. 2017), which will be a challenge further amplified for patients from the Balimo region undergoing treatment in Daru (Figure 4.6).

Figure 4.6: Undertaking an interview about TB treatment adherence during earlier research in the Balimo region. (Image credit: Jeffrey Warner)

The identification of RIF resistance in Balimo demonstrates a need for rapid identification of drug-resistant strains, as well as the resources, facilities, and training necessary to treat DR-TB patients at BDH. As such, implementation of a method such as the WHO-recommended Xpert MTB/RIF, which is capable of MTB diagnosis as well as detection of RIF resistance, should be considered. In addition, training of health workers already experienced in the management of TB to deliver and manage second-line treatment for DR-TB patients would be necessary, concurrent with ongoing supply of the required medications. In Western Province, a decentralised approach with improvement of resources and facilities at BDH could play a role in reducing the strain placed on Daru Hospital in the management of DR-TB patients, especially in this region where geographic and economic challenges are particularly relevant for TB patients.

4.4.3. Detection of *Mycobacterium tuberculosis* in follow-up samples

Detectable MTB DNA was present in all three of the follow-up sputum samples found to be smear-negative. Even though there was a reduction in detectable DNA between the initial 'new' sample and the follow-up sample from the same patient, the qPCR positivity of the

follow-up samples is of concern as these patients had been under treatment for two to four months at the time of the follow-up collection. It has been suggested that TB patients are non-infectious after two weeks of treatment, although this belief has been disputed (Ahmad & Morgan 2000; Schwartzman & Menzies 2000). Furthermore, smear-negative TB patients are often considered to be less infectious, although studies have demonstrated that they are still able to transmit TB, and indeed there is evidence of TB transmission from patients who are nucleic acid amplification test-negative (Behr et al. 1999; Hernández-Garduño et al. 2004; Tostmann et al. 2008; Xie et al. 2018). The presence of smear-negative/qPCR-positive samples supports results from previous research in the Balimo region, and demonstrates the inherent challenges of TB diagnosis in this setting where limited laboratory-based methods are available (Guernier et al. 2018).

4.4.4. Limitations

Laboratory registration of 'new' samples did not include information on whether a patient had previously been treated for TB. As a result, there may have been a higher risk of DR-TB for some patients. However, given only 7.5% of TB patients identified in Chapter 3 of this study had an incoming relapse, failure, or default status (see Table 3.4), the proportion of retreatment patients in this sample of TB investigation patients is likely to be small.

This was a small study, undertaken on samples collected on a passive case detection basis at BDH. Although the sample size was small, confidence intervals have been calculated to provide context for the proportion of DR-TB. However, a larger study would be necessary to provide greater understanding of the burden of DR-TB in the Balimo region.

In this study, samples were diagnosed with MTB based only on sputum smear microscopy and molecular assays, with no confirmation using culture-based assays. As a result, culture and subsequent DST would be necessary to confirm RIF resistance in samples where *rpoB* resistance-associated SNPs were identified.

Our investigation of possible INH resistance included only one of the several genes associated with INH resistance, with gene targets such as *inhA* and *ahpC* not investigated. As

such, there may have been INH-resistant samples in our study that were not identified. Further investigation including these other genes would be necessary to develop a complete picture of the burden of genotypic DR-TB in the Balimo region.

In the majority of samples, sequencing was performed only once, and in some samples only one sequence direction was able to be read. This approach is not problematic for SNPs identified at nucleotide and codon sites that have previously been described and associated with drug resistance. However, for the *katG* SNPs that have been described in this study, repeat sequencing would be necessary to confirm the results. In addition, DST would be required to determine drug resistance status.

4.5. Conclusion

This study has demonstrated the presence of RIF-resistant MTB strains in the Balimo region, based on the identification of resistance-associated mutations in the RRDR of the *rpoB* gene. Although *katG* mutations known to be associated with INH resistance were not identified in the study, the *inhA* and *ahpC* genes were not investigated, and thus MDR-TB cannot be ruled out. The results emphasise the need for an efficient method of diagnosing DR-TB patients and commencing them on treatment at BDH, without the potential delays caused by resistance identification being conducted only in the provincial capital of Daru, and the necessity of travel for DR-TB treatment and management. Furthermore, the presence of RIF resistance in this region distant from Daru emphasises the importance of understanding the burden and possible heterogeneity of DR-TB across Western Province.

Given the presence of DR-TB patients in the Balimo region, it is possible that DR-TB may be associated with factors such as particular villages or distance from a health facility. In addition, the presence of DR-TB in the Balimo region raises the question of whether some treatment failure TB cases are linked with drug resistance – a possibility which would require further investigation. Understanding the spatial distribution of TB in the Balimo region may provide insights into setting-specific factors that influence the development of DR-TB.

CHAPTER 5

THE SPATIAL DISTRIBUTION OF TUBERCULOSIS IN THE BALIMO REGION OF PAPUA NEW GUINEA

5.1. Introduction

In Chapter 3, the descriptive epidemiological analysis of tuberculosis (TB) patients diagnosed at Balimo District Hospital (BDH) showed that patients present from an extensive region of the Middle Fly District of Western Province, although primarily within the Balimo Urban, Gogodala Rural, and Bamu Rural local level government (LLG) areas. This vast region, situated on the Aramia, Fly, and Gama River floodplains, is geographically isolated due to a lack of roads, with transport predominantly by boat or foot (Figure 5.1). The majority of people in the region have subsistence-based livelihoods, and monetary income is limited. These factors can make travel over long distances prohibitively expensive, laborious, and time-consuming. In addition to the data from Chapter 3, Chapter 4 described the extent of DR-TB in a cohort of people investigated for TB at BDH. This analysis highlighted the potential link of DR-TB with treatment failure, but noted that poor access to health services may also play a role in treatment failure.

Figure 5.1: Floodplain region in the south of Western Province, PNG. (Image credit: David Plummer)

Health facilities in the Balimo region are limited. BDH is the only hospital in the Middle Fly District, and is the primary facility providing TB diagnosis and DOTS-based TB treatment in the region. Other smaller peripheral health facilities, including health clinics and aid posts, provide limited TB services (see Appendix 2 for details of peripheral health facilities known to be operating at the time of this study). The services provided include clinical extrapulmonary TB diagnosis and treatment, and pulmonary TB services only where a sputum sample is not able to be transferred to Balimo. All of these facilities are supplied with TB resources and medications by BDH. Although the TB services provided by peripheral health facilities have limited scope, the presence of these facilities in close proximity to villages is likely to increase TB case detection in that area, and to decrease the numbers of patients seen at BDH from those villages.

Previous research in the Balimo region has highlighted the extensive reach of TB, and local health workers have attested to the presence of TB cases in every village in the district (Diefenbach-Elstob, unpublished interview data). If this belief is indeed the case, understanding the geographic distribution of TB cases in relation to population distribution will provide insights into hotspots of TB infection. Villages with a lack of identified TB cases may be indicative of a need for active case-finding investigations in those areas, particularly as our earlier research has identified ease of travel and proximity to health services as important factors in accessing TB care (Diefenbach-Elstob et al. 2017).

This study aims to investigate the intersection between location and TB in the Balimo region. The objectives are:

1. To define the catchment region of BDH, based on the locations in Western Province from where TB patients seek and access services.
2. To describe the distribution of TB patients and determine if there is non-random clustering of TB in the BDH catchment region.
3. To describe the locations of DR-TB patients, and identify any clustering.

Investigation of these objectives will provide insight into the previously undescribed influence of geography, access, and proximity to health services on TB and DR-TB distribution in the Balimo region.

5.2. Materials and Methods

5.2.1. Study setting and patient cohort

The analysis of TB patients is based on the Balimo TB patient register data previously described in Chapter 3 (see Section 3.2.3). For analyses in this chapter, residence is defined as the first residential address provided by a TB patient, based on the assumption that the first address provided was more likely to reflect the patient's most recent place of residence. All patients included in the data presented in Chapter 3 were also included in the spatial and clustering analyses, with the exception of those from outside Western Province, and where a residential address was not able to be determined, as shown in Figure 5.2. The boundaries of the LLG areas of the Middle Fly District are shown in Figure 5.3. The cluster analyses focused only on TB patients from the Gogodala (Balimo Urban and Gogodala Rural LLGs) and Bamu (Bamu Rural LLG) regions, and thus a further eleven TB patients were excluded from these analyses, as described in Figure 5.2.

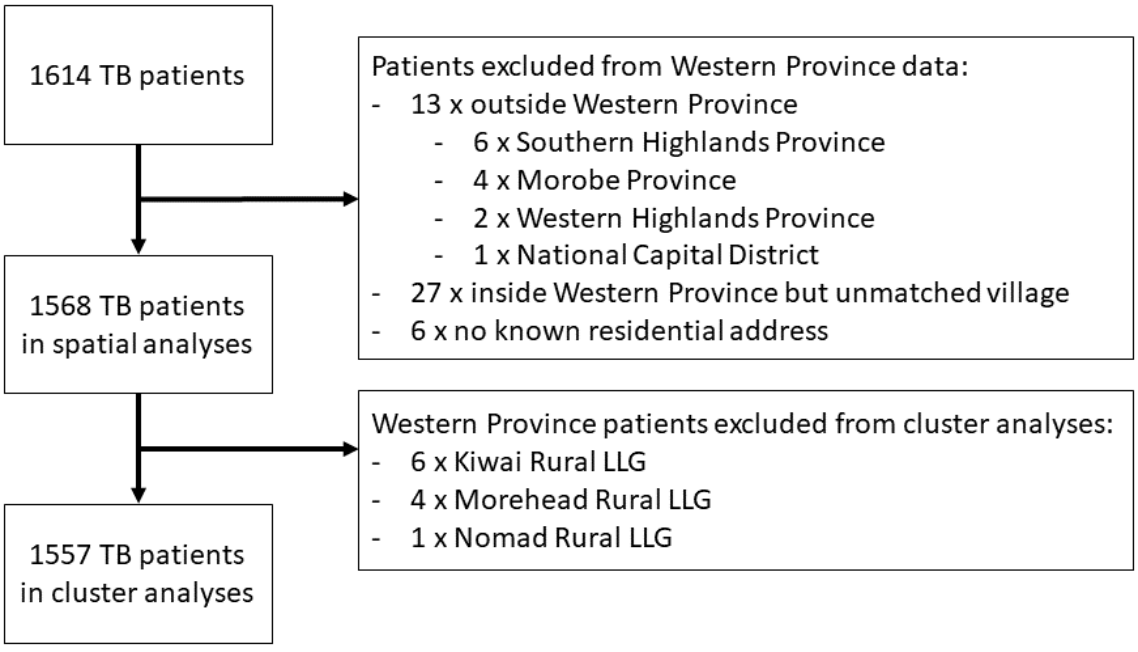


Figure 5.2: Flow diagram detailing TB patients excluded from the spatial and cluster analyses.

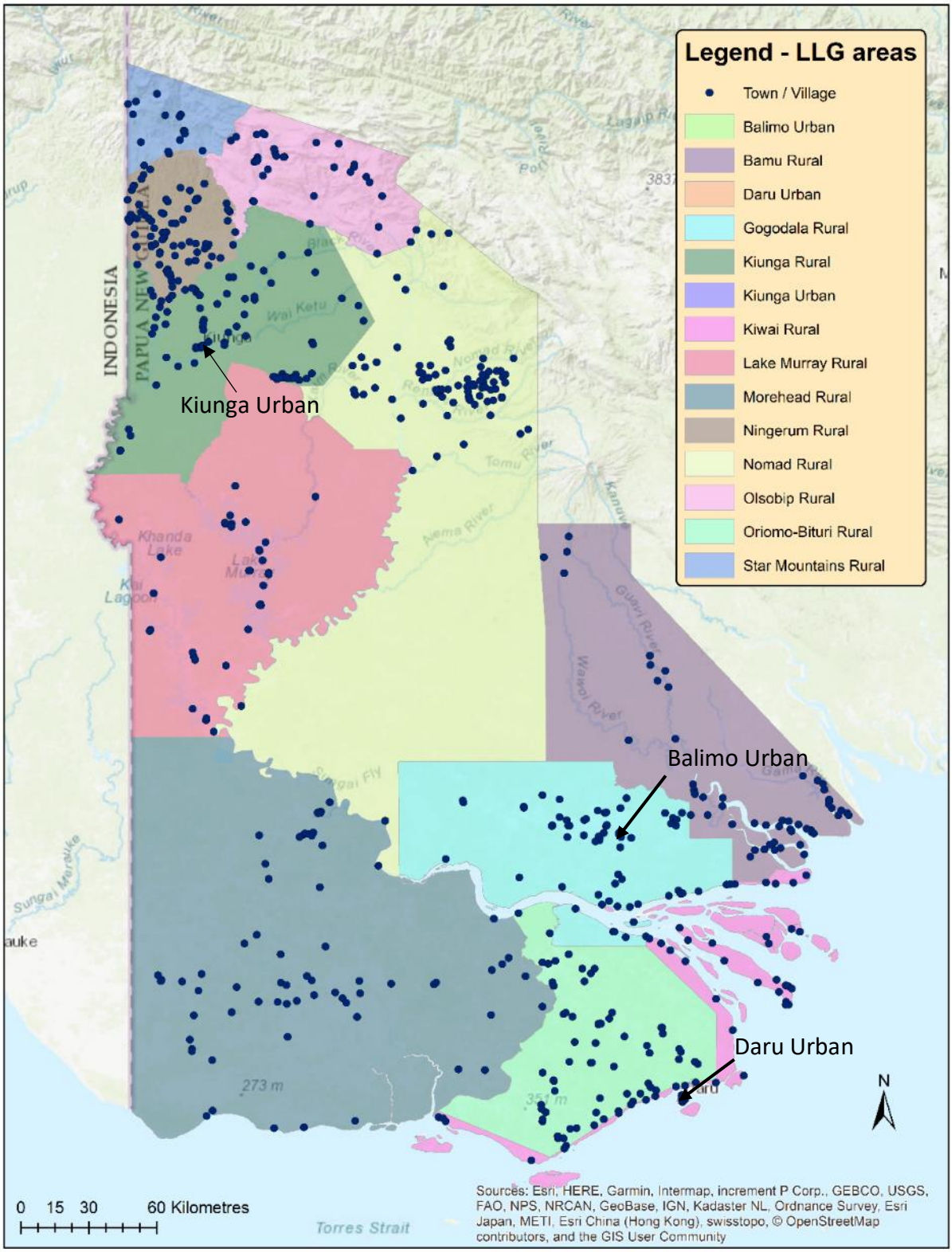


Figure 5.3: Map of towns, villages, and LLG areas in Western Province, PNG. (Map sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community)

5.2.2. Identification of drug-resistant tuberculosis patients

Drug-resistant TB patients were identified from the JCU laboratory database, which records results for all samples received and analysed at JCU Townsville. All patients who provided a clinical sample that was found to have genetic evidence of any drug resistance-associated mutation were considered to be DR-TB patients for the mapping analysis. This dataset included those patients described previously in Chapter 4, as well as DR-TB patients identified in earlier genetic analyses undertaken by our group (Diefenbach-Elstob and Moreau et al., unpublished data), which included samples collected from April 2012 to June 2017.

5.2.3. Geographic and population data

5.2.3.1. Papua New Guinea electoral and census divisions

PNG has 22 regional electorates, which includes 20 provinces, plus the Autonomous Region of Bougainville, and the National Capital District (NCD). Each province or region is sub-divided into one or more districts, with these districts further sub-divided into one or more LLG areas. For voting and census purposes, the LLG areas are sub-divided into rural wards or urban areas. These wards and urban areas are further sub-divided into census units, which approximately correlate to villages, or areas within larger towns (Figure 5.4).

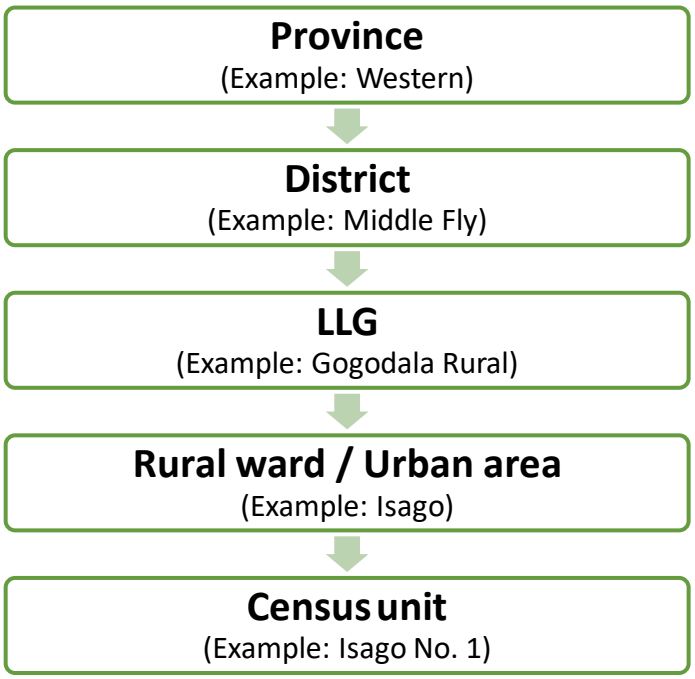


Figure 5.4: Flowchart detailing the structure of electoral and census divisions used in PNG.

5.2.3.2. Geographic divisions and population data

In this chapter, ward-level population data were used for all analyses, as current population data for census units were not available. Electoral wards were added to the TB patient register data for each patient, based on the first residential address recorded. This was performed by matching the first recorded residential address to a census unit, and from there to an electoral ward, based on PNG census data. Villages not matched based on this population data were matched to an electoral ward based on data obtained from the 2012 and 2017 PNG government election polling schedules (Papua New Guinea Electoral Commission 2012, 2017).

Population data for electoral wards were obtained from the final census figures released by the National Statistical Office (2014). These data are based on population figures from the 2011 national census. Current population numbers were calculated based on the most recent estimate of population growth, as described previously (see Section 3.2.6).

5.2.3.3. Location name discrepancies

A number of localities in the Balimo catchment region are known by alternate names or alternate spellings, with the census unit and/or ward name being different to the village name used locally and in the patient data. Potential village name discrepancies that were identified were checked and confirmed locally. Alternate names are shown in Table 5.1.

Table 5.1: BDH catchment region wards and census units known by alternate names.

Location name	Location type(s)	Alternate name
Bamustu / Bamutsa	Census unit / Ward	Aba
Dadi	Census unit / Ward	Widama
Dewara	Census unit / Ward	Dewala
Dogona	Census unit / Ward	Dogono
Gagori	Census unit / Ward	Gagoro-Matakaia
Ike	Census unit / Ward	Togowa
Kamusi	Ward	Kamusie
Kawiapo	Census unit / Ward	Kawiyapo
Kenewa	Census unit / Ward	Kaenewa
Sisiam	Ward	Sisiami
Uric	Census unit / Ward	Urio
Wasapea	Census unit / Ward	Wasapeya

5.2.3.4. Geographic coordinates

Latitude and longitude coordinates were obtained from census-level population data. Where a census ward included more than one census unit, the average ward coordinates were calculated from the latitude and longitude coordinates of all included census units. Average coordinates were calculated using the Geographic Midpoint Calculator available at http://www.geomidpoint.com/.

In a few cases, latitude and longitude data were not able to be obtained from the supplied population data. For these locations, Google was used to search for latitude and longitude data from alternate sources, as shown in Appendix 5.

The use of alternate coordinate sources meant that it was occasionally necessary to convert between the latitude-longitude and degrees-minutes-seconds (DMS) coordinate systems. All conversions were performed using the conversion calculator available at http://www.latlong.net/lat-long-dms.html.

Coordinate systems used for the data included in this analysis were based on the following systems:

- Projected Coordinate System: WGS_1984_Web_Mercator_Auxiliary_Sphere
- Geographic Coordinate System: GCS_WGS_1984
- Datum: D_WGS_1984

5.2.4. Sources for maps

The basemap layer was the 'World Topographic Map' provided within the Esri ArcGIS Online package. The data sources for this map include the following: Esri, HERE, DeLorme, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, MapmyIndia, © OpenStreetMap contributors, and the GIS User Community. Provincial, district, and LLG boundary data, and coordinates for populated places within Western Province, were sourced from the PNG National Statistical Office.

5.2.5. Data analysis

All data layers and maps were created and manipulated using ArcGIS – ArcMap 10.4.1 (Esri, Redlands, California, USA).

5.2.5.1. Calculation of specific case notification rates and standardised incidence ratios

Specific case notification rates and standardised incidence ratios were calculated for new TB patients in wards in the Balimo Urban and Gogodala Rural LLG areas. This included only new TB patients diagnosed during the three full years of the study period (2014 – 2016) (i.e. retreatment, transfer in, other, and unknown patients were excluded). Case numbers for the three full years were totalled, and then divided by three to determine an average number of cases per year. Population for each ward was calculated for the midpoint of the time period (i.e. 2015), using the 2011 census data (National Statistical Office 2014), and based on the method and growth rate described previously (see Section 3.2.6). Specific case notification rates for each ward were calculated using the average case number as the numerator, and the calculated population as the denominator, per 100,000 people. Standardised incidence ratios were calculated based on the expected rate of TB from the overall calculated incidence of 727 TB cases per 100,000 people per year (see Section 3.3.1). Calculations were undertaken using the 'Epidemiology/Biostatistics Tools' worksheets (LaMorte 2006).

5.2.5.2. Epidemiological mapping analyses

All TB patients registered at BDH from 26th April 2013 to 25th February 2017 were mapped, with the exception of the excluded cases described previously (see Section 5.2.1). The map depicting the locations of TB patients was based on census unit-level coordinates. However, patients from a number of localities were mapped based on the average coordinates for a number of census units within the town/village, as the precise census unit was rarely known for these patients (Figure 5.5). Localities where average coordinates were used are described in Table 5.2. All other epidemiological maps depicting descriptive and spatial statistics were based on ward-level coordinates.

Figure 5.5: Weekend football match in the town of Balimo. The Balimo Urban ward is comprised of six census units, however these individual census units are rarely recorded in the TB patient register. (Image credit: David Plummer)

Table 5.2: Localities where multiple census units were used to derive average ward-level coordinates.

Location	Census units	Average latitude	Average longitude
Adiba	Ago Alibi Lubi Sanabase	-8.058689	142.87474
Awaba	Awaba Community School Awaba Health Centre Awaba High School Awaba Mission	-8.009253	142.752998
Balimo	Balimo 01 Balimo 02 Balimo 03 Balimo 04 Balimo Village (1) Balimo Village (2)	-8.037795	142.957082
Bina	Bina No. 1 Bina No. 2	-8.103306	143.679974
Isago	Isago Community School Isago No. 1 Isago No. 2	-8.0097	142.687083
Pirupiru	Pirupiru No. 1 Pirupiru No. 2	-8.037183	143.721821
Sisiami	Sisiami No. 1 Sisiami No. 2	-8.094085	143.560867

5.2.5.3. Cluster analyses

Case, population, and coordinate data was tabulated in Microsoft Excel, and imported into SaTScan™. The spatial scan statistic was then calculated using SaTScan™ (versions 9.4.4 and 9.6), which is a trademark of Martin Kulldorff. The SaTScan™ software was developed under the joint auspices of (i) Martin Kulldorff, (ii) the National Cancer Institute, and (iii) Farzad Mostashari of the New York City Department of Health and Mental Hygiene (Kulldorff & Information Management Services 2009).

A discrete Poisson probability model was used because occurrence of the disease is rare (Kulldorff 1997). The data was scanned for areas with either high- or low-rate clusters. A circular spatial window was used, and the maximum spatial cluster size was set at the default size of 50% of the population at risk. The analysis was run with 999 replications, and statistical significance was set at $p < 0.05$. Secondary clusters that were significant were Gini clusters. These non-overlapping clusters are selected to maximise the Gini index, which is a measure of statistical dispersion, to ensure that there is a large difference between the cluster and non-cluster areas (Boscoe et al. 2003; Han et al. 2016; Kulldorff 2015). Shapefiles describing the cluster areas were generated as part of the SaTScan™ analyses, and overlaid on maps of the Gogodala and Bamu regions using ArcGIS – ArcMap 10.4.1 (Esri, Redlands, California, USA).

5.3. Results

Of the 1614 TB patients registered at BDH from 26th April 2013 to 25th February 2017, a total of 1568 were mapped following exclusion of the patients described previously (see Section 5.2.1).

5.3.1. The catchment area of Balimo District Hospital

The locations of towns and villages where TB patients were identified in this study are shown in Figure 5.6. As shown by the LLG boundaries on the map, Balimo Urban LLG and Gogodala Rural LLG are the primary catchment regions of BDH. However, the locations of some identified TB patients demonstrate that patients originate from across the region more broadly, particularly including a large part of the Bamu LLG, and to a limited extent areas to the north (Nomad Rural LLG), west (Morehead Rural LLG), and south (Kiwai Rural LLG) of the Gogodala region.

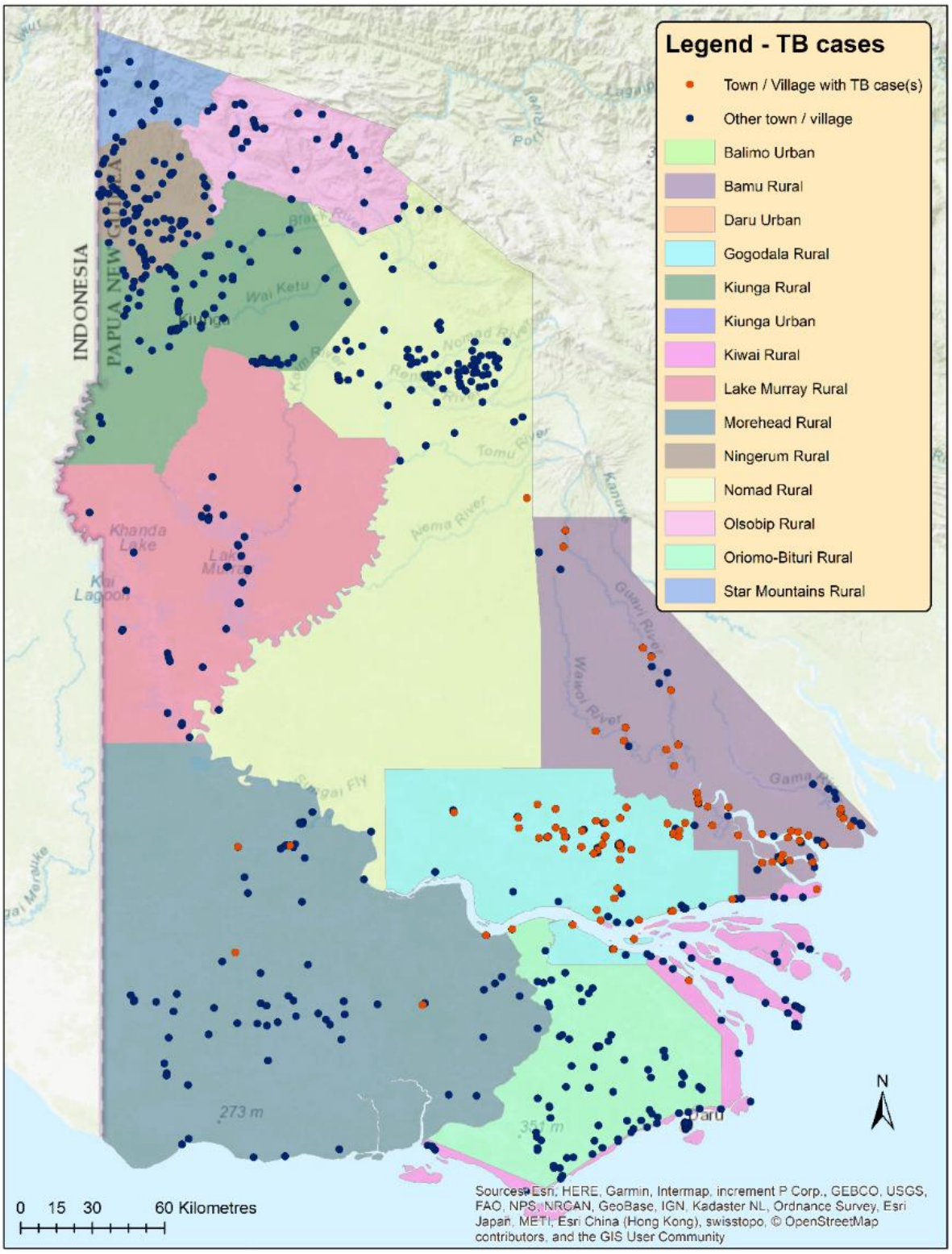


Figure 5.6: Map of Western Province showing all localities with TB patients identified in this study, differentiated by LLG area. (Map sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community)

5.3.2. Density of tuberculosis cases

The specific case notification rates and standardised incidence ratios (SIR) for ward are shown in Table 5.3. These case notification rates reflect the average number of new TB cases in the Gogodala region over the complete 2014 – 2016 period. Some wards were found to have extremely high rates of TB based on the standardised incidence rates. For example, the SIR of Kimama ward was 514% higher than that of the Gogodala region overall.

The density of TB cases was mapped in the Balimo region, depicting the number of TB cases in relation to ward-level population size (Figure 5.7). Overall, this map demonstrates lower case density in the peripheral regions, and higher case density in the area immediately surrounding Balimo, particularly along the river.

Table 5.3: Average case numbers, specific CNR (per 100,000 people), and SIR by ward for new TB patients in the Gogodala region over the 2014 – 2016 period. SIR calculations are based on the average incidence of 727 TB cases per 100,000 people per year.

Ward	Average cases (n)	Population	Specific CNR (per 100,000)	SIR (95% CI)
Adiba	15.7	1893	829	114 (65 – 186)
Aduru	0	800	0	0
Aketa	12.3	935	1316	181 (94 – 314)
Ali	7.7	1814	424	58 (25 – 117)
Awaba	1.7	1816	94	13 (1 – 51)
Balimo	63.7	4850	1313	181 (139 – 231)
Bamustu	9.3	380	2446	337 (156 – 633)
Baramula	0	899	0	0
Dadi	11.7	928	1261	173 (89 – 305)
Dede	1	1376	73	10 (0 – 56)
Dewara	0	952	0	0
Dogono	4	835	479	66 (18 – 169)
Duaba	0	744	0	0
Ike	8.3	665	1248	172 (75 – 334)
Isago	13.7	1154	1187	163 (89 – 276)
Kawiapo	5.7	1314	434	60 (21 – 132)
Kawito Station	0	166	0	0
Kenewa	5	760	658	90 (29 – 211)
Kewa	7	904	774	107 (43 – 219)
Kimama	34.7	778	4460	614 (427 – 854)
Kini	5.3	468	1131	156 (52 – 355)
Konedobu	0	355	0	0
Kotale	9.7	983	987	136 (64 – 252)
Kubu	1.7	130	1306	180 (15 – 713)
Lewada	0.3	1511	20	3 (0 – 39)
Makapa	9	2047	440	60 (28 – 115)
Pagona	1.3	962	135	19 (0 – 87)
Pikiwa	5	1040	481	66 (21 – 154)
Pisi	16.3	1235	1320	182 (104 – 294)
Semabo	1.3	513	254	35 (1 – 163)
Sialoa	0	716	0	0
Tai	9	504	1785	246 (112 – 466)
Tapila	0.3	562	53	7 (-1 – 105)
Ugu	12.3	1550	794	109 (57 – 189)
Uladu	7	492	1423	196 (78 – 403)
Urio	0.7	1609	43	6 (0 – 43)
Waligi	7.3	852	857	118 (48 – 239)
Wasapeya	4	344	1161	160 (43 – 409)
Waya	4	762	525	69 (19 – 178)
Yau	5	713	701	96 (31 – 225)
Total	**301**	**41311**	**729**	**100 (89 – 112)**

CI: confidence interval; CNR: case notification rate; n: number; SIR: standardised incidence ratio; TB: tuberculosis

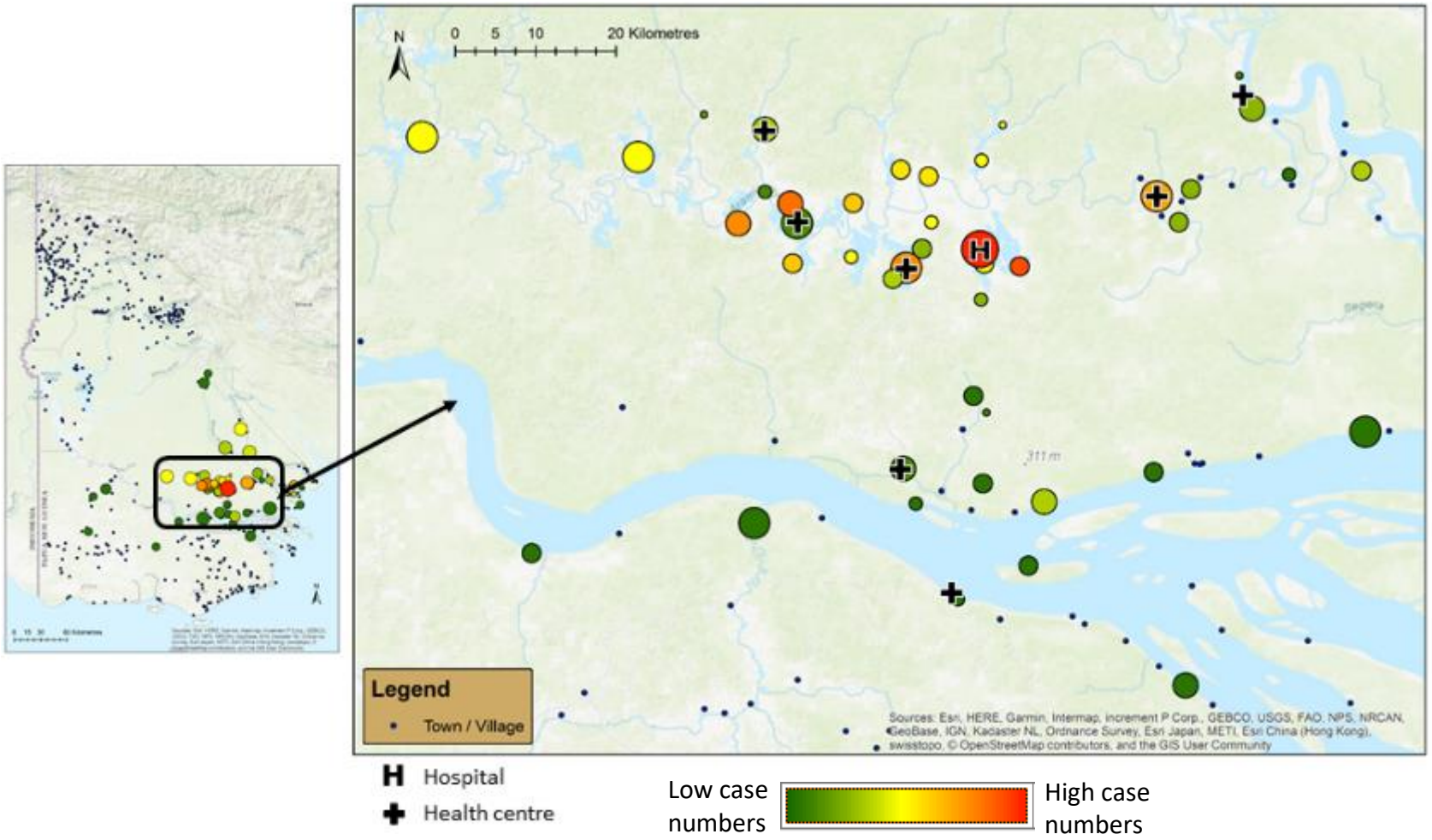


Figure 5.7: Case density map based on ward-level population data for TB cases identified in the Balimo region during the study period. Circle sizes reflect the population size of the villages, with the colour representing the number of TB cases. (Map sources: Esri, HERE, Garmin, Intermap, Increment IP Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User community)

5.3.3. Cluster analyses

TB patients in the Bamu (n = 295) and Gogodala (n = 1262) regions were analysed separately to investigate for TB case clusters. Clusters identified in the Gogodala region are described in Table 5.4 and depicted in Figure 5.8. The optimal Gini coefficient was found at 20%, so only clusters with less than 20% of the population at risk were reported. In general, high-rate clusters (more cases observed than expected given the population density) were identified close to Balimo, while low-rate clusters (less cases observed than expected given the population density) were seen on the outskirts of the region. However, a low-rate cluster was identified at Awaba ward, and high-rate clusters were identified in the individual wards of Kimama and Pisi.

Clusters identified in the Bamu region are described in Table 5.4. These are depicted in Figure 5.9, although only clusters that were statistically significant are shown. The optimal Gini coefficient was found at 12%, so only clusters with less than 12% of the population at risk were reported. Three high-rate clusters were identified in the lower regions of the Bamu and Gama Rivers; while low-rate clusters were identified further along the Gama River, and in the far north of the Bamu Rural LLG.

Table 5.4: High- and low-rate TB clusters identified in the Gogodala and Bamu regions using the spatial scan statistic.

No.	Locations in cluster	Gini cluster	Population	Observed cases	Expected cases	RR	p
Gogodala region							
1	Kimama	Yes	704	162	23.74	7.68	<0.01
2	Lewada, Dede, Konedobu, Tapila, Dewala, Pagona, Duaba	Yes	5854	20	197.39	0.09	<0.01
3	Bamustu, Uladu, Kewa, Kotale, Tai, Balimo Urban	Yes	7351	429	247.87	2.11	<0.01
4	Urio, Kenewa, Waya, Ugu, Kawiapo, Aduru, Baramula	Yes	6970	116	235.02	0.44	<0.01
5	Awaba	Yes	1646	6	55.50	0.10	<0.01
6	Ali, Makapa, Sialoa	Yes	4147	68	139.83	0.46	<0.01
7	Pisi	Yes	1119	73	37.73	1.99	<0.01
8	Ike, Yau, Aketa, Adiba, Kawito Station, Dadi	Yes	4801	212	161.88	1.37	0.01
Bamu region							
1	Sisiami	Yes	331	37	7.27	5.68	<0.01
2	Bamio	Yes	741	54	16.27	3.84	<0.01
3	Samakopa	Yes	1292	2	28.38	0.06	<0.01
4	Kawalasi	Yes	654	2	14.36	0.13	<0.01
5	Nemeti	Yes	229	15	5.03	3.09	0.02
6	Ukusi	Yes	293	0	6.44	0.00	0.03
7	Garu	No	549	3	12.06	0.24	0.09
8	Ibuo	No	385	2	8.46	0.23	0.27
9	Gagoro	No	184	8	4.04	2.01	0.92
10	Miruwo	No	789	22	17.33	1.29	1.00

RR: relative risk; TB: tuberculosis

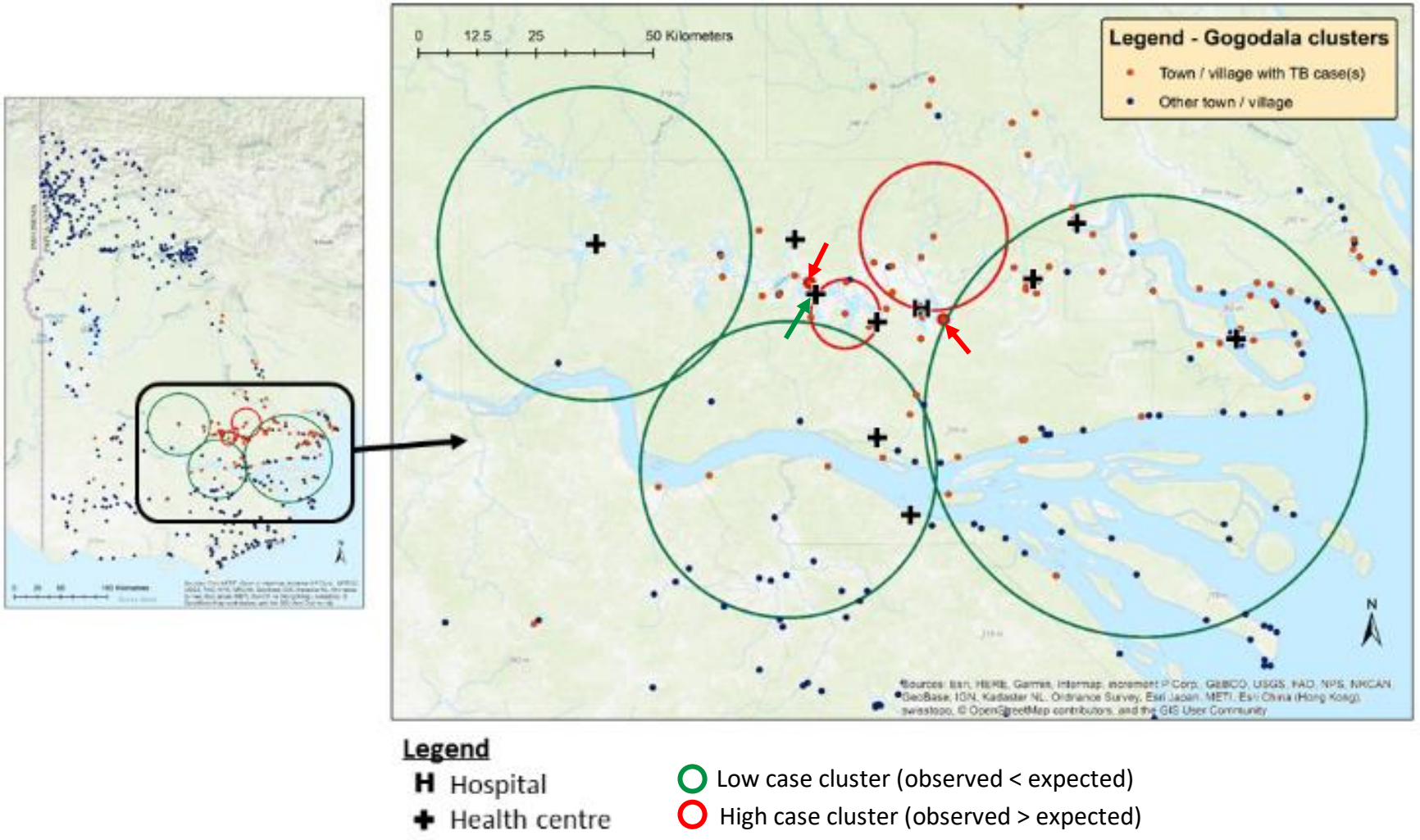


Figure 5.8: Geographic distribution of high- and low-rate TB clusters identified in the Gogodala region. (Map sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community)

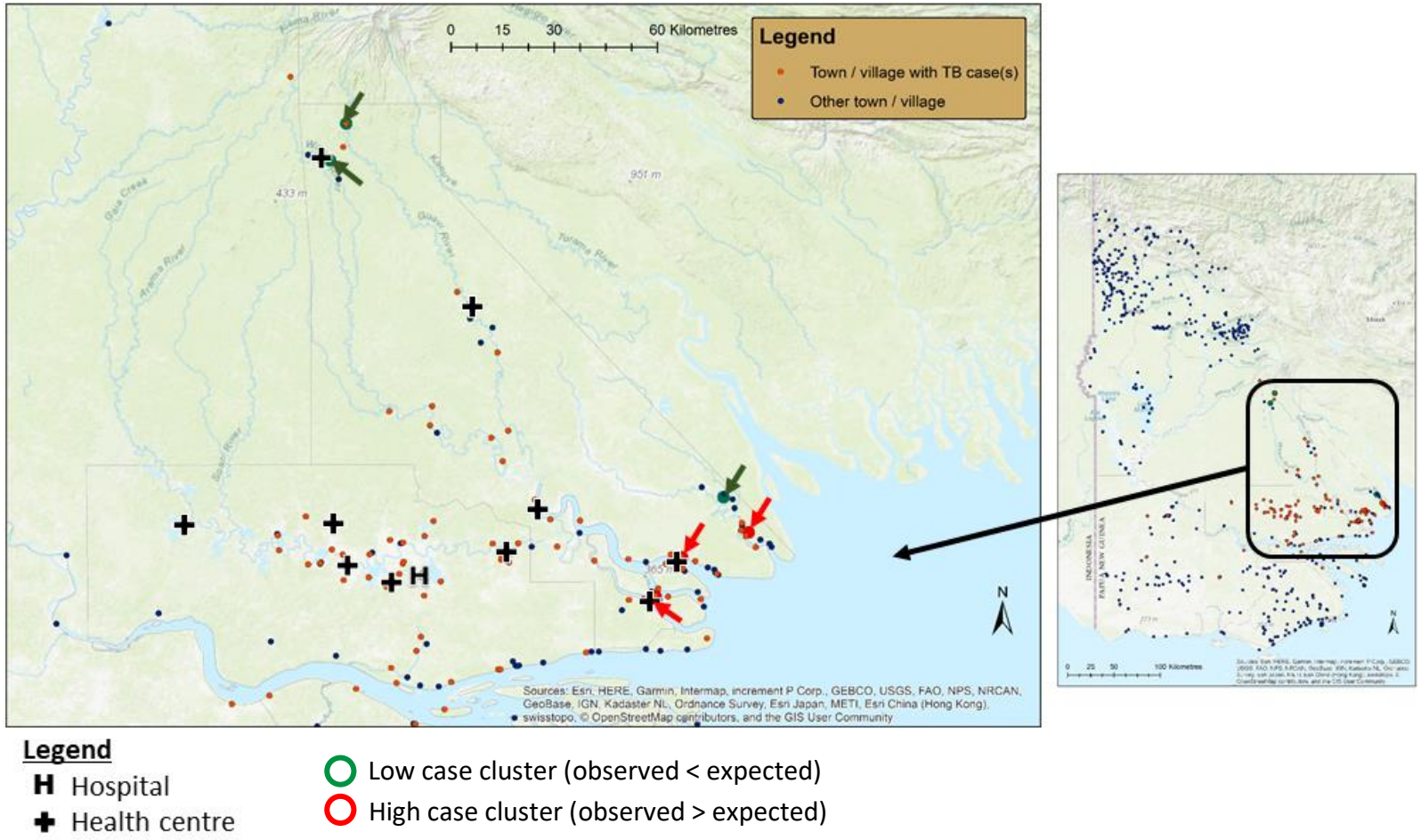
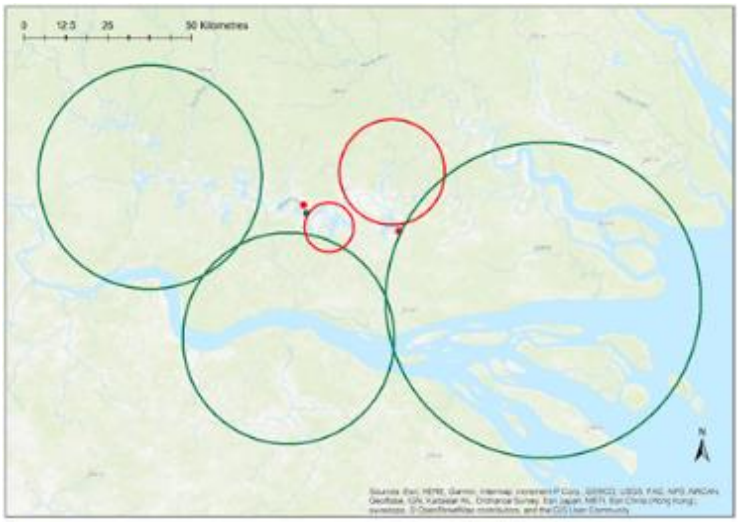


Figure 5.9: Geographic distribution of high- and low-rate TB clusters identified in the Bamu region. Only clusters that were statistically significant are shown. (Map sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community)

5.3.4. Cluster analyses of demographic variables

Comparative cluster analyses are shown for sex (Figure 5.10), age (Figure 5.11), and TB type (Figure 5.12). These analyses are consistent with the broader cluster analyses, and show that across the demographic variables there are consistently higher rates of TB in the Balimo region and immediate surrounds, while lower rates of TB are seen in the outlying areas.

(a)



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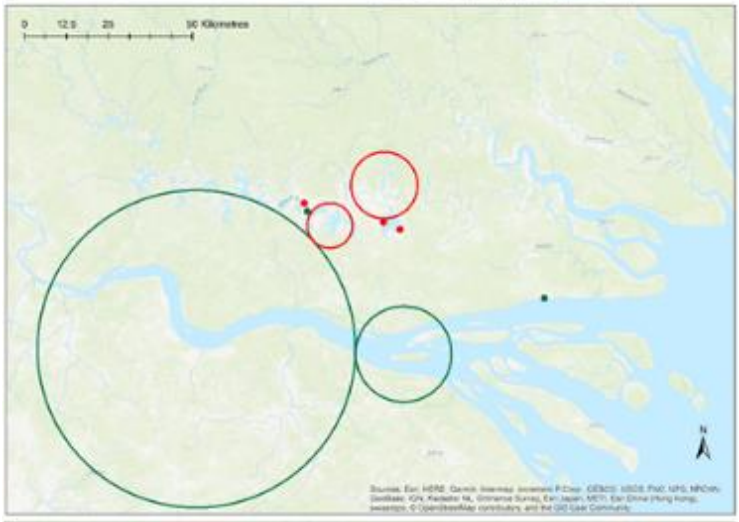

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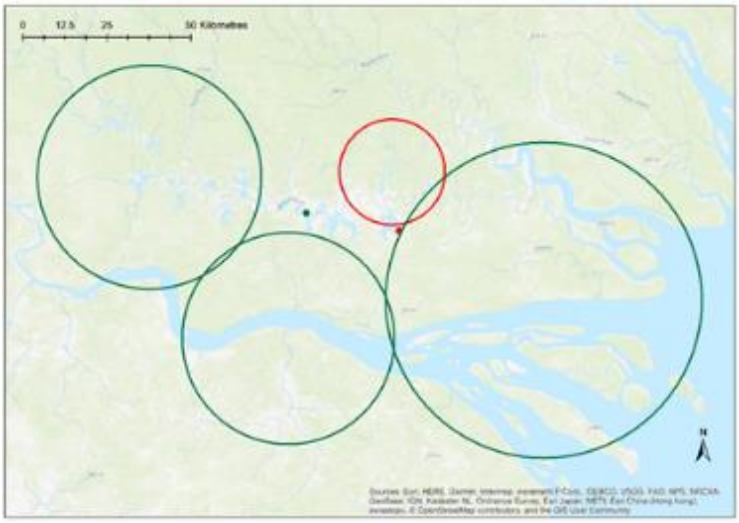
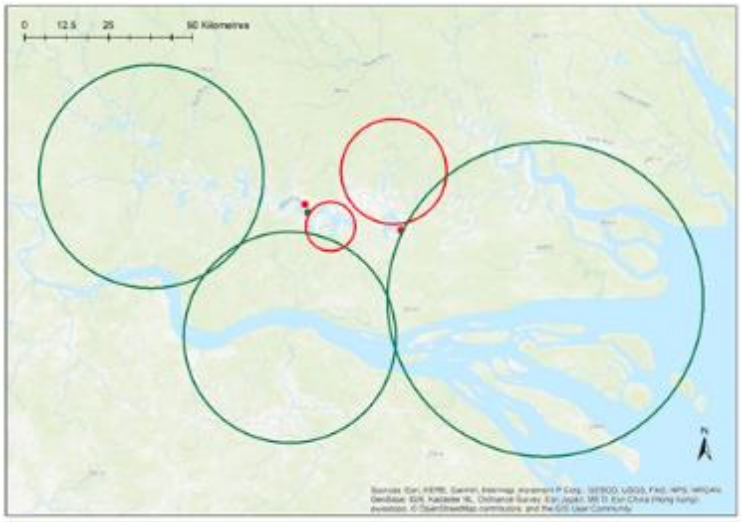


Figure 5.10: Cluster analyses for (a) all TB patients, (b) male TB patients, and (c) female TB patients in the Gogodala region. (Map sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community)

(a)



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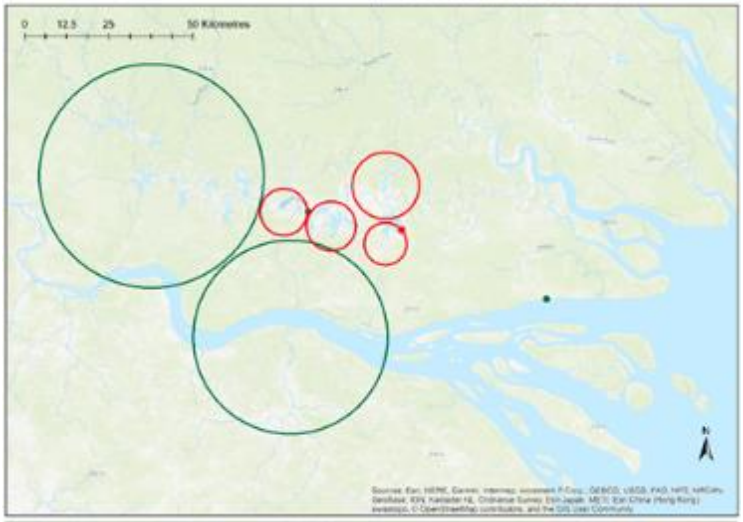

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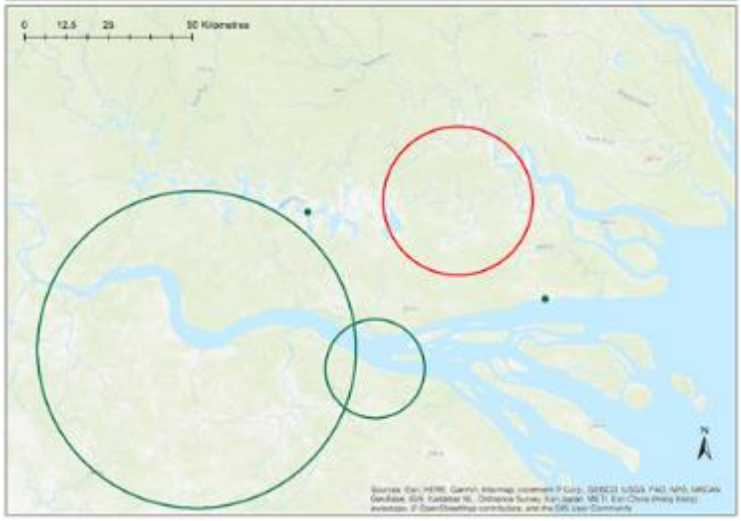
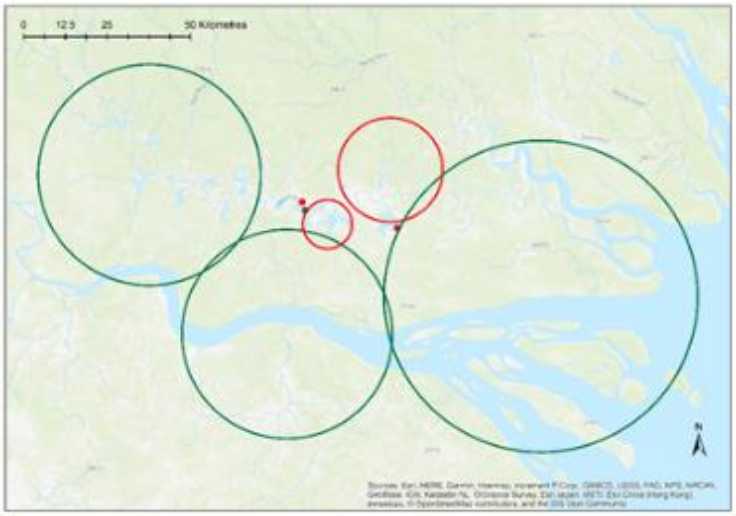


Figure 5.11: Cluster analyses for (a) all TB patients, (b) adult TB patients, and (c) child TB patients in the Gogodala region. (Map sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community)

(a)



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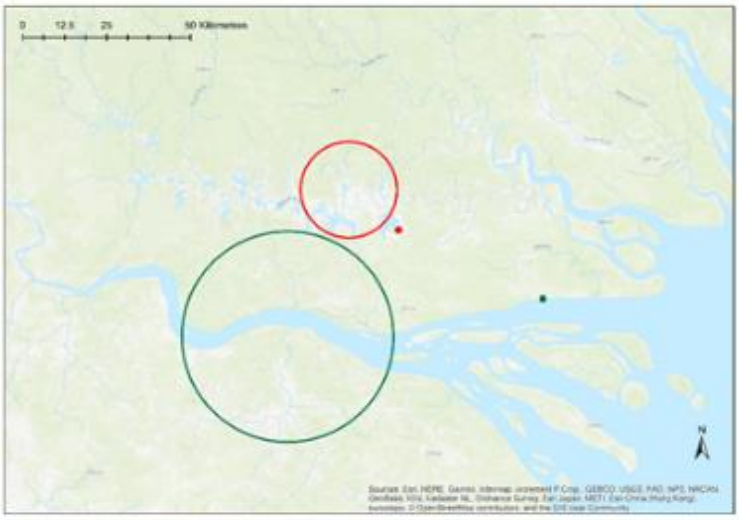

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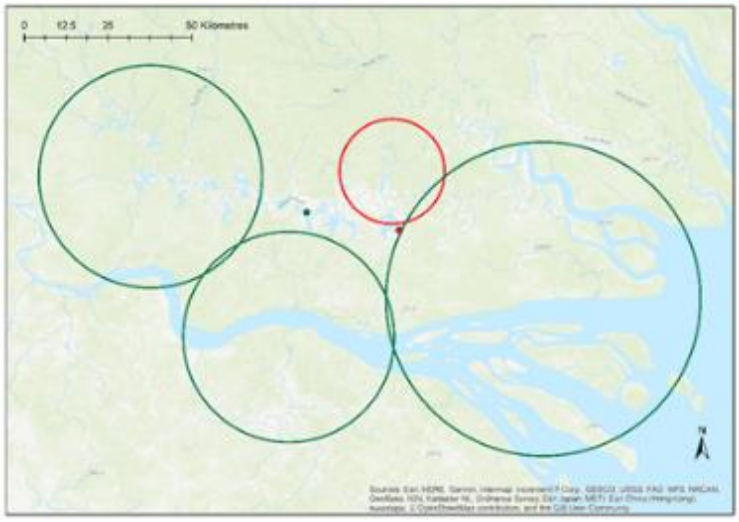


Figure 5.12: Cluster analyses for (a) all TB patients, (b) pulmonary TB patients, and (c) extrapulmonary TB patients in the Gogodala region. (Map sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community)

5.3.5. Drug-resistant tuberculosis in the Balimo region

The distribution of DR-TB is depicted based on the locations of 12 patients who had genetic evidence of TB drug resistance in any of the gene targets investigated (Figure 5.13). The ward-level locations of DR-TB patients were Adiba, Balimo, Bamustu, Bibisa, Dadi, Isago, Kawiyapo, Kewa, and Ugu. The majority of DR-TB cases were located along the river. The proportion of childhood TB and clustering results for these locations are shown in Table 5.5. There was no obvious association of DR-TB patients with overall high- or low-rate cluster locations, as patients were located in wards identified as low-rate, high-rate, and non-clustered. However, three DR-TB patients were located in Balimo, and two were located at the logging site of Panakawa in the Bibisa ward.

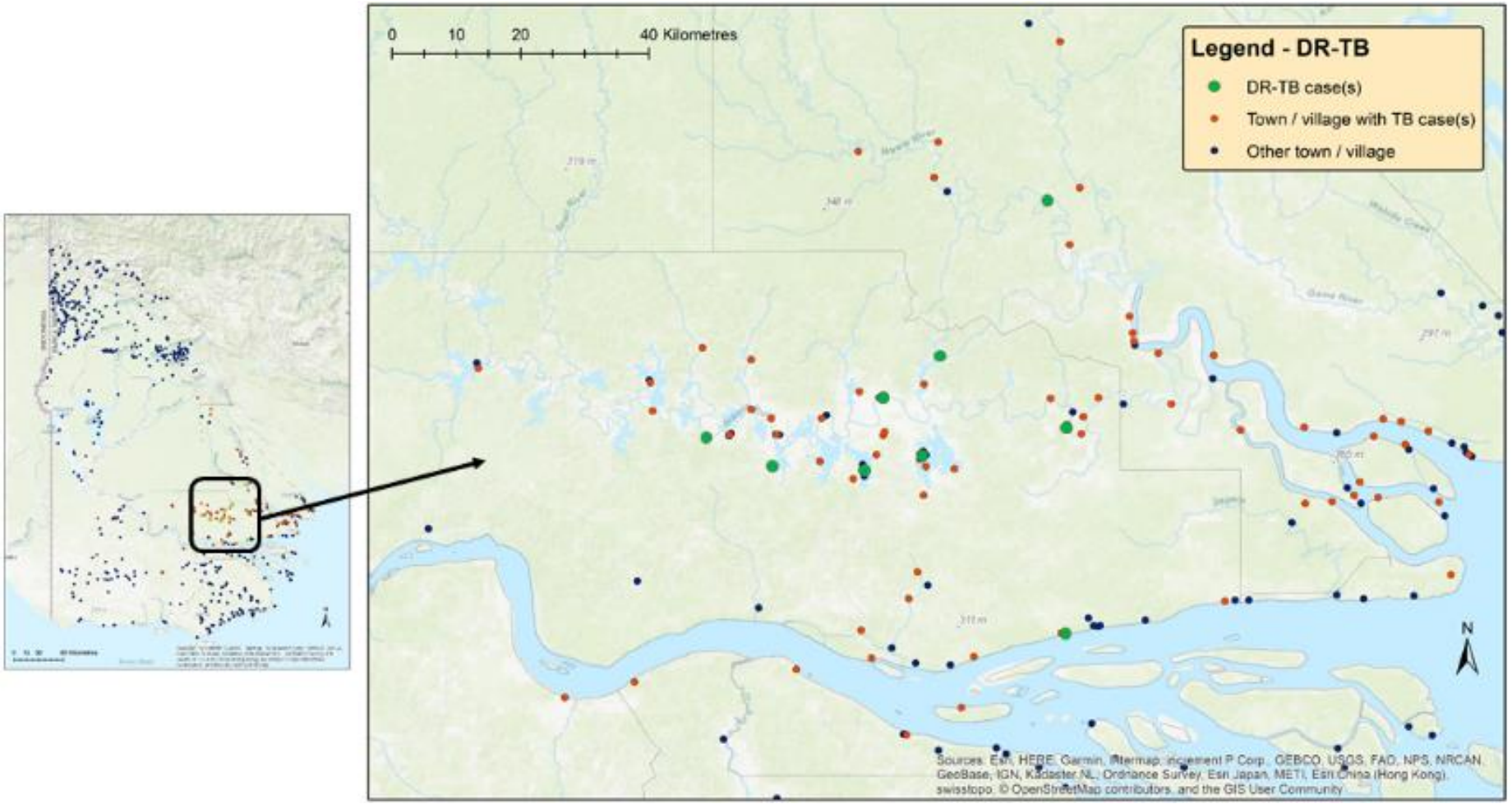


Figure 5.13: Locations where DR-TB patients were identified based on molecular characterisation of drug resistance-associated genes. (Map sources: Esri, HERE, Garmin, Intermap, increment P Corp., GEBCO, USGS, FAO, NPS, NRCAN, GeoBase, IGN, Kadaster NL, Ordnance Survey, Esri Japan, METI, Esri China (Hong Kong), swisstopo, © OpenStreetMap contributors, and the GIS User Community)

Table 5.5: Proportions of childhood TB and clustering (Gogodala region only) for ward-level locations that had DR-TB cases.

Location	No. DR-TB cases	Child TB %	Clustering
Adiba	1	13.8	H Overall H Adult H Pulmonary
Balimo	3	17.3	H Overall H Adult H Extrapulmonary H Pulmonary H Female H Male
Bamustu	1	38.9	H Overall H Child H Adult H Extrapulmonary H Pulmonary H Female H Male
Bibisa	2	28.0	n/a
Dadi	1	23.4	H Overall H Adult
Isago	1	10.7	H Adult
Kawiyapo	1	27.3	L Overall L Extrapulmonary L Female L Male
Kewa	1	28.6	H Overall H Adult H Extrapulmonary H Pulmonary H Female H Male
Ugu	1	42.9	L Overall H Child L Extrapulmonary L Female

DR-TB: drug-resistant tuberculosis; H: included in high-rate cluster area; L: included in low-rate cluster area; No.: number; TB: tuberculosis

5.4. Discussion

The results of this analysis describe the broad region of Western Province from which patients present at BDH. High-rate TB clusters were consistently seen closer to Balimo, while low-rate TB clusters occurred in the more remote areas.

5.4.1. The locations of tuberculosis patients demonstrate the broad Balimo District Hospital catchment region

Mapping of TB patients diagnosed at BDH demonstrates the broad catchment region served by the hospital. Although the majority of cases originate from the Gogodala region, a large number also present from the Bamu region to the north-east of Balimo. Understanding the reach of the BDH catchment region is important in the context of the broader Western Province TB control program, as the large number of TB patients presenting at BDH from outside the Gogodala region emphasises the importance of the hospital to the Middle Fly District more broadly. The broad catchment region also illustrates the considerable distances that people may need to travel to seek care for TB symptoms.

In the Gogodala region, most villages had TB cases identified during the study period, and villages where TB patients were not identified were predominantly located in the south of the region, along the Fly River. Villages within the Gogodala region where no TB was identified should be noted for future investigation. It would be necessary to identify if there are people symptomatic for TB in these locations, and if so, to determine if people with TB symptoms in these locations travel elsewhere for investigation (for example, to Daru in the south, which may particularly be the case for people located near the Fly River). Geographic challenges may be particularly important for people from the Fly River region, as travel to either Balimo or Daru will be lengthy, and as such TB cases may remain undiagnosed.

5.4.2. Higher tuberculosis case densities closer to Balimo may reflect access challenges and health-seeking behaviour

The challenges associated with travel in PNG, particularly in the context of TB care, have been described previously. Ongugo et al. (2011) noted this issue, stating that only 35% of the PNG population live within 10 kilometres of a major road, and 17% have no access to roads at all. This situation is indeed the case in the Gogodala and Bamu regions, where roads are limited to the Balimo town area, and in proximity to the logging camps (Figure 5.14). Travel in the region is primarily by foot or boat. Air travel is prohibitively expensive for most people, and limited to smaller prop-driven aircraft capable of landing on short, grass airstrips (Figure 5.15). Additionally, the airport at Balimo has been closed since 2013 (Orere 2017). This closure necessitates a journey of 30 to 60 minutes by motorised boat upstream to the closest airport at Kawito, which has scheduled flights limited to once or twice per week.

Figure 5.14: Logging road near Sasareme logging camp in Western Province, PNG. (Image credit: Tanya Diefenbach-Elstob)

Figure 5.15: A DHC-6 Twin Otter used for air travel from Port Moresby to Kawito Station, upstream of Balimo in Western Province, PNG. (Image credit: Tanya Diefenbach-Elstob)

The presence of high-rate clusters and higher densities of TB patients in the area immediately surrounding Balimo is not surprising, as access to care will be easier for people living closer to Balimo, and in villages adjacent to the Balimo lagoon and Aramia River (Figure 8.5). Such influences have also been described in Ethiopia, with studies finding higher TB density in regions with closer proximity to urbanised areas (Dangisso et al. 2015a), a greater risk of mortality in children under five living further away from a regional health facility (Okwaraji et al. 2012), and delayed treatment-seeking in people who had to travel to a health facility by foot (Robel et al. 2017). In addition, urbanisation is associated with higher rates of TB, as a result of factors such as overcrowding and increased TB transmission risk (Antunes & Waldman 2001; Oppong et al. 2015; Wanyeki et al. 2006).

Smaller villages with high TB patient numbers, particularly those within the immediate Gogodala area, should be noted for further investigation to identify the underlying causes of the high TB burden. In this context, both Kimama and Pisi were identified in this analysis. Neither of these villages had DR-TB patients identified in the analysis, and both had proportions of childhood TB comparable to the average proportion (17.6% and 26.4% respectively). Furthermore, villages with a low density of TB, particularly those with easier access to health services, should also be identified. Such villages may have their own locally-

implemented public health measures for management of TB that could provide useful approaches applicable in high-burden villages.

Other possible reasons for high case density in particular villages include the presence of an actively staffed aid post or health clinic that regularly refers presumptive TB patients to Balimo, or increased case-finding or awareness activities (by either health workers or community members) (Figure 5.16). Proximity to a health facility, health worker training, and local TB awareness activities are all factors that have been associated with increased TB notifications in other settings (Dangisso et al. 2015b; Datiko et al. 2017; Parija et al. 2014; Yassin et al. 2013). Furthermore, a study from South Africa has described a large proportion of people with TB symptoms being overlooked for further investigation, highlighting the importance of health worker awareness and education in the identification and diagnosis of TB (Chihota et al. 2015), which may play a role in the number of patients who are subsequently diagnosed with TB. Interestingly, neither Kimama nor Pisi were sites with active peripheral health facilities at the time of this study. If these villages reflect accurate rates of TB diagnosis, rather than comparatively high rates of disease, villages with low case densities may indicate TB under-diagnosis, and thus sites where active TB investigations should be undertaken.

Figure 5.16: Sister Bisato Gula providing TB awareness and education during a health worker patrol to a Gogodala-region village. (Image credit: Tanya Diefenbach-Elstob)

Lower TB patient density in the regions further from Balimo are likely to occur for two reasons. The greater distance from Balimo will result in access challenges for people who must travel further to access care (Diefenbach-Elstob et al. 2017). Indeed, distance and easier access as a factor in TB diagnostic delay and illness duration have been described in other resource-limited settings (Babatunde et al. 2015; Demissie et al. 2002; Lawn et al. 1998). The second reason likely to influence the locations of TB patients in the Balimo region is that people with TB symptoms who are located in villages in the south of the Gogodala Rural LLG, particularly along the Fly River, may be more likely to travel to Daru to seek care, rather than to Balimo. However, the presence of Fly River-region patients at Balimo indicates that choosing to walk to Balimo rather than travel by boat to Daru (a long and potentially rough journey) is still a preferred option for some people.

The low-rate cluster identified in Awaba will be a reflection of TB patient recording. Awaba is a larger village with a population of more than 1600 people. It has one of the few high schools in the region, and is the site of the largest health centre in the Gogodala region outside of Balimo. This low-rate cluster will reflect the separate registration of TB patients diagnosed and commenced on TB treatment at Awaba Health Centre. The Awaba TB register

was not available for this study, although TB incidence at the centre was estimated to be 381 cases per 100,000 people per year in the 2011 Western Province TB evaluation study (McBryde 2012). It would be interesting to investigate the Awaba TB register in the future to provide further understanding of the burden of TB disease present in the Gogodala region.

The reasons for high- and low-rate TB clustering in the Bamu region were not always obvious, as this region does not have an urban centre. However, the high-rate clusters located at Nemeti and Sisiami will be the result of non-routine active case-finding activities undertaken in March 2016. Another high-rate cluster was located at Bamio, which is the location of a peripheral health clinic (see Appendix 2), which may suggest an increased likelihood of symptomatic people being referred to Balimo from this health centre. The low-rate clusters in other areas of the Bamu Rural LLG (Samakopa, Kawalasi, and Ukusi) likely reflect a combination of access challenges, and travel to health centres other than Balimo for TB care, as these were the most geographically distant of the identified cluster locations.

Figure 5.17: People travelling by motorised dugout canoe in the Gogodala region. (Image credit: David Plummer)

There was no apparent clustering in the spatial distribution of DR-TB patients. However, the majority of DR-TB patients were located in river- or lagoon-based villages, which will have easier access to BDH. Given the limited amount of available data about DR-TB patients from the region, caution must be used in drawing interpretations. The presence of three DR-TB patients in Balimo likely reflects both ease of access and the high proportion of TB patients overall in Balimo. Notably, urbanisation has been associated with DR-TB elsewhere (Alene et al. 2017). Two DR-TB patients were located at the logging site of Panakawa, which is frequently referred to as a TB hotspot by health workers in the Balimo region (Diefenbach-Elstob et al, unpublished interview data). Overall, the presence of DR-TB in the area is worrying due to the potential for transmission of DR-TB strains across a broad geographic region.

The presence of low-rate clusters should also be considered in the context of the burden of TB across the Gogodala and Bamu regions. If high-rate clusters are actually representative of the true TB burden in the regions where they occur, then low-rate clusters may be indicative of TB under-diagnosis.

5.4.3. Non-routine case-finding may reflect the true tuberculosis burden

There are some issues of note with the 57 excluded patients (see Section 5.2.1). The 11 patients from within Western Province, but outside the Gogodala and Bamu regions, may be important when considering importation of TB into the Gogodala region. Seven of the 11 patients had alternative addresses recorded within the Gogodala and Bamu regions, including two at logging camps, and one at Awaba High School. These alternative addresses suggest mobility of people in the region, particularly in the context of education and employment. Such mobility is important when considering TB transmission, as movement of people will increase the number of contacts that TB patients have.

There were 96 TB patients diagnosed as a result of non-routine active case-finding activities (see Section 3.3.6), and it was noted in Section 5.4.2 that two of the Bamu-region high-rate clusters were likely to be associated with this activity. However, these patients also demonstrate the potentially even higher burden of TB in this region. During the active case-

finding activity, patients from eight villages (located in the Nemeti, Magiwe, Sisiami, Oropai, Bunigi, Torobina, Upati, and Bina wards) were diagnosed with TB (2 with pulmonary TB and 94 with extrapulmonary TB) in an eight-day period. By comparison, there were only 31 patients diagnosed from these eight villages over the remainder of the study period. Although the majority of these patients were extrapulmonary TB, this difference suggests a substantial proportion of people with TB living in remote and difficult-to-reach locations who may not otherwise be diagnosed with TB. In the case of pulmonary TB, this situation increases the likelihood of transmission to others, and in all cases, reflects diminished quality of life for these people due to potentially long-term morbidity from TB symptoms.

5.4.4. Limitations of the geographic analysis

There are some limitations to the spatial data that has been presented in this chapter. As noted in the methods, it can occasionally be difficult to determine the 'correct' residential address to use for a patient. People frequently have more than one address recorded in the TB register. However, in this analysis only the first address recorded was used, as it was assumed that this address was where the patient was most likely to be living at the time, and thus reflected their most recent residence. However, people with more than one address may be more mobile, particularly if travelling between their residential village and home village (i.e. their place of birth or family village), and if they work at a logging site.

Earlier in this thesis it was noted that there are a number of smaller health facilities in the region where TB patients may be registered and commenced on treatment (see Appendix 2). These are small facilities, but there will be an unknown proportion of TB patients who would influence these analyses to an extent that is difficult to determine. The overall cluster analyses attempt to account for this by showing the locations of the peripheral health facilities. However, peripheral facilities were located in regions of both high- and low-rate density, implying that these facilities do not have a substantial impact on the overall distribution of TB patients in the region.

5.5. Conclusion

This analysis has provided insight into the influence of geography on TB distribution in the BDH catchment region. The results include baseline data about TB distribution across the region, as well as targeted information that points to the need for village- and ward-specific TB investigations. The provision of TB services to people outside of the immediate Gogodala region highlights the importance of BDH in TB control in the Middle Fly District of Western Province, and emphasises the importance of investment in resources and facilities at the hospital.

In this region, TB clustering likely reflects the ease with which people are able to travel and seek treatment, demonstrating the importance of being able to access health services. Easier access to health services, through targeted case-finding activities, may reveal an undiagnosed TB burden in the region.

The lack of any clear association of DR-TB patients with particular factors such as childhood TB or case density raises the question of whether DR-TB in the region is less likely to be due to transmission, and more likely the result of de novo mutagenesis or acquired resistance as a result of insufficient treatment. For those patients where access is difficult and treatment-seeking is delayed, the time taken to be diagnosed and commenced on treatment will also play a role in the development of drug resistance. Further research is necessary to develop a better understanding of the epidemiology of DR-TB in this area, and research undertaken using techniques such as whole genome sequencing would be useful to demonstrate links between DR-TB strains. However, the wide distribution of DR-TB across the Balimo region is of particular concern due to the risk of transmission of these strains across an extensive geographic region. The management of geographically dispersed DR-TB patients will be challenging, from both a patient and provider viewpoint, due to the need for travel to Daru for diagnosis and treatment.

CHAPTER 6

VALIDATION OF SMEAR MICROSCOPY IN THE DIAGNOSIS OF TUBERCULOSIS IN A RESOURCE-LIMITED SETTING

6.1. Introduction

The results presented in this thesis have described a heavy burden of tuberculosis (TB), particularly extrapulmonary TB, as well as the presence of drug-resistant TB (DR-TB) in the Gogodala region. In Chapter 3 the proportion of bacteriologically-confirmed pulmonary TB diagnosed at Balimo District Hospital (BDH) was described, with 77.9% (250/321) of pulmonary TB patients having a smear result recorded, and 65.8% (211/321) being smear-positive. However, smear microscopy is a diagnostic method known to have low and variable sensitivity, which systematic reviews have found to range from 31 – 98%, depending on the smear processing method used (Steingart et al. 2006a; Steingart et al. 2006b). The proportion of bacteriologically-confirmed pulmonary TB seen at BDH is high in comparison to the Papua New Guinea (PNG) national average, where 25.9% of pulmonary TB cases were bacteriologically confirmed (Aia et al. 2018). The known variable sensitivity of smear microscopy combined with the high proportion of smear-positive pulmonary TB in the Balimo setting indicates the possibility of missed pulmonary TB cases, including smear-negative results considered not to be TB that are indeed smear-negative pulmonary TB. This background signals a need for better understanding of the utility of smear microscopy for TB diagnoses at BDH. This chapter will examine smear microscopy results obtained from BDH, and validate these results based on comparison to culture and molecular methods.

The difficulties associated with bacteriological confirmation of *Mycobacterium tuberculosis* (MTB) infection are reflected in the World Health Organization (WHO) definitions of a TB case, which include patients with either bacteriological confirmation or a clinical diagnosis (World Health Organization 2013a). Bacteriologically confirmed TB cases must have provided a clinical specimen, which is then found positive for the presence of MTB using smear microscopy, culture, or a WHO-approved rapid diagnostic such as the Xpert MTB/RIF or

Xpert MTB/RIF Ultra (World Health Organization 2013a, 2017d). A clinically diagnosed TB case is a patient who does not meet the criteria for bacteriological confirmation, but for whom a qualified practitioner has diagnosed active TB and decided to commence a full course of TB treatment (World Health Organization 2013a). These guidelines mean that where a diagnostic specimen is unavailable, the lack of bacteriological confirmation does not preclude the diagnosis of active TB. In practice, bacteriological confirmation of TB is often easily achieved only for pulmonary TB cases, as has been demonstrated in the BDH TB patient cohort where 77.9% of pulmonary TB patients had a smear result recorded, compared to only 8.8% of extrapulmonary TB patients.

Figure 6.1: Labelling sputum pots and collecting clinical information during a TB patrol to a rural village in the Balimo region. (Image credit: Mauri Amadu)

Other diagnostic methods used for the identification of pulmonary TB also rely on the examination of a sputum sample. Historically, culture has been considered the gold standard in the detection of MTB (Public Health Laboratory Network 2006). However liquid culture, often using mycobacteria growth indicator tubes (MGIT), has an increased chance of detecting a variety of other mycobacterial species, and thus requires differentiation of the MTBC from other mycobacterial growth, which can include a variety of non-tuberculous mycobacteria (NTM) (Chihota et al. 2010; Public Health Laboratory Network 2006). Both solid and liquid culture are time-consuming, with results taking about two weeks for liquid culture, and up to eight weeks for solid culture (Chihota et al. 2010). More recently, molecular methods are increasingly being used, and the Xpert MTB/RIF or MTB/RIF Ultra are both WHO-approved rapid diagnostics for active TB (World Health Organization 2017d). Molecular methods such as the Xpert platforms have advantages for both patients and health providers, requiring a single on-the-spot sputum sample, results available within two hours, and detection of drug resistance (World Health Organization 2013c).

The variety of methods available for TB diagnosis mean that in specific settings one or more techniques may be used depending on the available resources and facilities. Although microscopy is the only method available at BDH, clinical samples used for smear microscopy have also been analysed by our group at James Cook University (JCU) using culture and molecular techniques. The aim of this study is to support the BDH laboratory by validating the smear microscopy technique, through comparison of BDH smear microscopy results with JCU laboratory results from culture and molecular testing methods undertaken on clinical samples collected from people being investigated for TB. This will provide insight into the potential misdiagnosis and/or under-diagnosis of TB at BDH. The specific objectives are:

1. To describe microscopy, culture, and molecular diagnostic results in samples from patients being investigated for TB
2. To determine the diagnostic utility of smear microscopy at BDH, based on culture and real-time PCR (qPCR) as gold standards.
3. To compare diagnostic outcomes where two or more methods were used on the same sample.

6.2. Materials and Methods

6.2.1. Diagnostic methods

This study analysed the results of diagnostic tests undertaken on samples collected at BDH from people being investigated for TB. For each sample, one or more of three different laboratory-based methods were used to identify MTB – microscopy, culture, and a molecular TaqMan qPCR assay.

Microscopy was undertaken at BDH using techniques described previously (see Section 4.2.2). Smear slides were examined at BDH, and smear grades were provided for each slide.

Culture was undertaken in the PC3 laboratory at James Cook University, Townsville. Decontaminated sputum samples were inoculated into PANTA-supplemented MGITs (Becton, Dickinson, Australia), and incubated at 37^{o}C for up to seven weeks.

The qPCR assay results were the outcome of analyses undertaken on genomic DNA extracted from smeared sputum slides, culture supernatant, and direct from decontaminated sputum. For the sputum smear slides, the DNA extraction procedure was a modified version of a Chelex DNA extraction method (Guernier et al. 2018; Van Der Zanden et al. 2003). For culture, DNA was extracted from positive MGIT cultures using the High Pure PCR Template Preparation Kit (Roche, Germany). The method undertaken for DNA extracted directly from decontaminated sputum has been described in Section 4.2. All molecular results have been grouped together for the analyses in this chapter, regardless of the origin of the extracted DNA (microscope slide, culture supernatant, or sputum).

6.2.2. Sources of diagnostic data

This analysis focused only on ‘new’ samples, i.e. only samples collected from people undergoing initial investigations for TB. Follow-up samples were excluded as these were collected from people who had been commenced on or had completed TB treatment, and were thus being investigated to exclude rather than identify the MTB organism. Smear microscopy data were primarily obtained from the BDH laboratory database. For cases that

were able to be matched to the TB patient register, any additional smear results that were recorded were also included. The results of the culture and molecular methods were obtained from the JCU laboratory database.

Laboratory test results were categorised depending on the test used and the result reported. For microscopy, if multiple smears were produced and read, only the highest smear result was retained for the analysis. For example, a sputum sample with microscopy results of 3+ and 2+ was categorised as 3+, while a sputum sample with reads of 1+ and no acid-fast bacilli (NAFB) was categorised as 1+. Positivity in the molecular assay was based on analysis of the IS*6110* assay, which was considered reactive when the cycle threshold (Cq) was less than or equal to 40 cycles. Samples considered to be 'MTBC or NTM' were classified as positive for this analysis, as described previously in Section 4.2.4.

In cases where more than one sputum sample was collected from an individual, either on the same day or within four days of each either, only one sample was retained for the analysis, with this being the positive result if the results were discrepant. The diagnostic results were combined for one sample that had been split for processing.

6.2.3. Data analysis

Diagnostic methods were statistically analysed using 2x2 tables constructed as shown in Table 6.1.

Table 6.1: Format of 2x2 tables for calculation of diagnostic statistics.

		Gold standard		
		Positive	Negative	**Total**
Diagnostic method	Positive	a	c	**a + c**
	Negative	b	d	**b + d**
	Total	**a + b**	**c + d**	**a + b + c + d**

Based on the 2x2 tables, diagnostic statistics were calculated using MedCalc's diagnostic test evaluation calculator, according to the following formulae (MedCalc Software 2018):

$$sensitivity = \frac{a}{a+b}$$

$$specificity = \frac{d}{c+d}$$

$$positive\ predictive\ value\ (PPV) = \frac{a}{a+c}$$

$$negative\ predictive\ value\ (NPV) = \frac{d}{b+d}$$

$$pre-test\ proportion = \frac{a+b}{a+b+c+d}$$

$$positive\ likelihood\ ratio = \frac{sensitivity}{1-specificity}$$

$$negative\ likelihood\ ratio = \frac{1-sensitivity}{specificity}$$

Likelihood ratio nomograms showing the pre- and post-test probabilities of disease were calculated and constructed using an online diagnostic test calculator (Schwartz 2006). For these calculations, the pre-test probability (prevalence) was set at 10%. The kappa index was calculated using GraphPad QuickCalcs (GraphPad Software 2018).

6.3. Results

6.3.1. Overall sputum sample testing results

A total of 804 sputum samples were transferred to JCU during the period April 2012 to June 2017. Of these, 655 were retained as 'new' samples for this analysis. Results for microscopy, culture, and qPCR are shown in Table 6.2.

Table 6.2: Diagnostic results for sputum samples tested with smear microscopy, liquid culture, and qPCR. The '% of known' column shows the proportions of positive and negative results in each group with the unknown proportions excluded.

Method	Overall result	n (%)	% of known
Microscopy	Positive	142 (21.7)	142 (23.1)
	Negative	472 (72.1)	472 (76.9)
	Unknown	41 (6.3)	
	Total	**655 (100.0)**	**614 (100.0)**
Liquid culture	Positive	127 (19.4)	127 (29.1)
	Negative	310 (47.3)	310 (70.9)
	Unknown	218 (33.3)	
	Total	**655 (100.0)**	**437 (100.0)**
qPCR	Positive	232 (35.4)	232 (47.3)
	Negative	258 (39.4)	258 (52.7)
	Unknown	165 (25.2)	
	Total	**655 (100.0)**	**490 (100.0)**

n: number; qPCR: real-time polymerase chain reaction

6.3.2. Comparative results in samples tested with more than one diagnostic method

Comparative results were tabulated for sputum samples where two results were known. These comparisons included smear microscopy and liquid culture (n = 400) (Table 6.3); smear microscopy and qPCR (n = 457) (Table 6.4); and liquid culture and qPCR (n = 272) (Table 6.5). There was poor agreement between the diagnostic techniques when comparing smear microscopy with liquid culture (kappa = 0.02, 95% CI -0.08 – 0.12), and when comparing liquid culture with qPCR (kappa = -0.12, 95% CI -0.23 – 0.00); while there was

moderate agreement between the methods when comparing smear microscopy with qPCR (kappa = 0.50, 95% CI 0.43 – 0.57).

Table 6.3: Comparative results for sputum smear microscopy and liquid culture for sputum samples where both results were known.

		Liquid culture		
		Positive	Negative	**Total**
Smear microscopy	Positive	32 (8.0)	76 (19.0)	**108**
	Negative	81 (20.3)	211 (52.7)	**292**
	Total	**113**	**287**	**400**

Table 6.4: Comparative results for sputum smear microscopy and qPCR for sputum samples where both results were known.

		qPCR		
		Positive	Negative	**Total**
Smear microscopy	Positive	109 (23.9)	2 (0.4)	**111**
	Negative	109 (23.9)	237 (51.9)	**346**
	Total	**218**	**239**	**457**

qPCR: real-time polymerase chain reaction

Table 6.5: Comparative results for liquid culture and qPCR for sputum samples where both results were known.

		qPCR		
		Positive	Negative	**Total**
Liquid culture	Positive	62 (22.8)	57 (21.0)	**119**
	Negative	98 (36.0)	55 (20.2)	**153**
	Total	**160**	**112**	**272**

qPCR: real-time polymerase chain reaction

Based on the above results, the sensitivity, specificity, PPV, and NPV for each of the compared techniques are shown in Table 6.6, while disease prevalence and likelihood ratios are shown in Table 6.7. Based on the prevalence and likelihood ratios, nomograms of post-test probability of disease are shown for each of the compared techniques in Figure 6.2.

Table 6.6: Sensitivity, specificity, positive predictive value, and negative predictive value for the smear microscopy and liquid culture diagnostic techniques.

Technique	**Gold standard**	**Sensitivity % (95% CI)**	**Specificity % (95% CI)**	**PPV % (95% CI)**	**NPV % (95% CI)**
Microscopy	Liquid culture	28.3 (20.2 – 37.6)	73.5 (68.0 – 78.5)	29.6 (22. – 37.4)	72.3 (69.5 – 74.9)
Microscopy	qPCR	50.0 (43.2 – 56.8)	99.2 (97.0 – 99.9)	98.2 (93.2 – 99.5)	68.5 (65.6 – 71.3)
Liquid culture	qPCR	38.8 (31.2 – 46.8)	49.1 (39.5 – 58.7)	52.1 (45.5 – 58.7)	36.0 (30.9 – 41.3)

CI: confidence interval; NPV: negative predictive value; PPV: positive predictive value; qPCR: real-time polymerase chain reaction

Table 6.7: Prevalence and likelihood ratios for the smear microscopy and liquid culture diagnostic techniques.

Technique	**Gold standard**	**Pre-test proportion* % (95% CI)**	**+LR (95% CI)**	**-LR (95% CI)**
Microscopy	Liquid culture	28.3 (23.9 – 32.9)	1.1 (0.8 – 1.5)	1.0 (0.9 – 1.1)
Microscopy	qPCR	47.7 (43.0 – 52.4)	59.8 (14.9 – 239.1)	0.5 (0.4 – 0.6)
Liquid culture	qPCR	58.8 (52.7 – 64.7)	0.8 (0.6 – 1.0)	1.3 (1.0 – 1.6)

+LR: positive likelihood ratio; -LR: negative likelihood ratio; CI: confidence interval; qPCR: real-time polymerase chain reaction

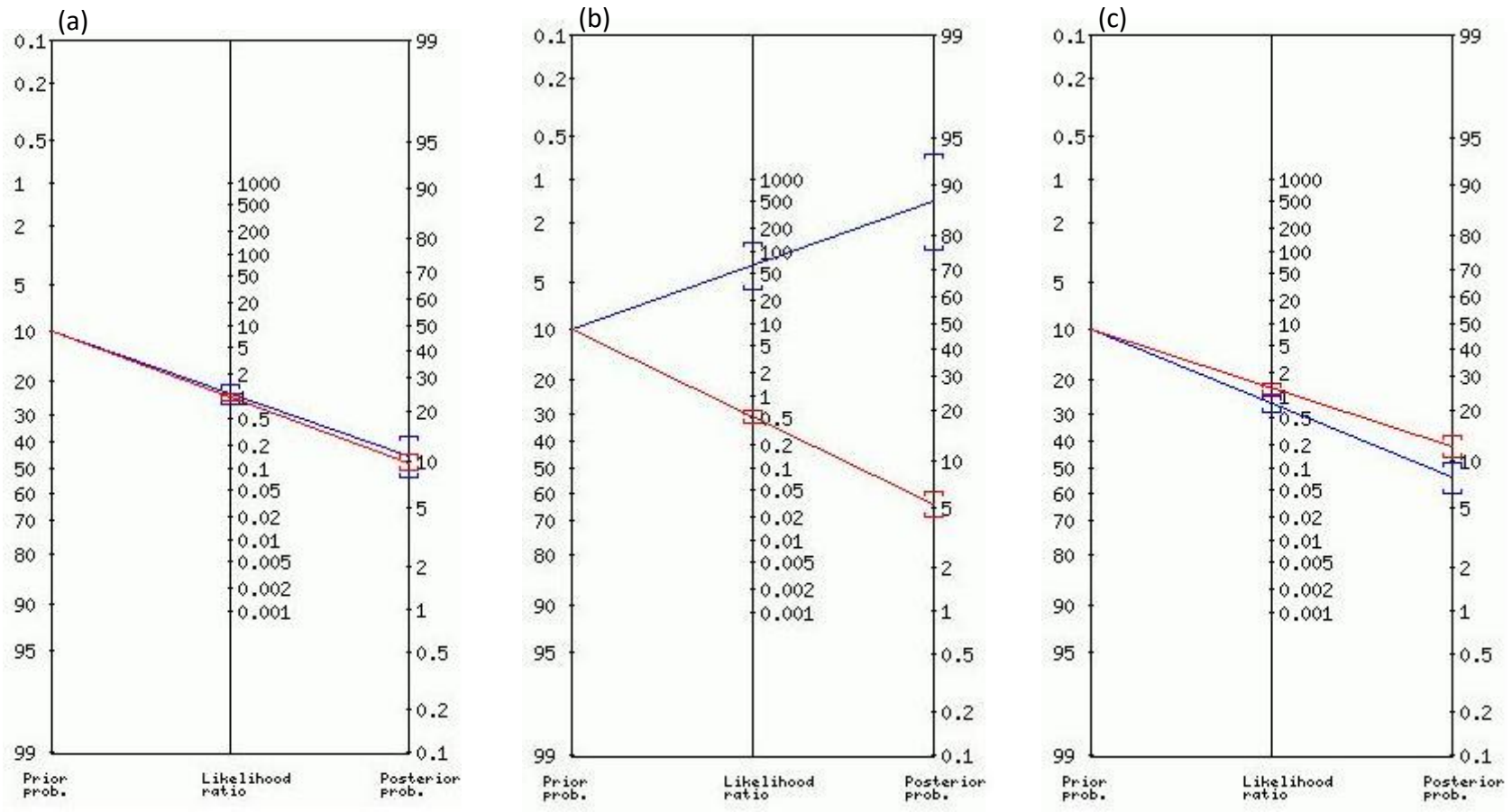


Figure 6.2: (a) Post-test probability of TB based on smear microscopy with liquid culture as the gold standard, indicating that culture was not useful as a gold standard in analysing sputum samples from this setting; (b) Post-test probability of TB based on smear microscopy with qPCR as the gold standard, indicating that a positive smear microscopy result had a high probability of indicating TB, while a negative smear microscopy result indicated only a moderate probability of the absence of TB; (c) Post-test probability of TB based on liquid culture with qPCR as the gold standard, indicating that culture results had little effect on either increasing or decreasing the probability of TB.

6.3.3. Diagnostic results in culture-negative samples

There were 153 sputum samples that were culture-negative, and that were also tested with both smear microscopy and qPCR. In these culture-negative samples, 89 (58.2%, 95% CI 50.3 – 65.7) samples were also smear-negative, and of these 43 (48.3%, 95% CI 38.2 – 58.6) were qPCR-negative while 46 (51.7%, 95% CI 41.5 – 61.8) were qPCR-positive (Figure 6.3). A total of 47 samples were culture-negative but both smear- and qPCR-positive. The remaining 17 culture-negative samples had been tested with all three methods, with 12 found to be qPCR-negative and five qPCR-positive, but the smear microscopy results were unknown. Overall 98/153 (64.1%, 95% CI 56.2 – 71.2) of the culture-negative samples tested with microscopy and qPCR were qPCR-positive.

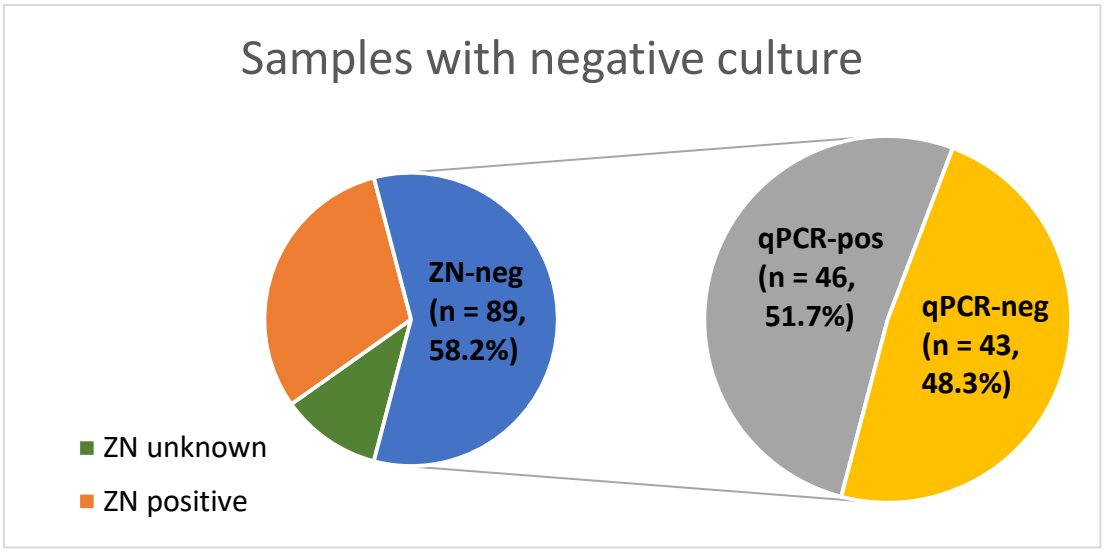


Figure 6.3: Smear microscopy and qPCR results in sputum samples that were culture-negative.

6.4. Discussion

This analysis focused on the utility of three laboratory-based techniques used in the diagnosis of TB, using sputum samples collected from people undergoing investigation for TB. The smear microscopy and sputum processing were undertaken in a resource-limited

laboratory in Balimo, while the liquid culture and TaqMan qPCR were undertaken in a well-resourced laboratory at JCU Townsville, using the same sputum samples that were originally processed on-site in Balimo. The comparative overall positive and negative proportions show that there is considerable variation between the three diagnostic methods, with microscopy detecting the lowest proportion of MTB and qPCR detecting the highest. Samples that were negative for all three methods will be partially explained by the unknown number of people who were tested but who did not actually have TB, although some samples may have returned false-negative results for all three methods.

In studies from many settings, culture results are considered the gold standard for comparison to microscopy and molecular assays (Afsar et al. 2018; Darban-Sarokhalil et al. 2013; Pinyopornpanish et al. 2015; Sharma et al. 2015). However in this study, the large proportion of negative culture results, and the poor agreement of culture with the other techniques, highlight the considerable challenges associated with the culture of samples collected and stored in resource-limited settings. In addition, the poor culture results limit the usefulness of comparisons to culture-based results in other settings. As a result, for this study the molecular technique was considered a more appropriate gold standard due to its increased ability to detect MTBC.

6.4.1. Diagnostic performance of smear microscopy at Balimo District Hospital

When considering the molecular method as the gold standard, microscopy undertaken at the BDH laboratory had limited sensitivity, but specificity was very high. These results emphasise the known variable sensitivity of smear microscopy as a diagnostic method for TB (Steingart et al. 2006b). Similarly for the likelihood ratios and nomograms, a positive smear indicated a high probability of TB, while a negative smear indicated only a moderate probability of the absence of TB. In this setting, smear microscopy is useful as a rule-in test for TB, but not as a rule-out test.

6.4.2. Diagnostic performance compared to previous analysis

In the analyses focused on direct comparison of two diagnostic methods on a single sample, the qPCR technique continued to detect the highest proportion of MTB. Earlier research from our group described qPCR and smear microscopy results in 309 samples from the Balimo region (Guernier et al. 2018), and that sub-set of samples was also incorporated in the sample set described here. In the more comprehensive analysis described here, the smear-negative/qPCR-negative group continued to contain the largest proportion of samples (51.9% compared to 38.5% previously). The increase in smear-negative/qPCR-negative results may have been due to the DNA extraction process used on more recently analysed samples, as spoligotyping analysis of these DNA extracts was also unsuccessful in comparison to the smear microscopy DNA (Guernier et al., unpublished data). Overall, the high proportion of smear-negative/qPCR-positive samples confirms that molecular methods have improved sensitivity over smear microscopy, as seen in other studies (Boehme et al. 2011; Ngabonziza et al. 2016; Opota et al. 2016; Theron et al. 2014).

Compared to the earlier study (Guernier et al. 2018), there was a smaller proportion of samples in the smear-positive/qPCR-positive group (23.9% compared to 31.7% previously), and in the smear-negative/qPCR-positive group (23.9% compared to 29.1% previously). Although the proportions of both of these groups decreased, it is of note that the proportions of the groups within each study remained similar to each other. The higher proportion of smear-positive/qPCR-positive samples in the earlier study may have been due to the inclusion of both new and follow-up samples, meaning that a higher proportion of samples from TB patients (rather than TB investigations) were included. Sampling bias may also have occurred, particularly if patients with certain clinical symptoms were more or less likely to have sputum samples collected and stored at certain times, thus affecting between-study results. The lower proportion of smear-negative/qPCR-negative samples in the earlier study may have been an outcome of sample collection, as trips were undertaken to Balimo more frequently from 2014 to 2017, meaning that the larger sample set may have included a broader selection of samples, and thus potentially less focus on TB patients or those suspected of DR-TB.

Figure 6.4: A Stop TB Kit used for treatment of drug-susceptible TB. (Image credit: Tanya Diefenbach-Elstob)

The comparison of diagnostic results between smear microscopy and qPCR identified two samples that were smear-positive but qPCR-negative. This very low proportion, as described previously, may have been due to a false-negative result with the qPCR assay due to poor quality of the DNA extraction, the presence of inhibitors in the sputum sample, or a low copy number of the IS*6110* target (Guernier et al. 2018; Lira et al. 2013). It is also possible that the two microscopy smear results were false-positives. This may be due to the presence of NTM, which have been seen in smear-positive samples in other settings (Buijtels et al. 2010; Desikan et al. 2017; Koh et al. 2003). The diagnostic primer sets used on these two samples (described previously in Chapter 4) have been tested against eight different mycobacterial species that are not part of the MTBC, and have demonstrated reactivity for seven of these species using the IS*6110* primer set, but no reactivity using the *senX3-regX3* primer set (Broccolo et al. 2003). Thus the detection of other NTM that have not been tested is possible. However, only a small number of NTM have been detected in PNG thus far (Aia et al. 2016; Ley et al. 2015), so their presence in these two samples is considered unlikely, although research is warranted. The presence of the *Nocardia* species, which were not

tested with these primer sets, may also result in false-positive microscopy results. The *Nocardia* species have a similar appearance to MTB when examined with sputum smear microscopy, and can present similarly to TB (Desikan et al. 2017; Muricy et al. 2014; Thirouvengadame et al. 2017). Further research would be necessary to determine the cause of smear-positive/qPCR-negative results.

6.4.3. Challenges associated with sample processing and transport

When considering sputum sample processing and storage in Balimo, the results obtained for each of the diagnostic methods are not surprising. In this setting, there are power outages daily, meaning that samples are subject to multiple freeze-thaw cycles prior to analysis at JCU. This has been shown to result in DNA of lesser quality in other organisms and sample types (Hasan et al. 2012; Ingersoll et al. 2008). Although smear microscopy is undertaken promptly on sputum samples in Balimo, the low proportion of positive results using this technique, based on the TaqMan qPCR assay as the gold standard, reflects the previously mentioned poor and variable sensitivity of smear microscopy as a diagnostic method (Steingart et al. 2006a; Steingart et al. 2006b).

The poor liquid culture results and comparatively better qPCR results will also be influenced by storage issues. As liquid culture requires growth of the MTB organism, if the organism is no longer viable due to unsatisfactory storage this growth will not be possible. The negative impact of storage and transport on MTB sputum culture yield and contamination has been described in other settings (Banda et al. 2000; Maharjan et al. 2016; Paramasivan et al. 1983; Šula et al. 1960). By contrast, the qPCR method that detects only DNA will have a greater chance of success even if organisms are undetectable or no longer viable, as seen in studies showing improved detection of MTB using molecular methods when compared to culture (Omar et al. 2016; Traore et al. 2006). Other research has demonstrated the challenges inherent in culturing MTB from sputum samples, with contamination due to other bacterial organisms known to be a problem in liquid culture even when decontamination of the sputum has been undertaken (Ahmad et al. 2017; Chang et al. 2002; Cornfield et al. 1997). In Balimo, for samples where both the collection and transport dates were known, the longest

storage time of a sample prior to transfer from Balimo to JCU Townsville was approximately 11 months. In addition, once samples are collected, the transit time is lengthy and may involve substantial temperature fluctuations, thus contributing to the discordant results seen in this study (Figure 6.5).

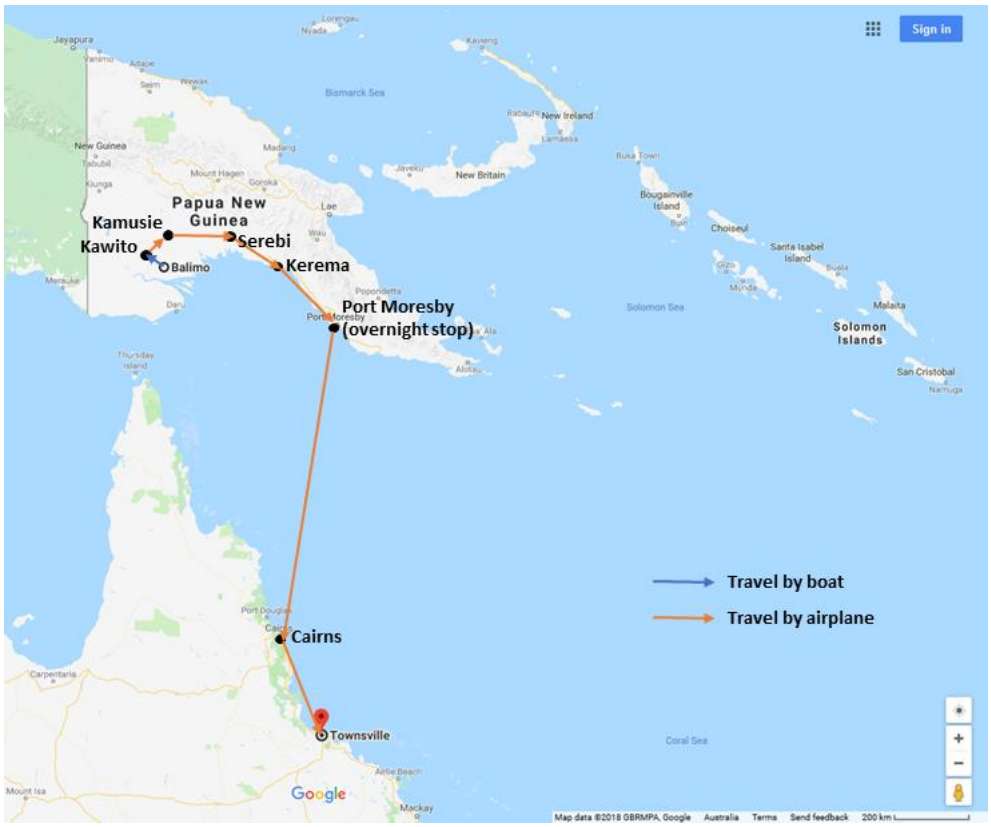


Figure 6.5: Itinerary of a trip undertaken from Balimo to Townsville in November 2017 (airport locations are approximate). (Image source: Google; Map data: ©2018 GBRMPA, Google)

Although molecular diagnostic methods are valuable, particularly in settings where sample storage is challenging and culture is not feasible, the successful culture of TB is important in the confirmation of DR-TB through drug susceptibility testing (DST) (World Health Organization 2018f). This method relies on the direct testing of the organism, rather than inferring drug resistance from genetic mutations, and is the gold standard in identifying DR-TB (World Health Organization 2018d). However, a culture-based approach is problematic when considering the testing of samples that have been stored in sub-optimal conditions,

and that may no longer contain viable organisms. In these cases, detection and testing of the organism may no longer be possible, and cases of both drug-susceptible and DR-TB may be missed.

The utility of various diagnostic techniques is particularly important in the Balimo setting when considering DR-TB. The Balimo region has been established as endemic for DR-TB, based on evidence of resistance-associated MTB gene mutations described earlier in this thesis (see Chapter 4), and discussions with health workers at BDH, who have identified DR-TB patients based on treatment response and subsequent confirmation at the provincial hospital in Daru. Given the presence of DR-TB in the Balimo region, the results described in this chapter emphasise the importance of the implementation of a rapid and achievable diagnostic method for TB in Balimo that is capable of both MTB disease and drug resistance detection. Such methods, particularly the WHO-endorsed Xpert MTB/RIF, are increasingly being implemented in resource-limited settings (Muyoyeta et al. 2015; Nakiyingi et al. 2013). Such methods also hold future promise in the identification of a broader range of drug resistance, with the ongoing development of molecular techniques and whole genome sequencing.

Molecular methods such as the Xpert MTB/RIF are also increasingly useful in the detection and confirmation of extrapulmonary TB. Published research has described confirmation of MTB infection based on Xpert MTB/RIF analysis of non-sputum bodily fluids, including urine, stool, cerebrospinal fluid, and tissue samples (Denkinger et al. 2014; Hillemann et al. 2011; Marouane et al. 2016; Maynard-Smith et al. 2014; Suzana et al. 2016; Zeka et al. 2011). Although extrapulmonary TB detection in Balimo would still be limited by the challenges associated with obtaining a clinical sample, a rapid method with improved sensitivity will still increase the number of bacteriologically confirmed cases, thus contributing to improved accuracy in extrapulmonary TB diagnoses in this setting.

6.4.4. Limitations

The molecular method used for the results presented in this study was undertaken according to a published protocol for identifying the *Mycobacterium* species and MTBC, as described in

Chapter 4. However, this method is different to the technique employed by Xpert MTB/RIF, and thus there may have been some differences in results if testing had been undertaken using Xpert MTB/RIF.

Diagnostic test results were not linked with TB patient register data, which could have provided further insight into discordant results, and the impact of smear-negative microscopy results on health worker decisions to commence empirical TB treatment.

HIV infection is known to negatively influence the sensitivity of smear microscopy for TB (Cattamanchi et al. 2009; Elliott et al. 1993), thus increasing the possibility of false-negative smear results. However, there is limited evidence suggesting a high burden of HIV in the Balimo region, and this factor is unlikely to influence a large proportion of samples.

6.5. Conclusion

This chapter has described the validation of smear microscopy used to detect MTB in sputum samples collected and processed at BDH, based on comparison to culture and molecular methods. Almost 35% of smear-negative samples were culture- and/or qPCR-positive. The results presented in this chapter did not include comparison to the TB patient register. However, the substantial proportion of smear-negative but culture- and/or qPCR-positive results, in combination with the low proportion of smear-negative pulmonary TB described earlier (see Table 3.5), continues to support the possibility that smear-negative pulmonary TB is under-diagnosed or misdiagnosed in Balimo (Guernier et al. 2018). This finding is particularly important because smear-negative results may complicate diagnostic protocols, especially in a setting where melioidosis and DR-TB are also endemic. In a setting such as Balimo, clinical response to treatment in the absence of bacteriological confirmation of MTB infection is often the only method of confirming a TB diagnosis. In cases of melioidosis and DR-TB there will not be positive response to treatment, although for DR-TB the diagnosis of TB will actually have been correct. Thus, a reliance on clinical outcomes is not sufficient to differentiate TB and other infections.

Overall, the qPCR molecular method had the greatest sensitivity in detecting MTB, while microscopy missed half of the qPCR-positive samples. However, microscopy was shown to be very useful as a rule-in test for TB. The high rate of qPCR-positive/smear-negative samples in a setting known to be endemic for DR-TB emphasises the benefit that would be gained from the implementation at the BDH laboratory of a rapid molecular MTB diagnostic with additional drug resistance detection abilities. In this resource-limited setting, the challenges associated with the diagnosis of pulmonary TB are amplified for presumptive extrapulmonary TB patients, where no clinical sample may be available. As a result, further investigation of extrapulmonary TB diagnoses in the Balimo region will provide greater insight into TB diagnoses at BDH.

CHAPTER 7

INVESTIGATION OF EXTRAPULMONARY AND LATENT TUBERCULOSIS IN THE BALIMO REGION

7.1. Introduction

The research previously presented in this thesis has described the very high burden of extrapulmonary tuberculosis (TB) in the Balimo region, occurring in more than 75% of all TB cases (see Chapter 3). This is a setting where diagnosis of TB is primarily clinical (based on the Papua New Guinea (PNG) guidelines), and where smear microscopy has a positive predictive value (PPV) of 98.2% and a negative predictive value (NPV) of 68.5% (based on a molecular method as the gold standard) (see Chapter 6). Extrapulmonary TB is known to present diagnostic challenges for clinicians for a number of reasons, including paucibacillary presentation, the large number of possible differential diagnoses, and the difficulties involved in obtaining suitable diagnostic specimens to achieve laboratory confirmation of infection (Purohit & Mustafa 2015). Furthermore, latent TB in a population represents a potential reservoir of infection that may reactivate into clinical disease, some of which will have pulmonary presentation and thus contribute to ongoing transmission of TB (Rangaka et al. 2015; Uplekar et al. 2015).

Symptomatic extrapulmonary TB patients may present similarly to those with pulmonary TB, with symptoms such as fever, night sweats, and weight loss, but with additional symptoms localised to the site of infection (Peirse & Houston 2017). Examples of localised symptoms include lymphadenitis (lymph node TB); bone and joint pain or swelling (bone or joint TB); and fatigue and headache (tuberculous meningitis) (Peirse & Houston 2017; Thwaites 2017). However, difficulty in reaching a diagnosis of extrapulmonary TB is not uncommon, and diagnostic delay may be lengthy. For example, one study describes a man from Guatemala with extrapulmonary TB presenting as masses in his neck, who was not diagnosed with TB until 10 months after he first presented at a healthcare provider (Shah et al. 2017). It should also be noted that even in a setting with a high level of resources, diagnosis can still be

challenging. This situation is demonstrated in a study of 117 extrapulmonary TB patients in Australia, where microbiological confirmation was achieved for only 58.1% of patients, 56.4% had histopathological confirmation, and 78.6% overall had microbiological or histopathological confirmation (Pollett et al. 2016).

These diagnostic challenges mean that diagnoses of extrapulmonary TB are frequently clinical, based solely on presenting signs and symptoms. Indeed, the World Health Organization (WHO) diagnostic guidelines include the classification of a TB case based solely on the decision of a clinician to commence a patient on TB treatment, without bacteriological confirmation of infection (World Health Organization 2013a).

Diagnostic challenges, and particularly the limited resources available to achieve laboratory confirmation of *Mycobacterium tuberculosis* (MTB) infection, are evident at Balimo District Hospital (BDH) (see Chapter 6). In this hospital setting, diagnoses of TB, and particularly extrapulmonary TB, are predominantly clinical. This situation was described earlier in this thesis, with sputum smear microscopy results recorded in 77.9% of pulmonary TB cases, while 91.2% of extrapulmonary TB patients did not have a smear result recorded (see Section 3.3.2). The high proportion of clinical diagnoses that occur in Balimo are primarily due to two reasons:

1. Laboratory facilities for TB diagnosis are limited to microscopy, which has been demonstrated in this study to have sensitivity of only 50% (based on a molecular method as the gold standard) (see Section 6.3.2).
2. More than 75% of TB cases are extrapulmonary, with a clinical sample frequently not able to be obtained.

The combination of these two factors results in a situation where positive response to treatment is often the only means of supporting or confirming a TB diagnosis. However, a positive response to treatment cannot be relied upon as proof of extrapulmonary TB infection (Colebunders & Bastian 2000). Extrapulmonary TB is frequently difficult to diagnose due to its non-specific presentation. Therefore, it is possible that some clinically diagnosed cases of extrapulmonary TB are actually misdiagnoses, and in some cases may have been self-limiting conditions which would have resolved regardless of the initiation of TB

treatment. In addition, the antibiotics contained within the four-drug combination used to treat TB may be effective against other infections, particularly in the case of rifampicin (RIF), which has broad antibacterial activity (McCabe & Lorian 1968; Thornsberry et al. 1983); again implying that resolution of symptoms does not provide proof of TB (Figure 7.1).

Figure 7.1: The four-drug combination tablets, including RIF, that are used in the two-month intensive phase of TB treatment. (Image credit: Tanya Diefenbach-Elstob)

In a setting where a lack of diagnostic facilities means that confirmation of extrapulmonary TB infection frequently relies on assessing the response to treatment, there are a number of potential problems:

- There is a reliance on patients being available for ongoing monitoring and assessment, which may be challenging due to factors such as limited access to a health facility. This situation may lead to delays in patient management and loss to follow-up.

- Non-response to treatment cannot be relied on as an indicator that a TB diagnosis was incorrect if the patient's region of origin is endemic for drug-resistant TB (DR-TB).
- For patients who do not have TB, there will be substantial delay in the provision of a correct diagnosis and treatment, and thus possible worsening of their condition.

For these reasons, it is important to attempt to confirm clinically-diagnosed TB cases, as well as to understand the clinical decision-making processes that are involved in making these TB diagnoses.

The laboratory confirmation of extrapulmonary TB relies on techniques such as microscopy, histology, culture, and molecular tests (Purohit & Mustafa 2015). However, all of these techniques are limited by the need for a diagnostic specimen on which to undertake the MTB-specific analysis.

Other methods can provide underlying evidence for TB infection by demonstrating immune reactivity. While not supported as a means of diagnosing active TB at an individual level, the tuberculin skin test (TST) and the interferon (IFN)-ɣ release assay (IGRA) can give valuable insight into the proportion of TB infection at a community level, and hence the rates of misclassification of TB in the setting described. Advantages of the IGRA over TST include the need for a single visit to a health facility for collection of a whole blood sample, and the ability to provide evidence of *M. tuberculosis* (MTBC) exposure without false-positive results due to prior Bacille Calmette-Guérin (BCG) vaccination (Division of Tuberculosis Elimination 2016; Katsenos et al. 2010).

In a setting where symptoms are the primary method of extrapulmonary TB diagnosis, immunological tests such as IGRAs may be able to provide evidence to support a TB diagnosis (Feng et al. 2012; Shin et al. 2015). The IGRA provides evidence of previous MTBC exposure through the detection and measurement of IFN-ɣ released by lymphocytes that have been exposed to MTBC-derived antigens including ESAT-6 and CFP-10 (Division of Tuberculosis Elimination 2016; QIAGEN 2016).

The IGRA is based on the collection of whole blood into four blood collection tubes. The nil (negative control) and mitogen (positive control) tubes validate the assay in two ways.

Measurement of IFN-ɣ in sample wells derived from the nil tube allows adjustment for any background IFN-ɣ that is produced but not associated with cellular responses to the assay's peptide antigens (QIAGEN 2016). The mitogen tube determines that there is an adequate immune response, as it contains a non-specific T-cell stimulator, thus demonstrating the ability of lymphocytes to generate IFN-ɣ (QIAGEN 2016; Quest Diagnostics™ 2014). The TB1 and TB2 tubes are the MTBC-specific tubes of the assay. The TB1 tubes are designed to elicit cell-mediated immune responses from CD4+ T-helper lymphocytes, while the TB2 tubes additionally target the CD8+ cytotoxic T lymphocytes (QIAGEN 2016). Furthermore, the CD8+ T lymphocytes that are specific for ESAT-6 and CFP-10 may be associated with active TB, or more recent exposure to MTB (Day et al. 2011; Nikolova et al. 2013; QIAGEN 2016; Rozot et al. 2013).

The IGRA assay is not able to differentiate active from latent TB, and is thus not a diagnostic for active TB (Division of Tuberculosis Elimination 2016). This limitation is because the assay detects an immune response that reflects prior T-cell exposure to the MTBC (QIAGEN 2016), and thus the assay may be positive in cases of both latent and active infection. However, on a population level, comparison of IGRA-positive and -negative proportions in patient and non-patient groups may provide insight into the accuracy of TB diagnoses in a setting where diagnoses are predominantly clinical. However, it is important to understand the burden of IGRA-positivity in the underlying general population, as this proportion will reflect the baseline positive proportion that should be expected regardless of active TB infection status in the patient group.

The aim of this chapter is to provide evidence to test the accuracy of clinical diagnoses of TB at BDH, with a particular focus on extrapulmonary TB, through the use of an IGRA. The specific objectives are:

1. To assess the burden of MTBC infection (latent TB) in the BDH catchment population, based on the IGRA; with comparison across community, health worker, and TB patient groups.
2. To inform the accuracy of clinical extrapulmonary TB diagnoses, based on the comparison of IGRA results in non-patient and extrapulmonary TB patient groups, with reference to the burden of latent TB.

7.2. Materials and Methods

7.2.1. Study setting and participant recruitment

The Balimo region has been previously described in Chapter 3. For this study, participant recruitment was undertaken in the immediate Balimo area. Participants were classified into five groups, as follows: general population, health worker, TB patients, TB investigation, and other.

People in the TB patient, TB investigation, and 'other' groups were inpatients at BDH, present at hospital clinics at the time of the study, or identified by health workers from BDH. Health workers were employees at BDH who volunteered to participate in the study. General population participants were community members who volunteered to participate following communication of the study by BDH staff. The groups will be described in greater detail in Section 7.2.4.

7.2.2. Collection of participant data and diagnostic specimens

Participants were recruited during three study visits, and identified and approached in collaboration with local health workers. A total of 220 participants were recruited across three study visits, as shown in Table 7.1. Recollections occurred for five participants – three at the request of the participants, and two due to borderline results (TB2 tube concentration within 0.15 IU/mL of the 0.35 IU/mL threshold) in an earlier run of the assay (initial collection occurring in the second visit).

Table 7.1: Timing of hospital visits and participant numbers across the study period.

Visit	Date	Participants	Recollections	Total collections
1	February 2017	25	0	25
2	June 2017	99	0	99
3	November 2017	96	5	101
Total		**220**	**5**	**225**

7.2.2.1. Participant data

Demographic and clinical data were collected for each participant, including age, sex, clinical signs and symptoms (if relevant), and current and past TB diagnosis and treatment details. The TB patient register (previously described in Chapter 3) was searched to identify diagnosis and treatment information for current patients and people who indicated a past history of TB.

7.2.2.2. Blood collection, incubation, and processing

A volume of 1 mL of whole blood was collected from each participant into the four labelled QuantiFERON® blood collection tubes (1 mL into each tube), in accordance with the package directions. Immediately following blood collection, the tubes were gently shaken to coat the inner surface of the tubes with blood. Tubes were then placed upright in a 37^{o}C incubator or water bath (Figure 7.2). Following incubation, tubes were refrigerated, and plasma was harvested. To do this, tubes were centrifuged at 2000 – 3000 g to separate the cells from the plasma, and plasma was harvested into cryotubes using a micropipette. Harvested plasma was stored at minus-20^{o}C in Balimo, and at minus-80^{o}C following transfer to JCU Townsville.

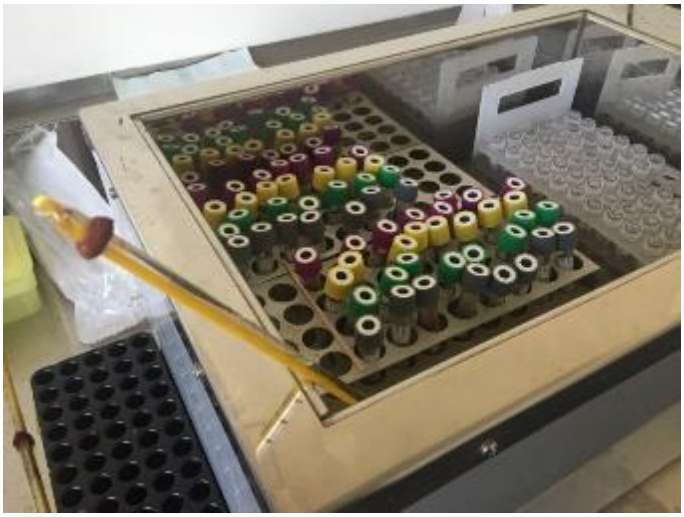


Figure 7.2: Incubating blood collection tubes in a water bath at the BDH laboratory. (Image credit: Tanya Diefenbach-Elstob)

7.2.3. Technical details of the interferon-ɣ release assay analysis

The QuantiFERON®-TB Gold Plus (QFT®-Plus) ELISA (QIAGEN, Hilden, Germany) was undertaken according to the manufacturer instructions (QIAGEN 2016). The optical density (OD) of each well was measured using a microplate reader fitted with a 450 nm filter and a 620 nm reference filter. Plates processed at JCU Townsville (sample numbers 1 – 125) were read using a SPECTROstar Nano (BMG LABTECH, Ortenberg, Germany), while plates processed at the BDH laboratory (sample numbers 126 – 225) were read using an EZ Read 400 (Biochrom Ltd., Cambridge, United Kingdom).

The ODs were measured, IFN-ɣ concentrations were calculated, and IGRA results were interpreted using the QuantiFERON-TB Gold Plus Analysis Software (version 2.71) (QIAGEN, Hilden, Germany). The measured ODs from the IGRAs were interpreted according to the package insert (QIAGEN 2016). For reference, the criteria for interpretation are shown in Table 7.2.

Table 7.2: Criteria for interpretation of QFT-Plus® results. (Source: QIAGEN 2016)

<table>
<tr><th>Nil (IU/mL)</th><th>TB1 minus Nil (IU/mL)</th><th>TB2 minus Nil (IU/mL)</th><th>Mitogen minus Nil (IU/mL)</th><th>QFT-Plus result</th><th>Interpretation</th></tr>
<tr><td rowspan="4">≤8.0</td><td>≥0.35 and ≥25% of Nil value</td><td>Any</td><td rowspan="2">Any</td><td rowspan="2">Positive</td><td rowspan="2">MTB infection likely</td></tr>
<tr><td>Any</td><td>≥0.35 and ≥25% of Nil value</td></tr>
<tr><td><0.35 or ≥0.35 and <25% of Nil value</td><td><0.35 or ≥0.35 and <25% of Nil value</td><td>≥0.5</td><td>Negative</td><td>MTB infection not likely</td></tr>
<tr><td><0.35 or ≥0.35 and <25% of Nil value</td><td><0.35 or ≥0.35 and <25% of Nil value</td><td><0.5</td><td rowspan="2">Indeterminate</td><td rowspan="2">Likelihood of MTB infection cannot be determined</td></tr>
<tr><td>>8.0</td><td colspan="3">Any</td></tr>
</table>

IU/mL: international units per millilitre; MTB: *Mycobacterium tuberculosis*

7.2.4. Allocation of participant groups

Participants were allocated into five primary groups based on their status at the time of sample collection, as described previously: general population, health worker, TB patient, TB investigation, and other. If a participant had a history of TB diagnosis and treatment they were also given a secondary allocation to the TB patient group. Allocation to the 'other' group was the result of being unwell but having symptoms not suspected to be TB by the recording health worker (backache, asthma), being a guardian of a patient admitted to the hospital ward, undertaking non-TB treatment, or not having a status recorded (i.e. unknown). Due to the very small number of these participants (n = 8), the 'other' group was excluded in the comparison of all patient groups. In the analyses, any person who had ever received TB treatment was classified as a TB patient, while any person who was undergoing investigation for TB was classified as TB investigation. In the comparison of the non-patient and extrapulmonary TB patient groups, the non-patient group included general population, health workers with no history of TB, and others; while the extrapulmonary TB patient group included all current and past extrapulmonary TB patients. By definition, this comparative analysis excluded all people being investigated for TB, and any TB patients with non-extrapulmonary TB diagnoses (pulmonary, concurrent, or unknown).

Five participants had two sets of IGRA results due to recollections, and were classified as follows:

- Two participants were IGRA-positive at both collections, and one participant was IGRA-negative at both collections. The IFN-ɣ concentrations from the most recent collections were analysed.
- One participant was initially IGRA-negative, but then IGRA-positive at recollection. The results from the IGRA-positive collection were analysed, as this participant also returned a positive molecular result in sputum testing undertaken external to this study.
- One participant was initially IGRA-positive, but then IGRA-negative at recollection. The results from the IGRA-negative collection were analysed, as the initial IGRA-positive concentration was very close to the assay threshold; the likelihood of

discordant results in borderline IGRA results have been described previously based on an earlier version of the QuantiFERON assay (Banaei et al. 2016).

In some analyses it was necessary to exclude results that were unable to be categorised. These are described below:

- When comparing IFN-ɣ concentrations across all participant groups:
    - One participant with discordant IGRA results (reversion) could not be classified – this was a TB investigation participant.
- When comparing non-patient and extrapulmonary TB patient groups:
    - In the non-patient group, one general population participant was included in the IFN-ɣ concentration analysis, but not the IGRA interpretation analysis, because they had a borderline IGRA result; while one 'other' participant was excluded from both analyses because they had an uncertain IGRA result due to IGRA reversion.
    - In the extrapulmonary TB patient group one patient was included in the IFN-ɣ concentration analysis, but not the IGRA interpretation analysis, because they had an indeterminate IGRA result (based on the criteria described in Table 7.2).

7.2.5. Statistical analyses

The IGRA interpretations and IFN-ɣ concentrations were analysed using GraphPad Prism version 7.03 and 7.04 (GraphPad Software, Inc., La Jolla (CA), USA). All test wells with an OD greater than 4.000 (i.e. an IFN-ɣ concentration greater than 10.0 IU/mL) were assigned an IFN-ɣ concentration of 10.0 IU/mL for the analyses, as with other published studies (Petruccioli et al. 2017; Xia et al. 2015). Fisher's exact test was used to test for differences between patient and non-patient groups. The Kruskal-Wallis test was used to examine IFN-ɣ concentrations between the four main participant groups (general population, health worker, TB investigation, and TB patient), with Dunn's multiple comparisons test used for pairwise comparisons. The Mann-Whitney test was used to analyse differences in IFN-ɣ concentrations between the non-patient and extrapulmonary TB patient groups. The

Wilcoxon matched-pairs signed rank test was used to test for differences between the TB1 and TB2 tubes, based on subtracting the TB1-Nil value from the TB2-Nil value. The threshold for statistical significance was set at $p \leq 0.05$ for all analyses. In the graphs, statistical significance was depicted as follows: **** (p-values < 0.0001); *** (p-values < 0.001); ** (p-values < 0.01); and * (p-values 0.01 to 0.05).

Figure 7.3: A meeting with local health authorities and health workers at BDH to discuss research in the region. (Image credit: David Plummer)

7.3. Results

7.3.1. Demographic characteristics of participant groups

Based on the categorisation of any patient with a history of TB treatment as a TB patient, participants were classified as follows:

- General population (n = 114)
- Health workers (n = 24)
- TB patients (n = 59)
- TB investigation (n = 15)
- Other (n = 8)

The TB patient group included 13 health workers, while the TB investigation group included two health workers. The age and sex distributions of each of the participant groups are shown in Table 7.3.

Table 7.3: Age and sex distributions of the participant groups.

	General population	Health worker	TB patient	TB investigation	Other	Total
Sex						
Male	53	11	19	9	3	**95**
Female	60	13	40	6	5	**124**
Unknown	1	0	0	0	0	**1**
Total	**114**	**24**	**59**	**15**	**8**	**220**
Age						
0 – 14 years	42	0	4	3	1	**50**
15 – 24 years	20	1	10	0	0	**31**
25 – 34 years	18	7	10	2	0	**37**
35 – 44 years	10	4	12	3	2	**31**
45 – 54 years	13	6	12	2	1	**34**
55 – 64 years	5	3	8	1	3	**20**
65+ years	3	0	1	0	0	**4**
Adult	3	3	1	3	1	**11**
Unknown	0	0	1	1	0	**2**
Total	**114**	**24**	**59**	**15**	**8**	**220**

TB: tuberculosis

7.3.2. Interferon-ɣ release assay interpretations by group

In the general population group, 24 participants (21.1%, 95% CI 14.6 – 29.4) were IGRA-positive. Health workers who had no history of TB had a higher proportion of IGRA-positive results than the general population (n = 12, 50%, 95% CI 31.4 – 68.6). In the TB patient group, 32 participants (54.2%, 95% CI 41.7 – 66.3) had an IGRA-positive result. Overall, three participants had uncertain IGRA results. These were due to a borderline result (IFN-ɣ concentration of 0.01 IU/mL above the threshold) in one general population participant, and reversions (IGRA-positive to IGRA-negative) in one TB investigation participant and one 'other' participant. There were also three indeterminate outcomes – two in TB patients and one in a TB investigation participant. A summary of IGRA results is shown in Table 7.4. Among the TB patients, there were 33 participants with a current diagnosis of TB, of whom 16 were IGRA-positive and 17 were IGRA-negative; and there were 24 participants with a past history of TB, of whom 16 were IGRA-positive and 8 were IGRA-negative. There was no significant difference in the odds of having a positive or negative IGRA result between the current and past TB patient groups (OR = 0.5, 95% CI 0.2 – 1.4).

Table 7.4: Interpretations of the IGRA analysis for all study participants by group.

	Negative, n (%)	**Positive, n (%)**	**Uncertain, n (%)**	**Indeterminate, n (%)**	**Total**
General population	89 (78.1)	24 (21.1)	1 (0.8)	0 (0.0)	**114**
Health worker	12 (50.0)	12 (50.0)	0 (0.0)	0 (0.0)	**24**
TB patient	25 (42.4)	32 (54.2)	0 (0.0)	2 (3.4)	**59**
TB investigation	5 (33.3)	8 (53.3)	1 (6.7)	1 (6.7)	**15**
Other	2 (25.0)	5 (62.5)	1 (12.5)	0 (0.0)	**8**
Total	**133**	**81**	**3**	**3**	**220**

n: number; TB: tuberculosis

7.3.3. Interferon-ɣ concentrations in participant groups

The distribution of IFN-ɣ concentrations across the participant groups are shown in Figure 7.4. There were no significant differences between the groups for the nil tubes ($H = 4.89$, $p = 0.18$). However, there was a significant difference between the groups for the mitogen tubes ($H = 13.86$, $p < 0.01$), with a significantly lower IFN-ɣ concentration in the TB investigation group compared to the general population group (Dunn's multiple comparisons test: $p < 0.01$). There was also a significant difference between the groups in the analysis of the TB1 ($H = 17.18$, $p < 0.001$) and TB2 tubes ($H = 15.34$, $p < 0.01$), with the IFN-ɣ concentration higher for the TB patient group when compared to the general population (Dunn's multiple comparisons test: TB1, $p < 0.001$; TB2, $p < 0.01$).

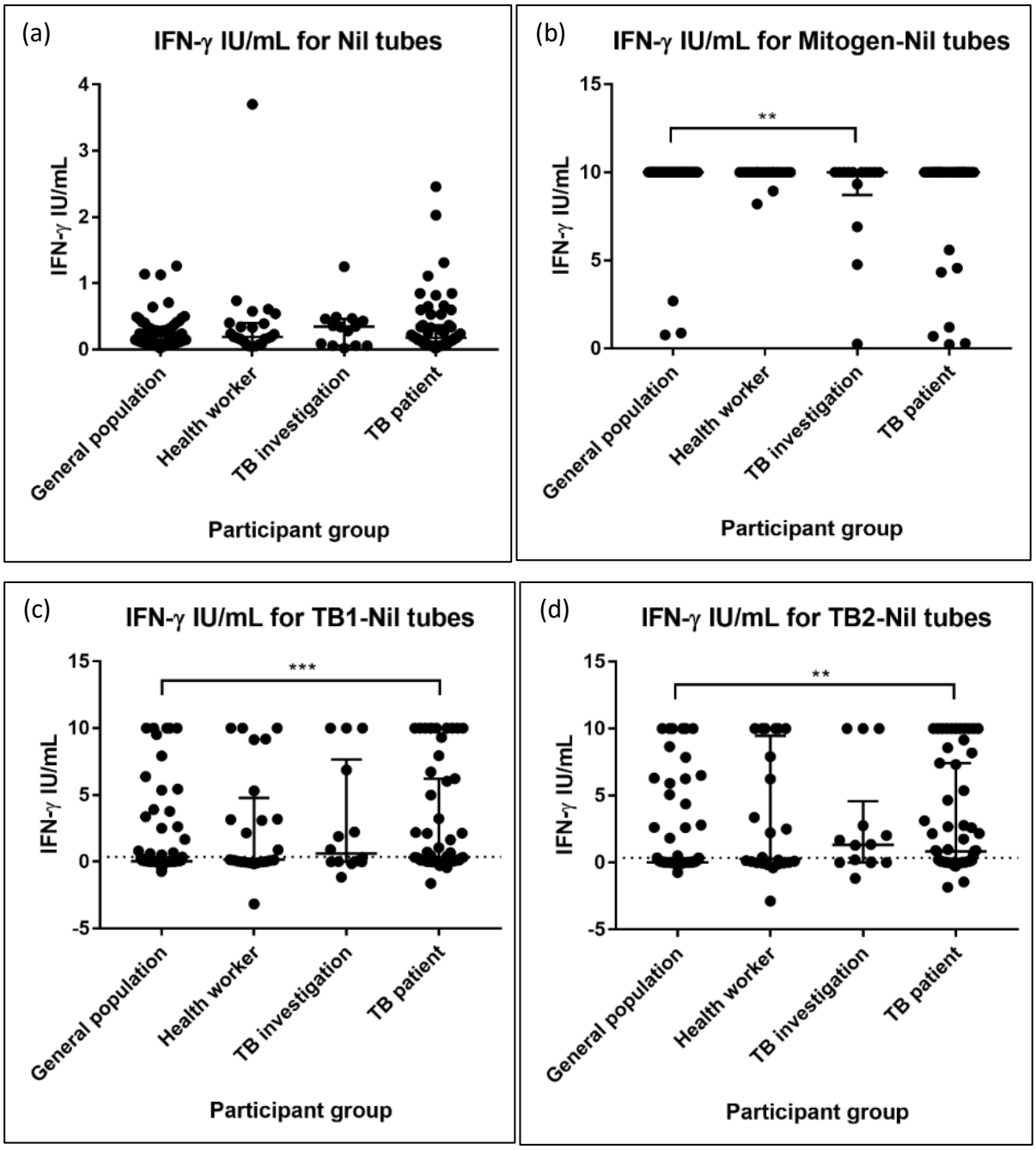


Figure 7.4: IFN-ɣ concentrations for the participant groups, as measured in the (a) Nil, (b) Mitogen-Nil, (c) TB1-Nil, and (d) TB2-Nil tubes. The plot lines indicate the median and interquartile ranges. The dotted lines on graphs (c) and (d) indicate the assay cut-off value of 0.35 IU/mL.

7.3.4. Comparison of the non-patient and extrapulmonary tuberculosis patient groups

The positive and negative IGRA results for the non-patient and extrapulmonary TB patient groups are shown in Table 7.5. There was a significant difference between these two participant groups (Fisher's exact test, $p < 0.01$). The extrapulmonary TB patient group had increased odds of a positive IGRA result (OR = 2.8, 95% CI 1.4 – 5.6) in comparison to the non-patient group.

Table 7.5: Distribution of positive and negative IGRA results in non-patients and extrapulmonary TB patients.

	Positive, n (%)	**Negative, n (%)**	**Total**
Extrapulmonary TB	22 (52.4)	20 (47.6)	**42**
Non-patient	41 (28.5)	103 (71.5)	**144**
Total	**63**	**123**	**186**

n: number; TB: tuberculosis

In the comparison of the non-patient and extrapulmonary TB patient groups, there was no difference between the groups for the nil and mitogen tubes. However, there was a significant difference between the two groups in both the TB1 (U = 2306, $p < 0.01$) and TB2 (U = 2378, $p = 0.02$) tubes. The IFN-ɣ concentration distributions are shown for the four tube sets in Figure 7.5.

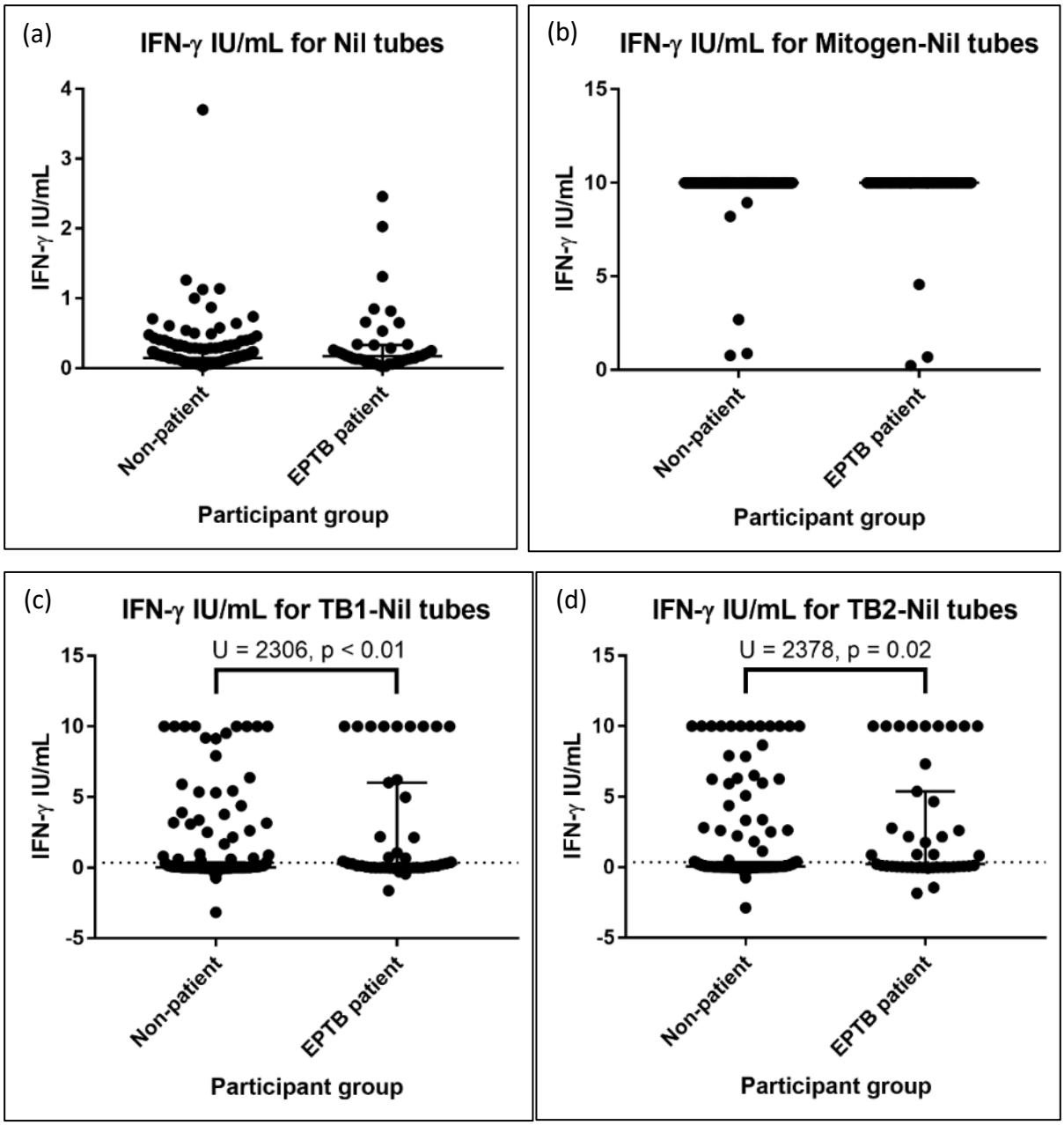


Figure 7.5: IFN-ɣ concentrations for the non-patient and extrapulmonary TB patient groups, for the (a) Nil, (b) Mitogen-Nil, (c) TB1-Nil, and (d) TB2-Nil tubes. The plot lines indicate the median and interquartile ranges. The dotted lines on graphs (c) and (d) indicate the assay cut-off value of 0.35 IU/mL.

The IFN-ɣ concentrations measured in the TB1 and TB2 tubes were also investigated within the non-patient and extrapulmonary TB groups. In the analysis of the paired tubes, there was a significant difference in the non-patient group ($p < 0.01$), but not the extrapulmonary

TB patient group (p = 0.68). The graphs of differences between the tubes for the two participant groups are shown in Figure 7.6.

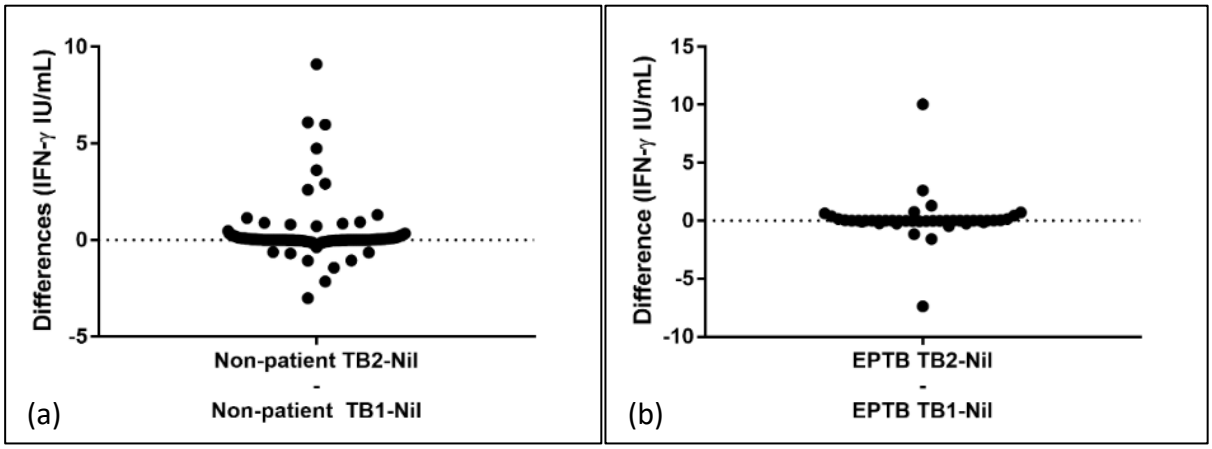


Figure 7.6: Differences in IFN-ɣ concentrations for the TB1-Nil and TB2-Nil tubes in the (a) non-patient and (b) extrapulmonary TB patient groups. The data points show the TB1-Nil tubes subtracted from the TB2-Nil tubes.

7.4. Discussion

This study examined the T-cell response to TB peptides, based on the QFT®-Plus IGRA, in samples collected from various participant groups in the TB-endemic Balimo region. In the general population, 21.1% (95% CI 14.6 – 29.4) of participants were IGRA-positive, compared to 54.2% (95% CI 41.7 – 66.3) of TB patient participants who were either being treated or had been treated previously. When comparing non-patients with people with a history of extrapulmonary TB, the patient group were significantly more likely to be IGRA-positive, and to have increased IFN-ɣ concentrations based on analysis of the TB1 and TB2 tubes.

7.4.1. Low proportion of latent tuberculosis in the general population

Historically, it has often been suggested that approximately one-third of the world's population has latent TB infection, based on data that was estimated in 1997 (Dye et al.

1999; Houben & White 2015). However, the global burden of latent TB has more recently been estimated to be lower, and is now suggested to be 23.0% globally and 27.9% in the Western Pacific region (Houben & Dodd 2016).

Due to the lack of a gold standard test for latent TB, studies investigating the sensitivity and specificity of assays such as the QFT®-Plus have focused on people with active TB (Pieterman et al. 2018). In such studies, sensitivity has been estimated to range from 83 – 99% (Hoffmann et al. 2016; Horne et al. 2018; Petruccioli et al. 2017; Takasaki et al. 2018; Telisinghe et al. 2017; Yi et al. 2016). Based on an approximate sensitivity of 90%, the 21.1% positivity identified in this study equates to a rate of IGRA positivity of approximately 23% (21.1% / 0.9 = 23.4%). Therefore, given the estimated global proportions of latent TB, and the high reported incidence of TB in the Balimo region, at approximately 23% the general population group in this study had an unexpectedly low proportion of IGRA-positivity.

There are some possible explanations for the relatively low proportion of latent TB in the general population. Potential over-diagnosis of TB in the Balimo setting has been discussed in previous chapters, which would support a lower than expected proportion of IGRA-positivity in the general population. The demographics of participants in this aim of the study may also be relevant, as participants were predominantly resident in the urban Balimo area. Although results are variable by region, studies in some settings have demonstrated that people resident in urban settings may have decreased diagnostic delay (Godfrey-Faussett et al. 2002; Mesfin et al. 2009). In Balimo, this possibility is supported by the results presented in Chapter 3 (see Section 3.3.5), which described a higher likelihood of treatment failure outcomes in people from rural areas. Our earlier research in the Balimo region has described the challenges faced in TB treatment adherence, including the costs and difficulties of travel (Diefenbach-Elstob et al. 2017). These factors will particularly influence people from rural and remote areas, and will impact all facets of TB management, including time to diagnosis, treatment adherence, and ongoing patient follow-up. The high incidence of extrapulmonary TB seen in this study of the Balimo region combined with the relatively low proportion of latent TB seen in the general population of the Balimo urban area raises the possibility that there is high awareness of TB in this region. This would reflect the lower level of latent TB seen in this predominantly urban population if TB symptoms are rapidly recognised and

treated. It would be necessary to undertake comparative investigation of latent TB in the rural and remote areas surrounding Balimo to determine if there is truly a difference between the urban and rural populations. Another possible explanation for the relatively low proportion of latent TB is the substantial proportion of younger people who were in the general population group. As the proportion of individuals with latent TB infection has been shown to increase with age (Ncayiyana et al. 2016; Wood et al. 2010), the younger general population age group in our study may partially explain the lower overall proportion of latent TB.

7.4.2. High proportion of interferon-ɣ release assay positivity in health workers

This study identified a high proportion of latent TB in health workers, even when only health workers without a history of TB diagnosis or treatment were investigated (Figure 7.7). Health workers are a group known to have a higher risk of MTB exposure and latent TB, as described in numerous published studies and reviews (Adams et al. 2015; Agaya et al. 2015; Alele et al. 2018; El-Sokkary et al. 2015; Malotle et al. 2017). Although not unexpected given their risk of TB exposure, the high proportion of latent TB in this group in the Balimo region has important implications for infection control and management of health facilities.

Figure 7.7: Health workers at BDH on International Day of the Tropics in 2016. (Image credit: Bisato Gula)

7.4.3. Interferon-ɣ release assay interpretations and concentrations in tuberculosis patients

The analysis of the IGRA interpretations and IFN-ɣ concentrations indicate that a substantial proportion of clinically-diagnosed extrapulmonary TB patients in the Balimo setting have evidence of MTBC infection. This conclusion is based on both the IGRA interpretations and the IFN-ɣ concentrations, with a higher proportion of IGRA-positive participants and significantly higher IFN-ɣ concentrations in the TB patient group. This trend continued when considering extrapulmonary TB patients in comparison to the non-patient group, with increased odds of an IGRA-positive result and significantly increased IFN-ɣ concentrations in the extrapulmonary TB patients. The greater likelihood of detectable TB1 and TB2 responses in the extrapulmonary TB patients provides evidence of the TB patient group being more likely to have had T-cell recognition of MTB, despite the substantial proportion of TB patients who had an IGRA-negative result.

There are a number of possible reasons for the large proportion of IGRA-negative results in the TB patient group, including false-negative results. The form of extrapulmonary TB may be linked with the likelihood of a positive or negative IGRA result. For example, studies of extrapulmonary TB patients have found proportions of IGRA-positive results of 81 – 93% in patients with tuberculous lymphadenitis (Kim et al. 2011; Shin et al. 2015; Song et al. 2009). However, other forms of extrapulmonary TB can have lower proportions of IGRA-positive results, including only 45% in patients with skeletal involvement, and 38.5% in pleural TB (Shin et al. 2015; Song et al. 2009). In a recent study from Indonesia, among 57 extrapulmonary TB patients with confirmed MTB infection (based on histopathology or smear microscopy), seven were IGRA-negative (Rumende et al. 2018). Although results vary across settings and studies, false-negative and indeterminate IGRA results in TB patients have been linked with factors including older age, being underweight or overweight, long diagnostic delay, bilateral disease, use of immunosuppressive drugs, lymphocytopenia, elevated C-reactive protein, and HIV co-infection (Azghay et al. 2016; de Visser et al. 2015; Hang et al. 2011; Kim et al. 2014; Kobashi et al. 2012; Kwon et al. 2015; Pan et al. 2015). False-negative IGRA results have also been seen in the presence of other underlying conditions, including cancer and diabetes mellitus (Kobashi et al. 2012; Kwon et al. 2015; Lai

et al. 2011). Furthermore, concurrent malaria has been associated with impaired mitogen responses (Drabe et al. 2016).

Given that a number of the factors that have been associated with false-negative and indeterminate IGRA results will be relevant in PNG, the possibility of false-negatives is particularly important in the context of the Balimo region population. A number of studies have described nutritional deficiencies in various groups in PNG, including mal- and under-nutrition, particularly in children, as well as insufficient protein intake (Goris et al. 2017; Morita et al. 2015; Samiak & Emeto 2017; Wand et al. 2012). Malaria is endemic across many parts of PNG, and although Western Province has low endemicity, malaria cases have been seen in the province (Bande et al. 2014; Hetzel et al. 2015).

The presence of other potentially immune-influencing factors should also be considered, such as parasite burden. Although burdens will undoubtedly vary over time and by region, various helminth species have been described in PNG. These include *Necator americanus, Ascaris lumbricoides, Trichuris trichiuria,* and *Strongyloides fuelleborni kellyi* (King & Mascie-Taylor 2004; Shield & Kow 2013). Although research in this field is ongoing, helminth infection has been linked with indeterminate IGRA results (Banfield et al. 2012; Lucas et al. 2010; Thomas et al. 2010).

Earlier results presented in this thesis suggest that the prevalence of TB-HIV coinfection in the Balimo region may be low (see Section 3.3.8), however HIV is endemic in PNG, including in Western Province and the Gogodala region (Dundon 2010; McBryde 2012; National Department of Health 2010; World Health Organization 2018b). In addition to HIV, human T-lymphotropic virus type 1 (HTLV-1) has been described as endemic in PNG and other countries in Melanesia (Asher et al. 1988; Cassar et al. 2007; Cassar et al. 2017; Kazura et al. 1987; Yanagihara et al. 1990a; Yanagihara et al. 1990b). HTLV-1 is primarily linked with adult T-cell leukaemia and HTLV-associated myelopathy/tropical spastic paraparesis (Willems et al. 2017), but studies have also linked the virus with conditions with diverse presentations, including uveitis, scabies, invective dermatitis, depression, and fibromyalgia (Blattner et al. 1990; Brites et al. 2002; Cruz et al. 2006; de Oliveira et al. 2005; Mochizuki et al. 1992; Stumpf et al. 2008).

Overall, there are numerous factors that should be considered in the context of false-negative IGRA results, particularly in a setting in which a variety of potentially immunosuppressing conditions are present. However, it is notable that in this study cohort, there was very little population-level evidence of immunosuppression. In the QFT®-Plus IGRA assay, the maximum concentration of IFN-ɣ is 10.0 IU/mL. In this study, three of the 114 general population participants had an IFN-ɣ concentration below 3.0 IU/mL in the analysis of the mitogen tubes, with the remainder having a mitogen response greater than 10.0 IU/mL (see Figure 7.4). Furthermore, there were only three indeterminate results overall – two in current TB patients and one in a TB investigation participant. Lower mitogen responses (below 8.0 IU/mL of IFN-ɣ) were measured in three IGRA-positive TB patients and two IGRA-negative TB patients; and in two IGRA-positive TB investigation participants and one IGRA-negative TB investigation participant. Thus, indeterminate and lower mitogen responses were seen more often in TB patients and TB investigation participants when compared to the general population, which is not surprising given that by definition they were more likely to be unwell at the time of the study. Although conditions such as malnutrition, parasites, and to a limited extent HIV are present in the Balimo region, and there is evidence of immunosuppression among people who are unwell or who have TB, the results of this analysis do not provide evidence of generalised immunosuppression in this population in the absence of broader immunological investigation.

The final consideration regarding IGRA-negative results in extrapulmonary TB patients is the possibility that some patients in this group did not actually have TB, even if symptoms resolved following therapy. Although the population-level results support the accuracy of clinical TB diagnoses in this region, there are almost certainly some misdiagnoses among clinically diagnosed TB patients.

7.4.4. Limitations

There are a number of limitations inherent in this study. Relatively low numbers of participants were investigated, particularly in the non-general population groups. It was not possible to bacteriologically confirm the majority of diagnosed TB patients, and thus the

majority of participants in the TB patient group were clinically diagnosed. Although the results support statistical differences between the patient and non-patient groups, the results should still be interpreted with caution.

This study also identified statistically significant differences in the analysis of the paired TB1 and TB2 tubes for the non-patient and extrapulmonary TB patient groups. This result conflicts with those expected by the assay, which is suggested to have a greater likelihood of an elevated TB2 response in active TB, based on more frequent detection of ESAT-6 and CFP-10 specific CD8+ cells in people with active TB compared to latent TB (Day et al. 2011; Nikolova et al. 2013; QIAGEN 2016; Rozot et al. 2013). However, this result may be influenced by the low sample numbers, particularly due to the substantially lower numbers of extrapulmonary TB participants when compared to the non-patient group. It should also be noted that there is other research that has not identified a difference between the TB1 and TB2 tube IFN-ɣ concentrations in latent and active TB (Hofland et al. 2018).

Finally, a number of studies have discussed the sources of variability that may influence the results of IGRA tests, with variability introduced in a number of the testing stages. In reviews of various IGRA tests, Banaei et al. (2016) and Pai et al. (2014) have described a number of sources of variability that could be important factors in the Balimo setting. These include blood collection time, adequacy of skin disinfection, blood volume, shaking of the blood tubes, time to incubation, and imprecision in the performance of the assay. Analytical factors are particularly notable close to the assay threshold, as they can give rise to discordant IGRA-positive and -negative results (Banaei et al. 2016). A number of studies using the earlier QFT-GIT assay have examined results close to the 0.35 IU/mL threshold. In one study, 19% of participants who underwent serial testing had discordant IGRA results, with IFN-ɣ variations as great as 11.1 IU/mL described (Detjen et al. 2009). Another study found 7% of participant samples that underwent duplicate testing had discordant results, with conversions (negative to positive) and reversions (positive to negative) significantly more likely in the borderline range of 0.25 – 0.80 IU/mL (Metcalfe et al. 2013). This study also suggested that variability of ±0.24 IU/mL could be expected for participants whose results fell in the borderline range (Metcalfe et al. 2013). In our study, some discordant

results were identified (see Section 7.2.4), and thus the possibility of result variability and false-positives and -negatives should be considered.

7.5. Conclusion

This study identified an unexpectedly low proportion of latent TB in the Balimo setting. Furthermore, there was little evidence of immunosuppression in the region's general population, based on the results of the mitogen control tubes in the IGRA tests. There was a high proportion of IGRA-negative, clinically diagnosed TB patients in the Balimo setting. However, on a population level extrapulmonary TB patients were more likely than the general population to be IGRA-positive, with an odds ratio of 2.8. The results provide evidence of MTBC infection in at least half of the extrapulmonary TB patients diagnosed in this setting. Although not definitive in the confirmation of extrapulmonary TB diagnoses, these results provide insight into the clinical identification of extrapulmonary TB in this setting.

The results emphasise the heavy burden of disease that is due to extrapulmonary TB in the Balimo region. Although extrapulmonary TB has limited influence on the transmission of TB, the large proportion of extrapulmonary TB patients in the Balimo region is a substantial contributor to the already considerable burden placed on health staff and facilities at BDH. As a result, reducing TB transmission is essential to the control of both pulmonary and extrapulmonary TB.

CHAPTER 8

GENERAL DISCUSSION

Tuberculosis (TB) in the Balimo region is contextualised by a number of health system factors – no resident physician, limited laboratory diagnostic facilities, and a high proportion of clinical diagnoses. These factors, while defining the TB epidemic in Balimo, also play a role in how TB patients are diagnosed, and the index of clinical suspicion with which symptomatic people are approached. These contextualising factors emphasise three defining aspects of the Balimo TB epidemic: (1) the influence of clinical non-laboratory confirmed diagnoses of TB, (2) the high proportion of extrapulmonary TB patients, and (3) the challenges associated with access to TB services.

8.1. Challenges in Clinical Tuberculosis Diagnosis at Balimo District Hospital

At BDH, a lack of advanced diagnostic facilities, as well as a high degree of suspicion of TB, likely contribute to the high proportion of clinical TB diagnoses. In this study, more than 90% of extrapulmonary TB diagnoses and about 20% of pulmonary TB diagnoses were clinical, based solely on presenting signs and symptoms. This situation is compounded by the inability to also identify other prevalent conditions that may present similarly to TB, such as HIV, malaria, and melioidosis. Rapid diagnostic kits for HIV are available in Balimo, although their use is complicated by the need for appropriate testing and counselling facilities that ensure patient privacy (McBryde 2012). Other febrile conditions have been identified in Western Province, including malaria, dengue fever, and Japanese E encephalitis (Bande et al. 2014; Mackenzie et al. 1998), and the province has faced other health challenges, including cholera (Horwood et al. 2014). The possibility of infection with non-tuberculous mycobacteria (NTM) should also be considered. Although only a small number of NTM have been identified in Papua New Guinea (PNG) to date (Aia et al. 2016; Ley et al. 2015), their presentation with TB-like symptoms including lymphadenitis should be considered in this setting where a high proportion of extrapulmonary TB has been described (Griffith et al.

2007; Haverkamp et al. 2004). Furthermore, melioidosis is endemic in the Balimo region (Diefenbach-Elstob et al. 2015; Warner et al. 2007; Warner et al. 2008), and can present similarly to TB (Warner et al. 2010). However, the laboratory resources required to identify melioidosis are only sporadically available (Figure 8.1), and differential diagnosis of other conditions is not likely to be achieved given the limited laboratory facilities (Saweri et al. 2017).

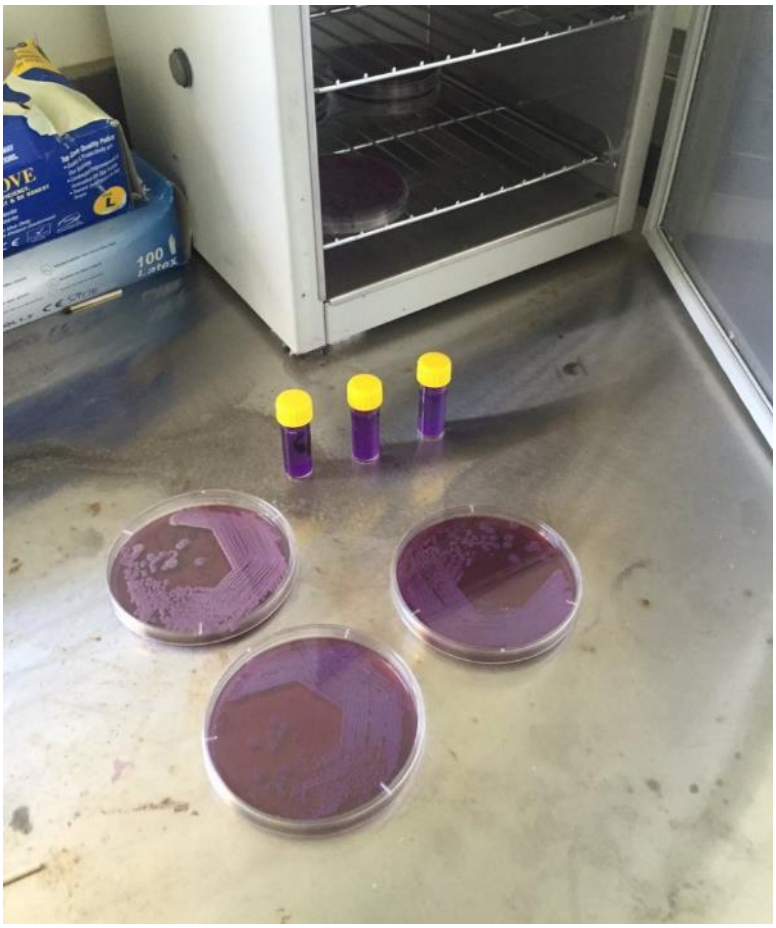


Figure 8.1: Laboratory identification of *Burkholderia pseudomallei*, the causative agent of melioidosis, in the BDH laboratory. (Image credit: Jeffrey Warner)

A reliance on clinical diagnoses of TB and treatment outcomes leads to important challenges in the context of drug resistance, particularly for patients who are smear-negative for TB, or for whom clinical samples are not able to be obtained. For such patients, it will be impossible

to differentiate between DR-TB and other potential conditions without the patient attending elsewhere for investigation – creating challenges for both the patient and health provider. In settings where laboratory-based diagnosis of TB is limited, it is not unusual for clinicians to use a treatment-as-confirmation-of-diagnosis approach, where patient improvement following initiation of a treatment regimen is considered to provide evidence of an accurate TB diagnosis, as has been described elsewhere in PNG (Kasa Tom et al. 2018). However, there is considerable uncertainty associated with this approach, especially given there are other conditions that may present similarly to TB with respiratory symptoms or lymphadenopathy, some of which will be self-limiting and resolve without treatment, and others which will respond to treatments such as rifampicin (Colebunders & Bastian 2000; Gaddey & Riegel 2016; Pandit et al. 2014; Thakkar et al. 2016).

8.1.1. Diagnosis of smear-negative pulmonary tuberculosis

In this region, bacteriological confirmation of TB, based on smear microscopy, occurred for a high proportion of about 65% of pulmonary TB patients recorded in the TB register. However, analysis of laboratory-based data, where microscopy and molecular results were compared for the same sample, found diagnostic sensitivity of the smear microscopy method to be only 50% (see Section 6.3.2). As already noted, a number of peripheral health facilities present in the Balimo region are able to clinically diagnose TB and commence people on TB treatment. The majority of these facilities maintain their own TB registers, which were not available for this study. These are small facilities, supplied with medication by BDH, and are thus unlikely to register a substantial number of TB patients. Furthermore, with the exception of very remote facilities such as Wawoi Falls, people with potential pulmonary TB should attend at Balimo for sputum examination (Figure 8.2). Therefore, although some smear-negative patients will be recorded elsewhere, the number is not likely to be substantial, and external registration of smear-positive patients will also occur. Hence, the data are unlikely to be substantially skewed by capture of a small proportion of TB patients outside of BDH.

Figure 8.2: Sister Bisato Gula, myself, Sister Keyanato Siwaeya, and Mr Robert Dowi, preparing to travel to a Gogodala-region village for a TB patrol, including collection of sputum samples. (Image credit: Mauri Amadu)

There is still a question of under-diagnosis of smear-negative pulmonary TB – an issue which has been raised in our earlier research in the Balimo region (Guernier et al. 2018). Although less infectious, smear-negative patients are still capable of transmitting infection at least intermittently, and are thus of concern if they are frequently missed and not commenced on treatment (Behr et al. 1999; Hernández-Garduño et al. 2004; Tostmann et al. 2008). There are clear implications for ongoing transmission of MTB in the region when considering a potentially substantial proportion of undiagnosed pulmonary TB.

8.2. Extrapulmonary Tuberculosis in the Balimo Region

The patterns of TB seen in the Balimo region, including factors such as the high proportion of extrapulmonary TB patients, the large proportion of childhood cases, and the approximately equal proportions of male and female TB patients, will reflect sociocultural, structural, geographic, and biomedical influences that are specific to the Balimo region. Understanding

the patterns of disease in relation to these influences will lead to a more informed TB control program.

Living in a rural setting with limited health facilities, people in the Balimo region have an increased likelihood of suffering from a variety of conditions that may increase susceptibility to infection or disease. These include immune-compromising conditions endemic to PNG such as mal- or under-nutrition, helminth and other parasite infections, and viral infections such as HIV and HTLV-1 (Asher et al. 1988; Dundon 2010; Goris et al. 2017; Kazura et al. 1987; McBryde 2012; Morita et al. 2015; Samiak & Emeto 2017; Wand et al. 2012; Yanagihara et al. 1990a; Yanagihara et al. 1990b). Although research is ongoing, all of these conditions have suggested links with TB, particularly in the case of HIV (Elias et al. 2006; Grassi et al. 2016; Jaganath & Mupere 2012; Marinho et al. 2005; World Health Organization 2014c). Other factors that may predispose to TB include exposure to smoke from cooking fires, and high housing density (i.e. high numbers of people per room), both of which have previously been associated with TB disease (Clark et al. 2002; Pérez-Padilla et al. 2001; Rahayu et al. 2015).

In addition to susceptibility to *Mycobacterium tuberculosis* (MTB) infection, the high proportion of extrapulmonary TB raises the question of why people have increased susceptibility to disseminated disease. The mechanisms underlying the dissemination of TB bacilli to extrapulmonary sites are not yet clearly defined, but numerous immune system components are thought to be important in both control and containment of the MTB organism, including CD4 and CD8 T-cells, interferon (IFN)-ɣ, and tumour necrosis factor (TNF) (Kaufmann 2001; Patel et al. 2007; Pawlowski et al. 2012; Roberts et al. 2007). Extrapulmonary TB is linked with diminished immunocompetence, and has been associated with immune-suppressing conditions including HIV/AIDS (Bates et al. 2015; Gounden et al. 2018). In the context of susceptibility to MTB infection and extrapulmonary dissemination of infection, immune dysregulation has been observed in many of the aforementioned conditions in the presence of MTB co-infection, including helminth infection, malaria, HIV, and HTLV-1 (Bastos et al. 2012; Carvalho et al. 2015; Chukwuanukwu et al. 2017; Day et al. 2017; George et al. 2014). The investigation of blood-based T-cell markers in MTB infection is

a field of ongoing research by our group, as immune factors may well play a role in the epidemiology of TB in the Balimo region.

8.2.1. Extrapulmonary tuberculosis in the context of clinical diagnoses

The high proportion of extrapulmonary TB seen in the Balimo region led to the question of whether these diagnoses are accurate, especially in this setting where more than 90% of extrapulmonary TB patients are diagnosed clinically, based only on presenting signs and symptoms. Although some diagnoses will undoubtedly be incorrect, data from the IFN-ɣ release assays (IGRA), and analysis of treatment outcomes, suggest that there is evidence of accurate diagnoses for many clinically-diagnosed extrapulmonary TB patients. Regardless of over-diagnosis or under-diagnosis, a setting with no resident medical officer, and with limited laboratory facilities, may see increased TB diagnoses if the setting has a high TB burden and there is a strong index of suspicion in the approach to symptomatic patients.

Figure 8.3: People travelling by dugout canoe in the Gogodala region of Western Province. (Image credit: David Plummer)

Given the difficulties associated with laboratory confirmation of extrapulmonary TB, as well as the non-specific nature of many extrapulmonary TB presentations, extrapulmonary TB cases are likely under-diagnosed in some settings. Indeed, the literature contains numerous case studies of extrapulmonary TB masquerading as other diseases for extended periods of time, particularly where the infection is localised to an unusual site. Examples include a case of pubic symphysis TB who was symptomatic for three years, as well as cases initially diagnosed as renal malignancy and leiomyoma (Kumar et al. 2014; Lal et al. 2013; Moore et al. 2008).

There has been some research into the possibility of under-diagnosis of smear-negative and extrapulmonary TB. The results of one study from Mozambique indicated this to be the case, particularly in settings where the only diagnostic test available is smear microscopy, and where HIV is prevalent (Brouwer et al. 2013). Furthermore, an autopsy study conducted in South Africa identified a high proportion of TB infection in HIV patients who had died. Autopsies were undertaken on 34 patients, of whom 16 had evidence of TB infection. Extrapulmonary TB infection was identified in 14 of those 16, but only 10 of the 16 had been commenced on TB treatment prior to their death (Karat et al. 2016). In PNG, it has been noted that both under- and over-diagnosis of extrapulmonary TB are possible outcomes in settings where diagnoses are predominantly symptom-based (Karki et al. 2017). However, the identification and diagnosis of extrapulmonary TB is complicated due to the resource-limited setting and lack of advanced specimen collection and diagnostic techniques.

Unlike in Mozambique and South Africa, HIV appears to be less common in TB patients in the Balimo region, although further research is required to understand the true TB-HIV burden. In this study, co-infection was identified in 5.4% (95% CI 2.1 – 13.1) of tested patients. Across PNG, 7% of TB patients with a known HIV status were HIV-positive in 2016 (World Health Organization 2017a). However, a recent study undertaken in Eastern Highlands Province that screened for TB among people living with HIV identified active TB in 82/532 adult patients (15.4%) – evidence of a substantial burden of undiagnosed TB-HIV coinfection (Carmone et al. 2017) (Figure 8.4). By comparison, TB-HIV coinfection in tested patients in Mozambique and South Africa is estimated to be 44% and 59% respectively (World Health Organization 2017a). However, in the Balimo region it is possible that HIV testing was more likely in

patients with certain risk factors, or increased clinical suspicion of HIV. These factors make it difficult to comment on the influence of HIV on TB, and particularly extrapulmonary TB, in this region.

Figure 8.4: Educational poster from PNG focusing on TB and HIV. (Image credit: David Plummer)

Figures from the World Health Organization (WHO) indicate that extrapulmonary TB occurs in 15% of notified TB patients globally, and only 8% of patients in the Western Pacific region, where PNG is located (World Health Organization 2017a). However, extrapulmonary TB proportions are known to be much higher in individual countries within the region, estimated at 46% in Vanuatu, 45% in Mongolia and New Zealand, 38% in the Solomon Islands, 37% in Australia, 33% in Cambodia, and 30% in Nauru (World Health Organization 2017a). The relatively high proportion of extrapulmonary TB patients in the high-income countries of Australia and New Zealand is notable, although extrapulmonary TB has been recognised as a concern in other low-incidence settings (Peto et al. 2009; Sandgren et al. 2013; Toms et al. 2017). However, Australia and New Zealand are not limited in laboratory

resources, and studies undertaken in Sydney and Auckland were able to confirm MTB infection based on bacteriology or histopathology in 78.6% and 97.0% of patients respectively (Herath & Lewis 2014; Pollett et al. 2016). However, the situation is likely to be different for the other countries listed with extrapulmonary TB proportions of 30% or more. Information about the proportion of laboratory-confirmed extrapulmonary TB cases was not able to be obtained for these countries, but it is likely that the proportion of laboratory-confirmed cases would be low, as these are resource-limited settings.

Published studies from other developing settings have found rates of extrapulmonary TB higher than expected based on comparison to countrywide proportions. Examples include 49.4% of TB cases in Van, Turkey; and 54.9% of TB cases in Kabul, Afghanistan (Fader et al. 2010; Sunnetcioglu et al. 2015). The authors of the Afghanistani study believed extrapulmonary TB to be erroneously over-represented in their study due to reporting anomalies (Fader et al. 2010). However, the authors of the Turkish study did not speculate as to the reason for their high proportion of extrapulmonary TB. There are additional characteristics of note in these studies. In the Turkish study, all cases were confirmed using laboratory methods and/or radiology (Sunnetcioglu et al. 2015). This result suggests that in a context where exhaustive diagnostic methods are available, cases of extrapulmonary TB may still be substantial. However, broader conclusions regarding the incidence of the two forms of active TB cannot be inferred from the study, as clinical diagnoses were not included. Clinical diagnoses of extrapulmonary TB were included in the study from Afghanistan (Fader et al. 2010). Contextual information in this study suggested many challenges similar to those found in Balimo. These include reporting difficulties, loss to follow-up of people following initial specimen collection and diagnosis, delays in recognition of potential extrapulmonary TB cases, uncertainty about age, and limited diagnostic facilities (Fader et al. 2010). In addition, HIV prevalence is thought to be low in the Afghanistani setting (Fader et al. 2010), as is also the case in Balimo.

8.3. The Influence of Health Systems and Access on Tuberculosis Diagnosis and Treatment

The results presented in this thesis emphasise the challenges associated with TB management in rural and remote areas. Notified TB case density was lower in the peripheral regions, and treatment outcomes were poorer for people from the rural local level government (LLG) areas when compared to the Balimo Urban LLG. However, there are contextual factors that should be considered in relation to these results. Lower recorded case density in the peripheral regions may be due to registration and treatment of some TB patients at peripheral health facilities instead of at Balimo, or could be because TB density is in fact lower in rural areas. If it is the former reason, and a substantial number of patients are diagnosed at the peripheral health facilities, then the burden of TB in the Balimo region is even higher than the results in this thesis suggest. Even if TB density is lower in the peripheral regions, in this setting there are likely to still be complications in management and the risk of ongoing TB transmission due to potentially lengthy time periods before TB is diagnosed, as a result of access challenges in the region. The difficulties associated with the diagnosis and management of TB in rural settings will also be important in the context of drug resistance. Although risk will be variable based on local epidemiological factors, a study from Ethiopia identified living in a rural region as a risk factor for the development of multidrug-resistant TB (MDR-TB), and suggested links with access and education as possible reasons for this association (Desissa et al. 2018). These are factors that will also exist in the Balimo region.

The diagnosis and treatment of TB in Balimo will be influenced by setting-specific factors, although there are a number of factors generalizable across PNG. The geographic diversity of PNG and large proportion of people living in rural and remote areas means that access challenges will be prevalent countrywide. There are challenges faced by PNG in the provision of health care services, with only 6.2 health workers (physicians, nurses, and midwives) per 10,000 people countrywide (World Health Organization Regional Office for the Western Pacific 2017b). As a result, health facilities operating under a non-doctor-led model of care, operated by health extension officers (HEO) and nurses, are the norm in many rural and remote settings.

Based on a situation analysis undertaken in 2001, PNG has been described as having 'a growing gap between the resources available for health personnel and the resources required to adequately staff health institutions and activities', influencing the abilities of health workers to initiate and maintain care (Aitken & Kolehmainen-Aitken 2009). At the time of the situation analysis, PNG had a staff-to-patient ratio of 5.9 health workers (physicians, nurses, and midwives) per 10,000 people, and this ratio had increased to only 6.2 per 10,000 people in 2010 (World Health Organization Regional Office for the Western Pacific 2017b). When also including HEOs, community health workers (CHW), dental officers, and other allied health workers, in 2008 the ratio was 18.3 per 10,000 people, still well below the WHO-recommended ratio of 23 health workers per 10,000 people (World Health Organization & National Department of Health 2012; World Health Organization Regional Office for the Western Pacific 2017b). However, the distribution of health workers is complex, and the full utilisation of health worker skills can be limited by a lack of health service resources (Aitken & Kolehmainen-Aitken 2009). Although difficult to quantify, TB epidemiology in the Balimo region (and undoubtedly other regions in PNG), will be influenced by the skills and experience of resident health workers, and by the availability of diagnostic tools and medications.

Figure 8.5: Overlooking the Balimo lagoon at sunset. (Image credit: David Plummer)

8.4. Implications for Practice and Public Health

The high burden of TB identified in the Balimo region emphasises the need for a rapid and achievable TB diagnostic that is capable of identifying both drug-susceptible and DR-TB. An example would be the implementation of a method such as the WHO-endorsed Xpert MTB/RIF. This approach would have the added benefit of contributing to the confirmation of some extrapulmonary TB diagnoses, as demonstrated in a number of studies using the Xpert MTB/RIF on non-sputum clinical samples (Denkinger et al. 2014; Hillemann et al. 2011; Marouane et al. 2016; Maynard-Smith et al. 2014; Suzana et al. 2016; Zeka et al. 2011). An essential component of implementation of the Xpert MTB/RIF would be the need for training and support for health workers in the Balimo region in the treatment of DR-TB. Expanding staffing at BDH to include a physician would also be essential, particularly in the context of the ongoing management of DR-TB patients. These recommendations are particularly important given that this study has identified rifampicin-resistant TB (RR-TB), with the possibility that MDR-TB is also present. The implementation of DR-TB diagnosis and treatment in Balimo would help to alleviate some of the TB burden seen at the hospital in Daru, which is the referral hospital for the province and that is known to face a considerable burden of DR-TB (Furin & Cox 2016; Kase et al. 2016; World Health Organization Representative Office: Papua New Guinea 2016).

Expansion of TB services at BDH would necessitate improvements to the current facilities, particularly the laboratory and TB ward. Furthermore, this study has described a considerable burden of latent TB infection in BDH health workers (see Chapter 7), which emphasises the importance of well-managed facilities and infection control practices in reducing this risk (Verkuijl & Middelkoop 2016). It is also important to ensure that lay health workers such as community-based treatment supporters, who are an important part of the Western Province TB Program (World Vision TB DOTS Project 2013), receive appropriate health education regarding TB risk and infection control. Additional investment in facilities and personnel at the BDH laboratory is critical to maintaining and improving TB diagnostic capacity, as well as expanding diagnostic capability to accurately diagnose other diseases endemic in Western Province.

Results from Chapter 5 of this thesis highlighted the potentially substantial burden of undiagnosed TB in the Balimo region, based on analysis of a non-routine active TB case-finding activity (see Section 5.4.3). This finding emphasises the need for finances and resources to undertake outreach activities. Given that increased active case-finding activities will lead to increased burden on the BDH health workers and facilities, the aforementioned investments in personnel, resources, and facilities would be necessary in combination with this approach.

8.5. Future Research Directions

This thesis has provided a broad description of the epidemiology of reported TB in the Balimo region. However, the limitations of the data, which have been described throughout this thesis, are an important consideration in the context of future research directions. These limitations highlight the importance of developing a greater understanding of TB in the Balimo region, and the representativeness of the data presented in this thesis. Further exploration in the near future of a number of aspects of this research would provide important additional detail to support TB control efforts in the region.

As one of the defining outcomes of this study, the heavy burden of extrapulmonary TB, particularly in the context of under- or over-diagnosis and the accuracy of clinical diagnoses, warrants further investigation. This approach should include focused investigation of decision-making and diagnostic protocols to understand how extrapulmonary TB diagnoses are reached without the benefit of laboratory confirmation.

The distribution of TB patients has highlighted the heterogeneity of case density across the Balimo region. It would be useful to undertake a more thorough investigation of select villages, particularly in the context of their proximity to peripheral sub-health centres and aid posts, to understand the influence of geography and access on TB distribution. In addition, investigation of TB in low- and high-burden villages could provide insights into structural and behavioural influences that influence the likelihood of developing TB. Analysis of the TB patient registers maintained at the peripheral health facilities would increase understanding of the epidemiology of TB in the Balimo region.

This study has described a number of influences that place people at greater risk of developing TB, or having poor TB treatment outcomes. These influences should be investigated, particularly regarding factors such as nutrition, cooking fires, housing density, and parasitic and viral infections. Furthermore, investigation of access-related challenges such as time to diagnosis and proximity to health services will be important to further the role of health promotion activities aimed at reducing TB transmission and enhancing community-based management of TB.

It would be worthwhile developing a greater understanding of the burden of latent TB in this region, especially in rural and remote areas, given the unexpectedly low proportion of latent TB identified in this study in the context of the high active TB burden. If the regional latent TB burden is indeed low, this relatively isolated region may benefit from active case-finding alongside prophylactic treatment. Such a response may be a viable option in reducing the overall TB burden in the broader Gogodala region.

Figure 8.6: Village houses located in the Aramia River floodplain area near Balimo. (Image credit: David Plummer)

8.6. Conclusion

This research has described and characterised the TB epidemic in the Balimo region of Western Province, PNG. The findings provide important information in the context of TB management in the region, and in providing evidence of the critical importance of BDH in TB control. Given the heterogeneity of TB distribution across Western Province, understanding TB epidemiology in the Balimo region is particularly important to contribute to focused and efficient distribution of limited resources across the province. In describing the high ongoing burden of TB in the Balimo region, the results of this thesis highlight the importance of ensuring that staff, resources, and facilities at BDH are capable of responding to the heavy burden of TB, particularly in light of the growing evidence of drug resistance. In conclusion, TB in this region is at a critical juncture, with systematic, concerted efforts in research and control essential to address the TB epidemic in the region.

REFERENCES

Abdallah T E M, Toum F E M, Bashir O H, Mansoor T I, Yuosif M M, Elkhawad M A-E, Okud I O, Mohammed A O and Ali A A A (2015) Epidemiology of extra pulmonary tuberculosis in Eastern Sudan. *Asian Pacific Journal of Tropical Biomedicine* 5(6): 505-508.

Adams S, Ehrlich R, Baatjies R, van Zyl-Smit R N, Said-Hartley Q, Dawson R and Dheda K (2015) Incidence of occupational latent tuberculosis infection in South African healthcare workers. *European Respiratory Journal* 45(5): 1364.

Adamu A L, Aliyu M H, Galadanci N A, Musa B M, Lawan U M, Bashir U and Abubakar I (2018) The impact of rural residence and HIV infection on poor tuberculosis treatment outcomes in a large urban hospital: a retrospective cohort analysis. *International Journal for Equity in Health* 17: 4.

Ade S, Harries A D, Trébucq A, Ade G, Agodokpessi G, Adjonou C, Azon S and Anagonou S (2014) National profile and treatment outcomes of patients with extrapulmonary tuberculosis in Bénin. *PLoS ONE* 9(4): e95603.

Afsar I, Gunes M, Er H and Sener A G (2018) Comparison of culture, microscopic smear and molecular methods in diagnosis of tuberculosis. *Revista Española de Quimioterapia* 31(5): 435-438.

Agaya J, Nnadi C D, Odhiambo J, Obonyo C, Obiero V, Lipke V, Okeyo E, Cain K and Oeltmann J E (2015) Tuberculosis and latent tuberculosis infection among healthcare workers in Kisumu, Kenya. *Tropical Medicine & International Health* 20(12): 1797-1804.

Ahmad D and Morgan W K C (2000) How long are TB patients infectious? *Canadian Medical Association Journal* 163(2): 157.

Ahmad V, Hanif M, Chopra K K, Sidiq Z, Dwivedi K K and Shrivastsava D (2017) Isolation and identification of *Mycobacterium tuberculosis* with mixed growth from positive MGIT 960 cultures by re-decontamination. *Journal of Biotechnology & Biomaterials* 7(3): 1000267.

Aia P, Kal M, Lavu E, John L N, Johnson K, Coulter C, Ershova J, Tosas O, Zignol M, Ahmadova S and Islam T (2016) The burden of drug-resistant tuberculosis in Papua New Guinea: results of a large population-based survey. *PLoS ONE* 11(3): e0149806.

Aia P, Wangchuk L, Morishita F, Kisomb J, Yasi R, Kal M and Islam T (2018) Epidemiology of tuberculosis in Papua New Guinea: analysis of case notification data and treatment-outcome data, 2008-2016. *Western Pacific Surveillance and Response* 9(2).

Aitken I W and Kolehmainen-Aitken R-L (2009) Human resource development: new assessments and new directions. *Papua New Guinea Medical Journal* 52(3-4): 139-158.

Alele F O, Franklin R C, Emeto T I and Leggat P (2018) Occupational tuberculosis in healthcare workers in sub-Saharan Africa: a systematic review. *Archives of Environmental & Occupational Health* In Press.

Alene K A, Viney K, McBryde E S and Clements A C A (2017) Spatial patterns of multidrug resistant tuberculosis and relationships to socio-economic, demographic and household factors in northwest Ethiopia. *PLoS ONE* 12(2): e0171800.

Ando H, Kondo Y, Suetake T, Toyota E, Kato S, Mori T and Kirikae T (2010) Identification of *katG* mutations associated with high-level isoniazid resistance in *Mycobacterium tuberculosis*. *Antimicrobial Agents and Chemotherapy* 54(5): 1793-1799.

Antunes J L F and Waldman E A (2001) The impact of AIDS, immigration and housing overcrowding on tuberculosis deaths in São Paulo, Brazil, 1994–1998. *Social Science & Medicine* 52(7): 1071-1080.

Arora V K and Gupta R (2006) Trends of extra-pulmonary tuberculosis under Revised National Tuberculosis Control Programme: a study from south Delhi. *Indian Journal of Tuberculosis* 53: 77-83.

Asher D M, Goudsmit J, Pomeroy K L, Garruto R M, Bakker M, Ono S G, Elliot N, Harris K, Askins H, Eldadah Z, Goldstein A D and Gajduek D C (1988) Antibodies to HTLV-I in populations of the southwestern Pacific. *Journal of Medical Virology* 26(4): 339-351.

Ates Guler S, Bozkus F, Inci M F, Kokoglu O F, Ucmak H, Ozden S and Yuksel M (2015) Evaluation of pulmonary and extrapulmonary tuberculosis in immunocompetent adults: a retrospective case series analysis. *Medical Principles and Practice* 24(1): 75-79.

Azghay M, Bouchaud O, Mechaï F, Nicaise P, Fain O and Stirnemann J (2016) Utility of QuantiFERON-TB Gold In-Tube assay in adult, pulmonary and extrapulmonary, active tuberculosis diagnosis. *International Journal of Infectious Diseases* 44: 25-30.

Babatunde O I, Bismark E C, Amaechi N E, Gabriel E I and Olanike A-U R (2015) Determinants of treatment delays among pulmonary tuberculosis patients in Enugu Metropolis, South-East, Nigeria. *Health* 7: 1506-1516.

Bainomugisa A, Lavu E, Hiashiri S, Majumdar S, Honjepari A, Moke R, Dakulala P, Hill-Cawthorne G A, Pandey S, Marais B J, Coulter C and Coin L (2018) Multi-clonal evolution of multi-drug-resistant/extensively drug-resistant *Mycobacterium tuberculosis* in a high-prevalence setting of Papua New Guinea for over three decades. *Microbial Genomics* 4(2).

Baird T, Donnan E, Coulter C, Simpson G, Konstantinos A and Eather G (2018) Multidrug-resistant tuberculosis in Queensland, Australia: an ongoing cross-border challenge. *The International Journal of Tuberculosis and Lung Disease* 22(2): 206-211.

Bala Raghu Raji V, Vasanthraj P K, Ramachandran R and Sai V (2018) Multi-system infection – tuberculosis or melioidosis? *The Egyptian Journal of Radiology and Nuclear Medicine* In Press.

Ballif M, Harino P, Ley S, Carter R, Coulter C, Niemann S, Borrell S, Fenner L, Siba P, Phuanukoonnon S, Gagneux S and Beck H-P (2012a) Genetic diversity of *Mycobacterium tuberculosis* in Madang, Papua New Guinea. *The International Journal of Tuberculosis and Lung Disease* 16(8): 1100-1107.

Ballif M, Harino P, Ley S, Coscolla M, Niemann S, Carter R, Coulter C, Borrell S, Siba P, Phuanukoonnon S, Gagneux S and Beck H-P (2012b) Drug resistance-conferring mutations in *Mycobacterium tuberculosis* from Madang, Papua New Guinea. *BMC Microbiology* 12: 191.

Banaei N, Gaur R L and Pai M (2016) Interferon gamma release assays for latent tuberculosis: what are the sources of variability? *Journal of Clinical Microbiology* 54(4): 845-850.

Banda H T, Harries A D, Boeree M J, Nyirenda T E, Banerjee A and Salaniponi F M L (2000) Viability of stored sputum specimens for smear microscopy and culture [Notes from the Field]. *The International Journal of Tuberculosis and Lung Disease* 4(3): 272-274.

Bande G, Hetzel M W, Iga J, Barnadas C, Mueller I, Siba P M and Horwood P F (2014) An investigation into febrile illnesses of unknown aetiology in Wipim, Papua New Guinea. *Papua New Guinea Medical Journal* 57(1-4): 52-58.

Banfield S, Pascoe E, Thambiran A, Siafarikas A and Burgner D (2012) Factors associated with the performance of a blood-based interferon-γ release assay in diagnosing tuberculosis. *PLoS ONE* 7(6): e38556.

Baral S C, Karki D K and Newell J N (2007) Causes of stigma and discrimination associated with tuberculosis in Nepal: a qualitative study. *BMC Public Health* 7: 211.

Bastos M d L, Santos S B, Souza A, Finkmoore B, Bispo O, Barreto T, Cardoso I, Bispo I, Bastos F, Pereira D, Riley L and Carvalho E M (2012) Influence of HTLV-1 on the clinical, microbiologic and immunologic presentation of tuberculosis. *BMC Infectious Diseases* 12: 199.

Basu S and Galvani A P (2008) The transmission and control of XDR TB in South Africa: an operations research and mathematical modelling approach. *Epidemiology & Infection* 136(12): 1585-1598.

Bates M, Mudenda V, Shibemba A, Kaluwaji J, Tembo J, Kabwe M, Chimoga C, Chilukutu L, Chilufya M, Kapata N, Hoelscher M, Maeurer M, Mwaba P and Zumla A (2015) Burden of tuberculosis at post mortem in inpatients at a tertiary referral centre in sub-Saharan Africa: a prospective descriptive autopsy study. *The Lancet Infectious Diseases* 15(5): 544-551.

Battaglioli T, Rintiswati N, Martin A, Palupi K R, Bernaerts G, Dwihardiani B, Ahmad R A, Matthys F, Mahendradhata Y and Van der Stuyft P (2013) Comparative performance of thin layer agar and Löwenstein–Jensen culture for diagnosis of tuberculosis. *Clinical Microbiology and Infection* 19: E502-E508.

Behr M A, Warren S A, Salamon H, Hopewell P C, de Leon A P, Daley C L and Small P M (1999) Transmission of *Mycobacterium tuberculosis* from patients smear-negative for acid-fast bacilli. *The Lancet* 353(9151): 444-449.

Benbaba S, Isaakidis P, Das M, Jadhav S, Reid T and Furin J (2015) Direct observation (DO) for drug-resistant tuberculosis: do we really DO? *PLoS ONE* 10(12): e0144936.

Blattner W, LaGrenade L, Hanchard B, Fletcher V and Cranston B (1990) Infective dermatitis of Jamaican children: a marker for HTLV-I infection. *The Lancet* 336(8727): 1345-1347.

Blouin Y, Hauck Y, Soler C, Fabre M, Vong R, Dehan C, Cazajous G, Massoure P-L, Kraemer P, Jenkins A, Garnotel E, Pourcel C and Vergnaud G (2012) Significance of the identification in the Horn of Africa of an exceptionally deep branching *Mycobacterium tuberculosis* clade. *PLoS ONE* 7(12): e52841.

Boehme C C, Nicol M P, Nabeta P, Michael J S, Gotuzzo E, Tahirli R, Gler M T, Blakemore R, Worodria W, Gray C, Huang L, Caceres T, Mehdiyev R, Raymond L, Whitelaw A, Sagadevan K, Alexander H, Albert H, Cobelens F, Cox H, Alland D and Perkins M D (2011) Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. *The Lancet* 377(9776): 1495-1505.

Bogale S, Diro E, Atsede Mazengia S and Yenit M K (2017) Factors associated with the length of delay with tuberculosis diagnosis and treatment among adult tuberculosis patients attending at public health facilities in Gondar town, Northwest, Ethiopia. *BMC Infectious Diseases* 17: 145.

Boscoe F P, McLaughlin C, Schymura M J and Kielb C L (2003) Visualization of the spatial scan statistic using nested circles. *Health & Place* 9(3): 273-277.

Brandis G and Hughes D (2013) Genetic characterization of compensatory evolution in strains carrying *rpoB* Ser531Leu, the rifampicin resistance mutation most frequently found in clinical isolates. *Journal of Antimicrobial Chemotherapy* 68: 2493-2497.

Brites C, Weyll M, Pedroso C and Badaró R (2002) Severe and Norwegian scabies are strongly associated with retroviral (HIV-1/HTLV-1) infection in Bahia, Brazil. *AIDS* 16(9): 1292-1293.

Brites D and Gagneux S (2015) Co-evolution of *Mycobacterium tuberculosis* and *Homo sapiens*. *Immunological Reviews* 264(1): 6-24.

Broccolo F, Scarpellini P, Locatelli G, Zingale A, Brambilla A M, Cichero P, Sechi L A, Lazzarin A, Lusso P and Malnati M S (2003) Rapid diagnosis of mycobacterial infections and quantitation of *Mycobacterium tuberculosis* load by two real-time calibrated PCR assays. *Journal of Clinical Microbiology* 41(10): 4565-4572.

Brossier F, Veziris N, Truffot-Pernot C, Jarlier V and Sougakoff W (2006) Performance of the Genotype MTBDR line probe assay for detection of resistance to rifampin and isoniazid in strains of *Mycobacterium tuberculosis* with low- and high-level resistance. *Journal of Clinical Microbiology* 44(10): 3659-3664.

Brouwer M, Gudo P S, Simbe C M, Perdigão P and van Leth F (2013) Benchmarking to assess potential under-diagnosis of smear-negative and extrapulmonary tuberculosis. A case study from Mozambique. *The Open Infectious Diseases Journal* 7: 1-5.

Buijtels P C, Iseman M D, Parkinson S, de Graaff C S, Verbrugh H A, Petit P L and van Soolingen D (2010) Misdiagnosis of tuberculosis and the clinical relevance of non-tuberculous mycobacteria in Zambia. *Asian Pacific Journal of Tropical Medicine* 3(5): 386-391.

Buregyeya E, Nuwaha F, Verver S, Criel B, Colebunders R, Wanyenze R, Kalyango J N, Katamba A and Mitchell E M H (2013) Implementation of tuberculosis infection control in health facilities in Mukono and Wakiso districts, Uganda. *BMC Infectious Diseases* 13: 360.

Buu T N, Lönnroth K and Quy H T (2003) Initial defaulting in the National Tuberculosis Programme in Ho Chi Minh City, Vietnam: a survey of extent, reasons and alternative actions taken following default. *The International Journal of Tuberculosis and Lung Disease* 7(8): 735-741.

Cardoso R F, Cooksey R C, Morlock G P, Barco P, Cecon L, Forestiero F, Leite C Q F, Sato D N, Shikama M d L, Mamizuka E M, Hirata R D C and Hirata M H (2004) Screening and characterization of mutations in isoniazid-resistant *Mycobacterium tuberculosis* isolates obtained in Brazil. *Antimicrobial Agents and Chemotherapy* 48(9): 3373-3381.

Carmone A, Rodriguez C A, Frank T D, Kiromat M, Bongi P W, Kuno R G, Palou T and Franke M F (2017) Increasing isoniazid preventive therapy uptake in an HIV program in rural Papua New Guinea. *Public Health Action* 7(3): 193-198.

Carvalho N B, Bastos M d L, Neves Y, Souza A, Netto E M, Santos S and Carvalho E M (2015) HTLV-1 infection interferes with immune responses to *Mycobacterium tuberculosis* antigens. *Retrovirology* 12(Suppl 1): O24.

Cassar O, Capuano C, Bassot S, Charavay F, Duprez R, Afonso P V, Abel M, Walter H, Mera W, Martin P M V, Chungue E and Gessain A (2007) Human T lymphotropic virus type 1 subtype C Melanesian genetic variants of the Vanuatu archipelago and Solomon Islands share a common ancestor. *The Journal of Infectious Diseases* 196(4): 510-521.

Cassar O, Charavay F, Touzain F, Jeannin P, Grangeon J-P, Laumond S, Chungue E, Martin P M V and Gessain A (2017) A novel human T-lymphotropic virus type 1c molecular variant in an indigenous individual from New Caledonia, Melanesia. *PLoS Neglected Tropical Diseases* 11(1): e0005278.

Cattamanchi A, Dowdy D W, Davis J L, Worodria W, Yoo S, Joloba M, Matovu J, Hopewell P C and Huang L (2009) Sensitivity of direct versus concentrated sputum smear microscopy in HIV-infected patients suspected of having pulmonary tuberculosis. *BMC Infectious Diseases* 9(53).

Cavalcante S C, Soares E C, Pacheco A G, Chaisson R E and Durovni B (2007) Community DOT for tuberculosis in a Brazilian favela: comparison with a clinic model. *Int J Tuberc Lung Dis* 11(5): 544-549.

Cave A J E (1939) The evidence for the incidence of tuberculosis in ancient Egypt. *The British Journal of Tuberculosis* 33(3): 142-152.

Centers for Disease Control and Prevention (2016) *Health disparities in TB,* Centers for Disease Control and Prevention, Atlanta (USA), viewed 6 June 2018, <https://www.cdc.gov/tb/topic/populations/HealthDisparities/default.htm>.

Chakravorty S, Sen M K and Tyagi J S (2005) Diagnosis of extrapulmonary tuberculosis by smear, culture, and PCR using universal sample processing technology. *Journal of Clinical Microbiology* 43(9): 4357-4362.

Chandler J (2016) *'Ebola with wings': expert raises alarm over deadly tuberculosis outbreak in PNG,* ABC RN, Sydney (Australia), viewed 5 June 2017, <http://www.abc.net.au/radionational/programs/backgroundbriefing/experts-raise-alarm-over-deadly-tuberculosis-outbreak-in-png/7327018>.

Chang C L, Park T S, Oh S H, Kim H H, Lee E Y, Son H C and Kim C M (2002) Reduction of contamination of mycobacterial growth indicator tubes with a modified antimicrobial combination. *Journal of Clinical Microbiology* 40(10): 3845-3847.

Chen L, Evans T, Anand S, Boufford J I, Brown H, Chowdhury M, Cueto M, Dare L, Dussault G, Elzinga G, Fee E, Habte D, Hanvoravongchai P, Jacobs M, Kurowski C, Michael S, Pablos-Mendez A, Sewankambo N, Solimano G, Stilwell B, de Waal A and Wibulpolprasert S (2004) Human resources for health: overcoming the crisis. *The Lancet* 364(9449): 1984-1990.

Chida N, Ansari Z, Hussain H, Jaswal M, Symes S, Khan A J and Mohammed S (2015) Determinants of default from tuberculosis treatment among patients with drug-susceptible tuberculosis in Karachi, Pakistan: a mixed methods study. *PLoS ONE* 10(11): e0142384.

Chien H P, Yu M C, Wu M H, Lin T P and Luh K T (2000) Comparison of the BACTEC MGIT 960 with Löwenstein-Jensen medium for recovery of mycobacteria from clinical specimens. *The International Journal of Tuberculosis and Lung Disease* 4(9): 866-870.

Chihota V N, Grant A D, Fielding K, Ndibongo B, van Zyl A, Muirhead D and Churchyard G J (2010) Liquid vs. solid culture for tuberculosis: performance and cost in a resource-constrained setting. *The International Journal of Tuberculosis and Lung Disease* 14(8): 1024-1031.

Chihota V N, Ginindza S, McCarthy K, Grant A D, Churchyard G and Fielding K (2015) Missed opportunities for TB investigation in primary care clinics in South Africa: experience from the XTEND trial. *PLoS ONE* 10(9): e0138149.

Chukwuanukwu R C, Onyenekwe C C, Martinez-Pomares L, Flynn R, Singh S, Amilo G I, Agbakoba N R and Okoye J O (2017) Modulation of the immune response to *Mycobacterium tuberculosis* during malaria/*M. tuberculosis* co-infection. *Clinical & Experimental Immunology* 187(2): 259-268.

Clark M, Riben P and Nowgesic E (2002) The association of housing density, isolation and tuberculosis in Canadian First Nations communities. *International Journal of Epidemiology* 31(5): 940-945.

Colebunders R and Bastian I (2000) A review of the diagnosis and treatment of smear-negative pulmonary tuberculosis. *The International Journal of Tuberculosis and Lung Disease* 4(2): 97-107.

Colijn C, Cohen T, Ganesh A and Murray M (2011) Spontaneous emergence of multiple drug resistance in tuberculosis before and during therapy. *PLoS ONE* 6(3): e18327.

Commonwealth, The (2018) *Papua New Guinea,* Commonwealth Secretariat, London (United Kingdom), viewed 6 June 2018, <http://thecommonwealth.org/our-member-countries/papua-new-guinea>.

Cornfield D B, Beavis K G, Greene J A, Bojak M and Bondi J (1997) Mycobacterial growth and bacterial contamination in the mycobacteria growth indicator tube and BACTEC 460 culture systems. *Journal of Clinical Microbiology* 35(8): 2068-2071.

Cowan J, Greenberg Cowan J, Barnhart S, Demamu S, Fiseha D, Graham W, Melese E, Reason L, Tefera Asfaw F, Feleke G and Feleke B (2013) A qualitative assessment of challenges to tuberculosis management and prevention in Northern Ethiopia. *The International Journal of Tuberculosis and Lung Disease* 17(8): 1071-1075.

Cox V, Brigden G, Crespo R H, Lessem E, Lynch S, Rich M L, Waning B and Furin J (2018) Global programmatic use of bedaquiline and delamanid for the treatment of multidrug-resistant tuberculosis. *The International Journal of Tuberculosis and Lung Disease* 22(4): 407-412.

Crofton J and Mitchison D A (1948) Streptomycin resistance in pulmonary tuberculosis. *British Medical Journal* 2: 1009-1015.

Cross G B, Coles K, Nikpour M, Moore O A, Denholm J, McBryde E S, Eisen D P, Warigi B, Carter R, Pandey S, Harino P, Siba P, Coulter C, Mueller I, Phuanukoonnon S and Pellegrini M (2014) TB incidence and characteristics in the remote gulf province of Papua New Guinea: a prospective study. *BMC Infectious Diseases* 14: 93.

Cruz B A, Catalan-Soares B and Proietti F (2006) Higher prevalence of fibromyalgia in patients infected with human T cell lymphotropic virus type I. *The Journal of Rheumatology* 33(11): 2300-2303.

Dangisso M H, Datiko D G and Lindtjørn B (2014) Trends of tuberculosis case notification and treatment outcomes in the Sidama Zone, southern Ethiopia: ten-year retrospective trend analysis in urban-rural settings. *PLoS ONE* 9(12): e114225.

Dangisso M H, Datiko D G and Lindtjørn B (2015a) Spatio-temporal analysis of smear-positive tuberculosis in the Sidama Zone, Southern Ethiopia. *PLoS ONE* 10(6): e0126369.

Dangisso M H, Datiko D G and Lindtjørn B (2015b) Accessibility to tuberculosis control services and tuberculosis programme performance in southern Ethiopia. *Global Health Action* 8: 29443.

Daniel T M (2006) The history of tuberculosis. *Respiratory Medicine* 100(11): 1862-1870.

Darban-Sarokhalil D, Fooladi A A I, Maleknejad P, Bameri Z, Aflaki M, Nomanpour B, Yaslianifard S, Modarresi M H and Feizabadi M M (2013) Comparison of smear microscopy, culture, and real-time PCR for quantitative detection of *Mycobacterium tuberculosis* in clinical respiratory specimens. *Scandinavian Journal of Infectious Diseases* 45(4): 250-255.

Datiko D G, Yassin M A, Theobald S J, Blok L, Suvanand S, Creswell J and Cuevas L E (2017) Health extension workers improve tuberculosis case finding and treatment outcome in Ethiopia: a large-scale implementation study. *BMJ Global Health* 2(4): e000390.

Day C L, Abrahams D A, Lerumo L, Janse van Rensburg E, Stone L, O'rie T, Pienaar B, de Kock M, Kaplan G, Mahomed H, Dheda K and Hanekom W A (2011) Functional capacity of *Mycobacterium tuberculosis*-specific T cell responses in humans is associated with mycobacterial load. *The Journal of Immunology* 187(5): 2222-2232.

Day C L, Abrahams D A, Harris L D, van Rooyen M, Stone L, de Kock M and Hanekom W A (2017) HIV-1 infection is associated with depletion and functional impairment of *Mycobacterium tuberculosis*-specific CD4 T cells in individuals with latent tuberculosis infection. *The Journal of Immunology* 199(6): 2069-2080.

de Oliveira M d F S P, Brites C, Ferraz N, Magalhães P, Almeida F and Bittencourt A L (2005) Infective dermatitis associated with the human T cell lymphotropic virus type I in Salvador, Bahia, Brazil. *Clinical Infectious Diseases* 40(11): e90-e96.

de Visser V, Sotgiu G, Lange C, Aabye M G, Bakker M, Bartalesi F, Brat K, Chee C B E, Dheda K, Dominguez J, Eyuboglu F, Ghanem M, Goletti D, Dilektasli A G, Guglielmetti L, Koh W-J, Latorre I, Losi M, Polanova M, Ravn P, Ringshausen F C, Rumetshofer R, de Souza-Galvão M L, Thijsen S, Bothamley G and Bossink A (2015) False-negative interferon-γ release assay results in active tuberculosis: a TBNET study. *European Respiratory Journal* 45(1): 279.

Demissie M, Lindtjørn B and Berhane Y (2002) Patient and health service delay in the diagnosis of pulmonary tuberculosis in Ethiopia. *BMC Public Health* 2: 23.

Demissie M, Getahun H and Lindtjørn B (2003) Community tuberculosis care through "TB clubs" in rural North Ethiopia. *Social Science & Medicine* 56: 2009-2018.

Denkinger C M, Schumacher S G, Boehme C C, Dendukuri N, Pai M and Steingart K R (2014) Xpert MTB/RIF assay for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysis. *European Respiratory Journal* 44(2): 435-446.

Department of Health (2011) *Papua New Guinea: national tuberculosis management protocol*, Department of Health, Port Moresby (Papua New Guinea), viewed 31 October 2017, <http://www.adi.org.au/wp-content/uploads/2016/11/National-Tuberculosis-Management-Protocol-PNG-2011.pdf>.

Desikan P, Tiwari K, Panwalkar N, Khaliq S, Chourey M, Varathe R, Mirza S B, Sharma A, Anand S and Pandey M (2017) Public health relevance of non-tuberculous mycobacteria among AFB positive sputa. *Germs* 7(1): 10-18.

Desissa F, Workineh T and Beyene T (2018) Risk factors for the occurrence of multidrug-resistant tuberculosis among patients undergoing multidrug-resistant tuberculosis treatment in East Shoa, Ethiopia. *BMC Public Health* 18: 422.

Detjen A K, Loebenberg L, Grewal H M S, Stanley K, Gutschmidt A, Kruger C, Du Plessis N, Kidd M, Beyers N, Walzl G and Hesseling A C (2009) Short-term reproducibility of a commercial interferon gamma release assay. *Clinical and Vaccine Immunology* 16(8): 1170-1175.

Dheda K, Gumbo T, Maartens G, Dooley K E, McNerney R, Murray M, Furin J, Nardell E A, London L, Lessem E, Theron G, van Helden P, Niemann S, Merker M, Dowdy D, Van Rie A, Siu G K H, Pasipanodya J G, Rodrigues C, Clark T G, Sirgel F A, Esmail A, Lin H-H, Atre S R, Schaaf H S, Chang K C, Lange C, Nahid P, Udwadia Z F, Horsburgh C R, Churchyard G J, Menzies D, Hesseling A C, Nuermberger E, McIlleron H, Fennelly K P, Goemaere E, Jaramillo E, Low M, Jara C M, Padayatchi N and Warren R M (2017) The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. *The Lancet Respiratory Medicine* 5(4): 291-360.

Diefenbach-Elstob T, Plummer D, Dowi R, Wamagi S, Gula B, Siwaeya K, Pelowa D, Siba P and Warner J (2017) The social determinants of tuberculosis treatment adherence in a remote region of Papua New Guinea. *BMC Public Health* 17: 70.

Diefenbach-Elstob T R, Graves P M, Burgess G W, Pelowa D B and Warner J M (2015) Seroepidemiology of melioidosis in children from a remote region of Papua New Guinea. *International Health* 7(5): 332-338.

Dimitrova B, Balabanova D, Atun R, Brobniewski F, Levicheva V and Coker R (2006) Health service providers' perceptions of barriers to tuberculosis care in Russia. *Health Policy and Planning* 21(4): 265-274.

Division of Tuberculosis Elimination (2016) *Interferon-gamma release assays (IGRAs) - blood tests for TB infection,* Centers for Disease Control and Prevention, Atlanta (USA), viewed 11 January 2018, <https://www.cdc.gov/tb/publications/factsheets/testing/igra.htm>.

Drabe C H, Vestergaard L S, Helleberg M, Nyagonde N, Rose M V, Francis F, Theilgaard O P, Asbjørn J, Amos B, Bygbjerg I C, Ruhwald M and Ravn P (2016) Performance of interferon-gamma and IP-10 release assays for diagnosing latent tuberculosis infections in patients with concurrent malaria in Tanzania. *The American Journal of Tropical Medicine and Hygiene* 94(4): 728-735.

Dundon A (2010) AIDS and 'building a wall' around Christian country in rural Papua New Guinea. *The Australian Journal of Anthropology* 21(2): 171-187.

Dye C, Scheele S, Dolin P, Pathania V, Raviglione M C and the WHO Global Surveillance and Monitoring Project (1999) Global burden of tuberculosis: estimated incidence, prevalence, and mortality by country. *The Journal of the American Medical Association* 282(7): 677-686.

Eccles G (2016) 'Papua New Guinea's tuberculosis pandemic', *The Diplomat*, 28 March 2016, viewed 2 August 2018, <https://thediplomat.com/2016/03/papua-new-guineas-tuberculosis-pandemic/>.

Egwaga S, Mkopi A, Range N, Haag-Arbenz V, Baraka A, Grewal P, Cobelens F, Mshinda H, Lwilla F and van Leth F (2009) Patient-centred tuberculosis treatment delivery under programmatic conditions in Tanzania: a cohort study. *BMC Medicine* 7: 80.

El-Sokkary R H, Abu-Taleb A M, El-Seifi O S, Zidan H E, Mortada E M, El-Hossary D and Farag S E (2015) Assessing the prevalence of latent tuberculosis among health care providers in Zagazig City, Egypt using tuberculin skin test and QuantiFERON-TB Gold In-Tube test. *Central European Journal of Public Health* 23(4): 324-330.

Elias D, Mengistu G, Akuffo H and Britton S (2006) Are intestinal helminths risk factors for developing active tuberculosis? *Tropical Medicine & International Health* 11(4): 551-558.

Elliott A M, Namaambo K, Allen B W, Luo N, Hayes R J, Pobee J O and McAdam K P (1993) Negative sputum smear results in HIV-positive patients with pulmonary tuberculosis in Lusaka, Zambia. *Tubercle and Lung Disease* 74(3): 191-194.

Engel N, Davids M, Blankvoort N, Pai N P, Dheda K and Pai M (2015) Compounding diagnostic delays: a qualitative study of point-of-care testing in South Africa. *Tropical Medicine & International Health* 20(4): 493-500.

Fader T, Parks J, Khan N U, Manning R, Stokes S and Nasir N A (2010) Extrapulmonary tuberculosis in Kabul, Afghanistan: a hospital-based retrospective review. *International Journal of Infectious Diseases* 14(2): e102-e110.

Feng Y, Diao N, Shao L, Wu J, Zhang S, Jin J, Wang F, Weng X, Zhang Y and Zhang W (2012) Interferon-gamma release assay performance in pulmonary and extrapulmonary tuberculosis. *PLoS ONE* 7(3): e32652.

Figueroa-Munoz J, Palmer K, Dal Poz M R, Blanc L, Bergström K and Raviglione M (2005) The health workforce crisis in TB control: a report from high-burden countries. *Human Resources for Health* 3: 2.

Figueroa-Munoz J I and Ramon-Pardo P (2008) Tuberculosis control in vulnerable groups. *Bulletin of the World Health Organization* 86(9): 733-735.

Floyd K, Glaziou P, Houben R M G J, Sumner T, White R G and Raviglione M (2018) Global tuberculosis targets and milestones set for 2016–2035: definition and rationale. *The International Journal of Tuberculosis and Lung Disease* 22(7): 723-730.

Forrellad M A, Klepp L I, Gioffré A, Sabio y García J, Morbidoni H R, de la Paz Santangelo M, Cataldi A A and Bigi F (2013) Virulence factors of the *Mycobacterium tuberculosis* complex. *Virulence* 4(1): 1-64.

Forssbohm M, Zwahlen M, Loddenkemper R and Rieder H L (2008) Demographic characteristics of patients with extrapulmonary tuberculosis in Germany. *European Respiratory Journal* 31(1): 99.

Foster N, Vassall A, Cleary S, Cunnama L, Churchyard G and Sinanovic E (2015) The economic burden of TB diagnosis and treatment in South Africa. *Social Science & Medicine* 130: 42-50.

Fox W, Ellard G A and Mitchison D A (1999) Studies on the treatment of tuberculosis undertaken by the British Medical Research Council Tuberculosis Units, 1946–1986, with relevant subsequent publications. *The International Journal of Tuberculosis and Lung Disease* 3(10): S231-S279.

Francis J R, Manchikanti P, Blyth C C, Denholm J, Lowbridge C, Coulter C, Donnan E, Stapledon R, Krause V L and Waring J (2018) Multidrug-resistant tuberculosis in Australia, 1998-2012. *The International Journal of Tuberculosis and Lung Disease* 22(3): 294-299.

Furin J and Cox H (2016) Outbreak of multidrug-resistant tuberculosis on Daru Island. *The Lancet Respiratory Medicine* 4(5): 347-349.

Gaddey H L and Riegel A M (2016) Unexplained lymphadenopathy: evaluation and differential diagnosis *American Family Physician* 94(11): 896-903.

Gagneux S, DeRiemer K, Van T, Kato-Maeda M, de Jong B C, Narayanan S, Nicol M, Niemann S, Kremer K, Gutierrez M C, Hilty M, Hopewell P C and Small P M (2006) Variable host-pathogen compatibility in *Mycobacterium tuberculosis*. *Proceedings of the National Academy of Sciences* 103(8): 2869-2873.

Gagneux S and Small P M (2007) Global phylogeography of *Mycobacterium tuberculosis* and implications for tuberculosis product development. *The Lancet Infectious Diseases* 7(5): 328-337.

García-Rodríguez J F, Álvarez-Díaz H, Lorenzo-García M V, Mariño-Callejo A, Fernández-Rial Á and Sesma-Sánchez P (2011) Extrapulmonary tuberculosis: epidemiology and risk factors. *Enfermedades Infecciosas y Microbiología Clínica* 29(7): 502-509.

Gebreegziabher S B, Bjune G A and Yimer S A (2016) Total delay is associated with unfavorable treatment outcome among pulmonary tuberculosis patients in West Gojjam Zone, northwest Ethiopia: a prospective cohort study. *PLoS ONE* 11(7): e0159579.

Gebremariam M K, Bjune G A and Frich J C (2010) Barriers and facilitators of adherence to TB treatment in patients on concomitant TB and HIV treatment: a qualitative study. *BMC Public Health* 10: 651.

Gebrezgabiher G, Romha G, Ejeta E, Asebe G, Zemene E and Ameni G (2016) Treatment outcome of tuberculosis patients under directly observed treatment short course and factors affecting outcome in southern Ethiopia: a five-year retrospective study. *PLoS ONE* 11(2): e0150560.

George P J, Anuradha R, Kumar N P, Sridhar R, Banurekha V V, Nutman T B and Babu S (2014) Helminth infections coincident with active pulmonary tuberculosis inhibit mono- and multifunctional CD4+ and CD8+ T cell responses in a process dependent on IL-10. *PLoS Pathogens* 10(9): e1004375.

Gilpin C M, Simpson G, Vincent S, O'Brien T P, Knight T A, Globan M, Coulter C and Konstantinos A (2008) Evidence of primary transmission of multidrug-resistant tuberculosis in the Western Province of Papua New Guinea. *Medical Journal of Australia* 188(3): 148-152.

Godfrey-Faussett P, Kaunda H, Kamanga J, van Beers S, van Cleeff M, Kumwenda-Phiri R and Tihon V (2002) Why do patients with a cough delay seeking care at Lusaka urban health centres? A health systems research approach. *The International Journal of Tuberculosis and Lung Disease* 6(9): 796-805.

Golub J E, Bur S, Cronin W A, Gange S, Baruch N, Comstock G W and Chaisson R E (2006) Delayed tuberculosis diagnosis and tuberculosis transmission. *The International Journal of Tuberculosis and Lung Disease* 10(1): 24-30.

Gomes T, Reis-Santos B, Bertolde A, Johnson J L, Riley L W and Maciel E L (2014) Epidemiology of extrapulmonary tuberculosis in Brazil: a hierarchical model. *BMC Infectious Diseases* 14(9).

Gonzalez O Y, Adams G, Teeter L D, Bui T T, Musser J M and Graviss E A (2003) Extra-pulmonary manifestations in a large metropolitan area with a low incidence of tuberculosis. *The International Journal of Tuberculosis and Lung Disease* 7(12): 1178-1185.

Goris J M, Zomerdijk N and Temple V J (2017) Nutritional status and dietary diversity of Kamea in Gulf Province, Papua New Guinea. *Asia Pacific Journal of Clinical Nutrition* 26(4): 665-670.

Gothi D and Joshi J M (2004) Clinical and laboratory observations of tuberculosis at a Mumbai (India) clinic. *Postgraduate Medical Journal* 80: 97-100.

Gounden S, Perumal R and Magula N P (2018) Extrapulmonary tuberculosis in the setting of HIV hyperendemicity at a tertiary hospital in Durban, South Africa. *Southern African Journal of Infectious Diseases* 33(3): 57-64.

Government of Papua New Guinea (2011a) *National health service standards for Papua New Guinea 2011-2020: volume 1*, National Department of Health, Port Moresby (Papua New Guinea), viewed 5 March 2018, <https://www.mindbank.info/item/1670>.

Government of Papua New Guinea (2011b) *National health service standards for Papua New Guinea 2011-2020: volume 2*, National Department of Health, Port Moresby (Papua New Guinea), viewed 8 March 2018, <https://www.mindbank.info/item/1670>.

Grandjean L, Crossa A, Gilman R H, Herrera C, Bonilla C, Jave O, Cabrera J L, Martin L, Escombe A R and Moore D A J (2011) Tuberculosis in household contacts of multidrug-resistant tuberculosis patients. *The International Journal of Tuberculosis and Lung Disease* 15(9): 1164-1169.

GraphPad Software (2018) *Quantify agreement with kappa,* GraphPad Software, La Jolla (USA), viewed 11 March 2018, <https://www.graphpad.com/quickcalcs/kappa1/>.

Grassi M F R, dos Santos N P, Lírio M, Kritski A L, Chagas Almeida M d C, Santana L P, Lázaro N, Dias J, Netto E M and Galvão-Castro B (2016) Tuberculosis incidence in a cohort of individuals infected with human T-lymphotropic virus type 1 (HTLV-1) in Salvador, Brazil. *BMC Infectious Diseases* 16: 491.

Griffith D E, Aksamit T, Brown-Elliott B A, Catanzaro A, Daley C, Gordin F, Holland S M, Horsburgh R, Huitt G, Iademarco M F, Iseman M, Olivier K, Ruoss S, von Reyn C F, Wallace Jr. R J and Winthrop K (2007) An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *American Journal of Respiratory and Critical Care Medicine* 175(4): 367-416.

Guernier V, Diefenbach-Elstob T, Pelowa D, Pollard S, Burgess G, McBryde E S and Warner J (2018) Molecular diagnosis of suspected tuberculosis from archived smear slides from the Balimo region, Papua New Guinea. *International Journal of Infectious Diseases* 67: 75-81.

Han J, Zhu L, Kulldorff M, Hostovich S, Stinchcomb D G, Tatalovich Z, Lewis D R and Feuer E J (2016) Using Gini coefficient to determining optimal cluster reporting sizes for spatial scan statistics. *International Journal of Health Geographics* 15: 27.

Hang N T L, Lien L T, Kobayashi N, Shimbo T, Sakurada S, Thuong P H, Hong L T, Tam D B, Hijikata M, Matsushita I, Hung N V, Higuchi K, Harada N and Keicho N (2011) Analysis of factors lowering sensitivity of interferon-γ release assay for tuberculosis. *PLoS ONE* 6(8): e23806.

Hasan M R, Tan R, Al-Rawahi G N, Thomas E and Tilley P (2012) Short-term stability of pathogen-specific nucleic acid targets in clinical samples. *Journal of Clinical Microbiology* 50(12): 4147-4150.

Haverkamp M H, Arend S M, Lindeboom J A, Hartwig N G and van Dissel J T (2004) Nontuberculous mycobacterial infection in children: a 2-year prospective surveillance study in the Netherlands. *Clinical Infectious Diseases* 39(4): 450-456.

Heep M, Brandstätter B, Rieger U, Lehn N, Richter E, Rüsch-Gerdes S and Niemann S (2001) Frequency of *rpoB* mutations inside and outside the cluster I region in rifampin-resistant clinical *Mycobacterium tuberculosis* isolates. *Journal of Clinical Microbiology* 39(1): 107-110.

Helb D, Jones M, Story E, Boehme C, Wallace E, Ho K, Kop J, Owens M R, Rodgers R, Banada P, Safi H, Blakemore R, Lan N T N, Jones-López E C, Levi M, Burday M, Ayakaka I, Mugerwa R D, McMillan B, Winn-Deen E, Christel L, Dailey P, Perkins M D, Persing D H and Alland D (2010) Rapid detection of *Mycobacterium tuberculosis* and rifampin resistance by use of on-demand, near-patient technology. *Journal of Clinical Microbiology* 48(1): 229-237.

Herath S and Lewis C (2014) Pulmonary involvement in patients presenting with extra-pulmonary tuberculosis: thinking beyond a normal chest x-ray. *Journal of Primary Health Care* 6(1): 64-68.

Hernández-Garduño E, Cook V, Kunimoto D, Elwood R K, Black W A and FitzGerald J M (2004) Transmission of tuberculosis from smear negative patients: a molecular epidemiology study. *Thorax* 59(4): 286.

Hershberg R, Lipatov M, Small P M, Sheffer H, Niemann S, Homolka S, Roach J C, Kremer K, Petrov D A, Feldman M W and Gagneux S (2008) High functional diversity in *Mycobacterium tuberculosis* driven by genetic drift and human demography. *PLoS Biology* 6(12): e311.

Hetzel M W, Morris H, Tarongka N, Barnadas C, Pulford J, Makita L, Siba P M and Mueller I (2015) Prevalence of malaria across Papua New Guinea after initial roll-out of insecticide-treated mosquito nets. *Tropical Medicine & International Health* 20(12): 1745-1755.

Hillemann D, Rusch-Gerdes S, Boehme C and Richter E (2011) Rapid molecular detection of extrapulmonary tuberculosis by the automated GeneXpert MTB/RIF system. *Journal of Clinical Microbiology* 49(4): 1202-1205.

Hoffmann H, Avsar K, Göres R, Mavi S C and Hofmann-Thiel S (2016) Equal sensitivity of the new generation QuantiFERON-TB Gold plus in direct comparison with the previous test version QuantiFERON-TB Gold IT. *Clinical Microbiology and Infection* 22(8): 701-703.

Hofland R W, Bossink A W J, Nierkens S, Paardekooper S P A, van den Broek B T A, Lammers J-W J, van Haeften I and Thijsen S F T (2018) QuantiFERON-plus does not discriminate between active and latent tuberculosis. *Infectious Diseases* 50(6): 479-482.

Horne D J, Jones B E, Kamada A, Fukushima K, Winthrop K L, Siegel S A R, Kovacs A, Anthony P, Meekin K A, Bhat S, Kerndt P, Chang A, Koelle D M and Narita M (2018) Multicenter study of QuantiFERON®-TB Gold Plus in patients with active tuberculosis. *The International Journal of Tuberculosis and Lung Disease* 22(6): 617-621.

Horwood P F, Karl S, Mueller I, Jonduo M H, Pavlin B I, Dagina R, Ropa B, Bieb S, Rosewell A, Umezaki M, Siba P M and Greenhill A R (2014) Spatio-temporal epidemiology of the cholera outbreak in Papua New Guinea, 2009-2011. *BMC Infectious Diseases* 14: 449.

Houben R and White R (2015) *Assessing the global burden of LTBI - need and considerations*, World Health Organization, Geneva (Switzerland), viewed 5 April 2018, <http://www.who.int/tb/advisory_bodies/impact_measurement_taskforce/meetings/global_consultation_doc09_ltbi_burden.pdf>.

Houben R M G J and Dodd P J (2016) The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. *PLoS Medicine* 13(10): e1002152.

Huang W-L, Chen H-Y, Kuo Y-M and Jou R (2009) Performance assessment of the GenoType MTBDR*plus* test and DNA sequencing in detection of multidrug-resistant *Mycobacterium tuberculosis*. *Journal of Clinical Microbiology* 47(8): 2520-2524.

Ilgazli A, Boyaci H, Basyigit İ and Yildiz F (2004) Extrapulmonary tuberculosis: clinical and epidemiologic spectrum of 636 cases. *Archives of Medical Research* 35(5): 435-441.

Ingersoll J, Bythwood T, Abdul-Ali D, Wingood G M, Diclemente R J and Caliendo A M (2008) Stability of *Trichomonas vaginalis* DNA in urine specimens. *Journal of Clinical Microbiology* 46(5): 1628-1630.

Ioannidis P, Papaventsis D, Karabela S, Nikolaou S, Panagi M, Raftopoulou E, Konstantinidou E, Marinou I and Kanavaki S (2011) Cepheid GeneXpert MTB/RIF assay for *Mycobacterium tuberculosis* detection and rifampin resistance identification in patients with substantial clinical indications of tuberculosis and smear-negative microscopy results. *Journal of Clinical Microbiology* 49(8): 3068-3070.

IRIN (2010) *MDR-TB an emerging "health emergency",* IRIN Association, Geneva (Switzerland), viewed 2 August 2018, <http://www.irinnews.org/report/91096/papua-new-guinea-mdr-tb-emerging-%E2%80%9Chealth-emergency%E2%80%9D>.

Jackson S, Sleigh A C, Wang G J and Liu X L (2006) Poverty and the economic effects of TB in rural China. *The International Journal of Tuberculosis and Lung Disease* 10(10): 1104-1110.

Jaganath D and Mupere E (2012) Childhood tuberculosis and malnutrition. *The Journal of Infectious Diseases* 206(12): 1809-1815.

Jamtsho T, Harries A D, Malhotra S, Wangchuk D, Dophu U, Dorji T and Dendup T (2013) The burden and treatment outcomes of extra-pulmonary tuberculosis in Bhutan. *Public Health Action* 3(1): 38-42.

Jennifery (2012) *Along the Bamu River... stories of new mums,* YWAM Medical Ships, Townsville (Australia), viewed 20 June 2017, <https://ywamships.org.au/along-the-bamu-river-stories-of-new-mums/>.

Kanabus A (2016) *Totally drug resistant TB - or extremely, XXDR, TDR,* Global Health Education, West Sussex (United Kingdom), viewed 1 November 2016, <http://www.tbfacts.org/xxdr-tb/>.

Karat A S, Omar T, von Gottberg A, Tlali M, Chihota V N, Churchyard G J, Fielding K L, Johnson S, Martinson N A, McCarthy K, Wolter N, Wong E B, Charalambous S and Grant A D (2016) Autopsy prevalence of tuberculosis and other potentially treatable infections among adults with advanced HIV enrolled in out-patient care in South Africa. *PLoS ONE* 11(11): e0166158.

Karki B, Kittel G, Bolokon I and Duke T (2017) Active community-based case finding for tuberculosis with limited resources: estimating prevalence in a remote area of Papua New Guinea. *Asia Pacific Journal of Public Health* 29(1): 17-27.

Kasa Tom S, Welch H, Kilalang C, Tefuarani N, Vince J, Lavu E, Johnson K, Magaye R and Duke T (2018) Evaluation of Xpert MTB/RIF assay in children with presumed pulmonary tuberculosis in Papua New Guinea. *Paediatrics and International Child Health* 38(2): 97-105.

Kase P, Dakulala P and Bieb S (2016) Outbreak of multidrug-resistant tuberculosis on Daru Island: an update. *The Lancet Respiratory Medicine* 4: e40.

Katsenos S, Nikolopoulou M, Konstantinidis A K, Gartzonika C, Gogali A, Margelis I, Tatsioni A, Mavridis A, Constantopoulos S H and Daskalopoulos G (2010) Interferon-gamma release assay clarifies the effect of bacille Calmette-Guérin vaccination in Greek army recruits. *The International Journal of Tuberculosis and Lung Disease* 14(5): 545-550.

Kaufmann S H E (2001) How can immunology contribute to the control of tuberculosis? *Nature Reviews Immunology* 1: 20.

Kazura J W, Saxinger W C, Wenger J, Forsyth K, Lederman M M, Gillespie J A, Carpenter C C and Alpers M A (1987) Epidemiology of human T cell leukemia virus type I infection in East Sepik Province, Papua New Guinea. *The Journal of Infectious Diseases* 155(6): 1100-1107.

Kennedy M (ca.1978) *Standard management of tuberculosis and leprosy in Papua New Guinea*. Tuberculosis/Leprosy Control Section, Division of Health Improvement, Health Department, Konedobu (Papua New Guinea).

Kent P T and Kubica G P (1985) *Public health mycobacteriology: a guide for the level III laboratory*, U.S. Department of Health and Human Services - Centers for Disease Control, Atlanta (USA), viewed 28 March 2018, <https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB86216546.xhtml>.

Keshavjee S and Farmer P E (2012) Tuberculosis, drug resistance, and the history of modern medicine. *New England Journal of Medicine* 367(10): 931-936.

Ketema K H, Raya J, Workineh T, Klinkenberg E and Enquselassie F (2014) Does decentralisation of tuberculosis care influence treatment outcomes? The case of Oromia Region, Ethiopia. *Public Health Action* 4(Suppl 3): S13-S17.

Khan M S, Khan M S, Sismanidis C and Godfrey-Faussett P (2012) Factors influencing sex differences in numbers of tuberculosis suspects at diagnostic centres in Pakistan. *The International Journal of Tuberculosis and Lung Disease* 16(2): 172-177.

Khan M S, Hasan R and Godfrey-Faussett P (2013) Unusual sex differences in tuberculosis notifications across Pakistan and the role of environmental factors. *Eastern Mediterranean Health Journal* 19(9): 821-825.

Khan W M, Smith H, Qadeer E and Hassounah S (2016) Knowledge and perceptions of national and provincial tuberculosis control programme managers in Pakistan about the WHO Stop TB strategy: a qualitative study. *Journal of the Royal Society of Medicine Open* 8(1): 1-9.

Kim C H, Lim J K, Yoo S S, Lee S Y, Cha S I, Park J Y and Lee J (2014) Diagnostic performance of the QuantiFERON-TB Gold In-Tube assay and factors associated with nonpositive results in patients with miliary tuberculosis. *Clinical Infectious Diseases* 58(7): 986-989.

Kim Y K, Uh Y, Lee N S, Cho M Y, Eom M and Kim H Y (2011) Whole-blood interferon-gamma release assay for diagnosis of tuberculous lymphadenitis. *The Tohoku Journal of Experimental Medicine* 224(3): 189-193.

King S E and Mascie-Taylor C G N (2004) *Strongyloides fuelleborni kellyi* and other intestinal helminths in children from Papua New Guinea: associations with nutritional status and socioeconomic factors. *Papua New Guinea Medical Journal* 47(3-4): 181-191.

Kipp A M, Stout J E, Hamilton C D and Van Rie A (2008) Extrapulmonary tuberculosis, human immunodeficiency virus, and foreign birth in North Carolina, 1993 – 2006. *BMC Public Health* 8: 107.

Knechel N A (2009) Tuberculosis: pathophysiology, clinical features, and diagnosis. *Critical Care Nurse* 29: 34-43.

Kobashi Y, Abe M, Mouri K, Obase Y, Miyashita N and Oka M (2012) Usefulness of tuberculin skin test and three interferon-gamma release assays for the differential diagnosis of pulmonary tuberculosis. *Internal Medicine* 51: 1199-1205.

Koh W J, Kwon O J, Yu C M, Jeon K M, Suh G Y, Chung M P, Kim H J, Han S W, Park S Y and Lee N Y (2003) Recovery rate of nontuberculous mycobacteria from acid-fast-bacilli smear-positive sputum specimens. *Tuberculosis and Respiratory Diseases* 54(1): 22-32.

Kolappan C and Subramani R (2009) Association between biomass fuel and pulmonary tuberculosis: a nested case–control study. *Thorax* 64(8): 705-708.

Kruijshaar M E and Abubakar I (2009) Increase in extrapulmonary tuberculosis in England and Wales 1999–2006. *Thorax* 64(12): 1090.

Kulldorff M (1997) A spatial scan statistic. *Communication in Statistics - Theory and Methods* 26(6): 1481-1496.

Kulldorff M and Information Management Services, Inc., (2009) *SaTScan™ v8.0: software for the spatial and space-time scan statistics*, SaTScan™, Boston (USA), viewed 4 July 2017, <http://www.satscan.org/>.

Kulldorff M (2015) *SaTScan™ user guide for version 9.4*, SaTScan™, Boston (USA), viewed 10 May 2018, <http://www.satscan.org>.

Kumar S, Shankaregowda S, Choudhary G and Singla K (2014) Rare presentation of genitourinary tuberculosis masquerading as renal cell carcinoma: a histopathological surprise. *Journal of Clinical Imaging Science* 4: 26.

Kwon Y-S, Kim Y H, Jeon K, Jeong B-H, Ryu Y J, Choi J C, Kim H C and Koh W-J (2015) Factors that predict negative results of QuantiFERON-TB Gold In-Tube test in patients with culture-confirmed tuberculosis: a multicenter retrospective cohort study. *PLoS ONE* 10(6): e0129792.

Lai C-C, Tan C-K, Lin S-H, Liao C-H, Huang Y-T and Hsueh P-R (2011) Diagnostic performance of whole-blood interferon-γ assay and enzyme-linked immunospot assay for active tuberculosis. *Diagnostic Microbiology and Infectious Disease* 71(2): 139-143.

Lal H, Jain V K and Kannan S (2013) Tuberculosis of the pubic symphysis: four unusual cases and literature review. *Clinical Orthopaedics and Related Research®* 471(10): 3372-3380.

LaMorte W W (2006) *Epidemiology/biostatistics tools,* Boston University School of Public Health, Boston (USA), viewed 29 August 2017, <http://sphweb.bumc.bu.edu/otlt/mph-modules/ep/ep713_randomerror/Epi_Tools.xlsx>.

Lawn S D, Afful B and Acheampong J W (1998) Pulmonary tuberculosis: diagnostic delay in Ghanaian adults. *The International Journal of Tuberculosis and Lung Disease* 2(8): 635-640.

Lawn S D and Zumla A I (2011) Tuberculosis. *The Lancet* 378: 57-72.

Lee J Y (2015) Diagnosis and treatment of extrapulmonary tuberculosis. *Tuberculosis and Respiratory Diseases* 78(2): 47-55.

Lepowsky M (1990) Sorcery and penicillin: treating illness on a Papua New Guinea island. *Social Science & Medicine* 30(10): 1049-1063.

Levy M H, Dakulala P, Koiri J B, Stewart G and Krause V (1998) Tuberculosis control in Papua New Guinea. *Papua New Guinea Medical Journal* 41(2): 72-76.

Lewis M P (2009) *Ethnologue: languages of the world - languages of Papua New Guinea*, 16th edn, SIL International, Dallas (USA), viewed 12 January 2017, <http://www.ethnologue.com/16/show_country/PG/>.

Ley S, Carter R, Millan K, Phuanukoonnon S, Pandey S, Coulter C, Siba P and Beck H-P (2015) Non-tuberculous mycobacteria: baseline data from three sites in Papua New Guinea, 2010–2012. *Western Pacific Surveillance and Response Journal* 6(4): 24-29.

Ley S D, Harino P, Vanuga K, Kamus R, Carter R, Coulter C, Pandey S, Feldmann J, Ballif M, Siba P M, Phuanukoonnon S, Gagneux S and Beck H-P (2014a) Diversity of *Mycobacterium tuberculosis* and drug resistance in different provinces of Papua New Guinea. *BMC Microbiology* 14: 307.

Ley S D, Riley I and Beck H-P (2014b) Tuberculosis in Papua New Guinea: from yesterday until today. *Microbes and Infection* 16(8): 607-614.

Lira L A S, Santos F C F, Carvalho M S Z, Montenegro R A, Lima J F C, Schindler H C and Montenegro L M L (2013) Evaluation of a IS6110-Taqman real-time PCR assay to detect *Mycobacterium tuberculosis* in sputum samples of patients with pulmonary TB. *Journal of Applied Microbiology* 114(4): 1103-1108.

Long Q, Li Y, Wang Y, Yue Y, Tang C, Tang S, Squire S B and Tolhurst R (2008) Barriers to accessing TB diagnosis for rural-to-urban migrants with chronic cough in Chongqing, China: a mixed methods study. *BMC Health Services Research* 8: 202.

Lorent N, Choun K, Malhotra S, Koeut P, Thai S, Khun K E, Colebunders R and Lynen L (2015) Challenges from tuberculosis diagnosis to care in community-based active case finding among the urban poor in Cambodia: a mixed-methods study. *PLoS ONE* 10(7): e0130179.

Lucas M, Nicol P, McKinnon E, Whidborne R, Lucas A, Thambiran A, Burgner D, Waring J and French M (2010) A prospective large-scale study of methods for the detection of latent *Mycobacterium tuberculosis* infection in refugee children. *Thorax* 65(5): 442.

Luke L, Phuanukoonnon S, Suarkia D, Pangoa H, Gilpin C, Coulter C, McCarthy J, Usurup J and Siba P (2008) Distribution of pulmonary tuberculosis and TB drug resistance in a hospital setting in Papua New Guinea. *The International Journal of Infectious Diseases* 12: e336.

Macfarlane J (2009) Common themes in the literature on traditional medicine in Papua New Guinea. *Papua New Guinea Medical Journal* 52(1-2): 44-53.

Macfarlane J E and Alpers M P (2009) Treatment-seeking behaviour among the Nasioi people of Bougainville: choosing between traditional and western medicine. *Ethnicity & Health* 14(2): 147-168.

Mackenzie J S, Broom A K, Hall R A, Johansen C A, Lindsay M D, Phillips D A, Ritchie S A, Russell R C and Smith D W (1998) Arboviruses in the Australian region, 1990 to 1998. *Communicable Diseases Intelligence* 22(6): 93-100.

MacPherson P, Houben R M G J, Glynn J R, Corbett E L and Kranzer K (2014) Pre-treatment loss to follow-up in tuberculosis patients in low- and lower-middle-income countries and high-burden countries. *Bulletin of the World Health Organization* 92(2): 126-138.

Maddocks I, Anders E M and Dennis E (1976) Donovanosis in Papua New Guinea. *The British Journal of Venereal Diseases* 52: 190-196.

Maharjan B, Shrestha B, Weirich A, Stewart A and Kelly-Cirino C D (2016) A novel sputum transport solution eliminates cold chain and supports routine tuberculosis testing in Nepal. *Journal of Epidemiology and Global Health* 6(4): 257-265.

Makhani L, Dennis K, Nguyen J, Jon F and Barnes E (2013) Extrapulmonary tuberculosis mimicking widespread metastatic disease: a case report. *Journal of Pain Management* 6(3): 253-256.

Makwakwa L, Sheu M-l, Chiang C-Y, Lin S-L and Chang P W (2014) Patient and health system delays in the diagnosis and treatment of new and retreatment pulmonary tuberculosis cases in Malawi. *BMC Infectious Diseases* 14: 132.

Malotle M M, Spiegel J M, Yassi A, Ngubeni D, O'Hara L M, Adu P A, Bryce E A, Mlangeni N, Gemell G S M and Zungu M (2017) Occupational tuberculosis in South Africa: are health care workers adequately protected? *Public Health Action* 7(4): 258-267.

Manson A L, Cohen K A, Abeel T, Desjardins C A, Armstrong D T, Barry III C E, Brand J, TBResist Global Genome Consortium, Chapman S B, Cho S-N, Gabrielian A, Gomez J, Jodals A M, Joloba M, Jureen P, Lee J S, Malinga L, Maiga M, Nordenberg D, Noroc E, Romancenco E, Salazar A, Ssengooba W, Velayati A A, Winglee K, Zalutskaya A, Via L E, Cassell G H, Dorman S E, Ellner J, Farnia P, Galagan J E, Rosenthal A, Crudu V, Homorodean D, Hsueh P-R, Narayanan S, Pym A S, Skrahina A, Swaminathan S, Van der Walt M, Alland D, Bishai W R, Cohen T, Hoffner S, Birren B W and Earl A M (2017) Genomic analysis of globally diverse *Mycobacterium tuberculosis* strains provides insights into the emergence and spread of multidrug resistance. *Nature Genetics* 49(3): 395-402.

Marais B J, Obihara C C, Warren R M, Schaaf H S, Gie R P and Donald P R (2005) The burden of childhood tuberculosis: a public health perspective. *The International Journal of Tuberculosis and Lung Disease* 9(12): 1305-1313.

Marais B J (2014) Tuberculosis in children. *Journal of Paediatrics and Child Health* 50(10): 759-767.

Marinho J, Galvão-Castro B, Rodrigues L C and Barreto M L (2005) Increased risk of tuberculosis with human T-lymphotropic virus-1 infection: a case-control study. *Journal of Acquired Immune Deficiency Syndromes* 40(5): 625-628.

Marouane C, Smaoui S, Kammoun S, Slim L and Messadi-Akrout F (2016) Evaluation of molecular detection of extrapulmonary tuberculosis and resistance to rifampicin with GeneXpert® MTB/RIF. *Medecine et Maladies Infectieuses* 46(1): 20-24.

Mason P H, Roy A, Spillane J and Singh P (2016) Social, historical and cultural dimensions of tuberculosis. *Journal of Biosocial Science* 48(2): 206-232.

Mason P H, Snow K, Asugeni R, Massey P D and Viney K (2017) Tuberculosis and gender in the Asia-Pacific region. *Australian and New Zealand Journal of Public Health* 41(3): 227-229.

Massey P D, Viney K, Kienene T, Tagaro M, Itogo N, Ituaso-Conway N and Durrheim D N (2011) Ten years on: highlights and challenges of directly observed treatment short-course as the recommended TB control strategy in four Pacific island nations. *Journal of Rural and Tropical Public Health* 10: 44-47.

Massey P D, Wakageni J, Kekeubata E, Maena'adi J, Laete'esafi J, Waneagea J, Fangaria G, Jimuru C, Houaimane M, Talana J, MacLaren D and Speare R (2012) TB questions, East Kwaio answers: community-based participatory research in a remote area of Solomon Islands. *Rural and Remote Health* 12: 2139.

Massey P D, Asugeni R, Wakageni J, Kekeubata E, Maena'aadi J, Laete'esafi J, Waneagea J, Harrington H, Fangaria G, MacLaren D and Speare R (2013) Progress towards TB control in East Kwaio, Solomon Islands. *Rural and Remote Health* 13: 2555.

Mavila R, Kottarath M, Nair S and Thaha M M (2015) Site predilection of extrapulmonary tuberculosis: study from a tertiary care centre. *International Journal of Research in Medical Sciences* 3(11): 3386-3390.

Maynard-Smith L, Larke N, Peters J A and Lawn S D (2014) Diagnostic accuracy of the Xpert MTB/RIF assay for extrapulmonary and pulmonary tuberculosis when testing non-respiratory samples: a systematic review. *BMC Infectious Diseases* 14: 709.

McBryde E (2012) *Evaluation of risks of tuberculosis in Western Province Papua New Guinea*, Department of Foreign Affairs and Trade, Barton (Australia), viewed 31 October 2017, <https://www.burnet.edu.au/system/publication/file/3606/2012_Evaluation_of_Risks_of_Tuberculosis_in_Western_Province_PNG.pdf>.

McCabe W R and Lorian V (1968) Comparison of the antibacterial activity of rifampicin and other antibiotics. *American Journal of Medical Sciences* 256(4): 255-265.

MedCalc Software (2018) *MedCalc's diagnostic test evaluation calculator,* MedCalc Software, Ostend (Belgium), viewed 27 April 2018, <https://www.medcalc.org/calc/diagnostic_test.php>.

Mehra D, Kaushik R M, Kaushik R, Rawat J and Kakkar R (2013) Initial default among sputum-positive pulmonary TB patients at a referral hospital in Uttarakhand, India. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 107(9): 558-565.

Memish Z A, Bamgboye E A, Abuljadayel N, Smadi H, Abouzeid M S and Hakeem R F A (2014) Incidence of and risk factors associated with pulmonary and extra-pulmonary tuberculosis in Saudi Arabia (2010-2011). *PLoS ONE* 9(5): e95654.

Mesfin M M, Newell J N, Walley J D, Gessessew A and Madeley R J (2009) Delayed consultation among pulmonary tuberculosis patients: a cross sectional study of 10 DOTS districts of Ethiopia. *BMC Public Health* 9: 53.

Metcalfe J Z, Cattamanchi A, McCulloch C E, Lew J D, Ha N P and Graviss E A (2013) Test variability of the QuantiFERON-TB gold in-tube assay in clinical practice. *American Journal of Respiratory and Critical Care Medicine* 187(2): 206-211.

Migliori G B, Matteelli A, Cirillo D and Pai M (2008) Diagnosis of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis: current standards and challenges. *Canadian Journal of Infectious Diseases and Medical Microbiology* 19(2): 169-172.

Mitchison D A (1998) How drug resistance emerges as a result of poor compliance during short course chemotherapy for tuberculosis. *The International Journal of Tuberculosis and Lung Disease* 2(1): 10-15.

Mochizuki M, Watanabe T, Yamaguchi K, Tajima K, Yoshimura K, Nakashima S, Shirao M, Araki S, Miyata N, Mori S and Takatsuki K (1992) Uveitis associated with human T lymphotropic virus type I: seroepidemiologic, clinical, and virologic studies. *The Journal of Infectious Diseases* 166(4): 943-944.

Moore A R, Rogers F, Dietrick D and Smith S (2008) Extrapulmonary tuberculosis in pregnancy masquerading as a degenerating leiomyoma. *Obstetrics & Gynecology* 111(2, Part 2): 550-552.

Morita A, Natsuhara K, Tomitsuka E, Odani S, Baba J, Tadokoro K, Igai K, Greenhill A R, Horwood P F, Soli K W, Phuanukoonnon S, Siba P M and Umezaki M (2015) Development, validation, and use of a semi-quantitative food frequency questionnaire for assessing protein intake in Papua New Guinean Highlanders. *American Journal of Human Biology* 27: 349-357.

Morris I P and Somanathan A (2011) *PNG health workforce crisis: a call to action*, The World Bank, Washington, D.C. (USA), viewed 3 August 2018, <http://documents.worldbank.org/curated/en/216511468332461651/Papua-New-Guinea-PNG-health-workforce-crisis-a-call-to-action>.

Muricy E C M, Lemes R A, Bombarda S, Ferrazoli L and Chimara E (2014) Differentiation between *Nocardia* spp. and *Mycobacterium* spp.: critical aspects for bacteriological diagnosis. *Revista do Instituto de Medicina Tropical de São Paulo* 56(5): 397-401.

Murray J F, Schraufnagel D E and Hopewell P C (2015) Treatment of tuberculosis. A historical perspective. *Annals of the American Thoracic Society* 12(12): 1749-1759.

Muttamba W, Ssengooba W, Sekibira R, Kirenga B, Katamba A and Joloba M (2018) Accuracy of different Xpert MTB/Rif implementation strategies in programmatic settings at the regional referral hospitals in Uganda: evidence for country wide roll out. *PLoS ONE* 13(3): e0194741.

Muyoyeta M, Moyo M, Kasese N, Ndhlovu M, Milimo D, Mwanza W, Kapata N, Schaap A, Godfrey-Faussett P and Ayles H (2015) Implementation research to inform the use of Xpert MTB/RIF in primary health care facilities in high TB and HIV settings in resource constrained settings. *PLoS ONE* 10(6): e0137934.

Naidoo P, Theron G, Rangaka M X, Chihota V N, Vaughan L, Brey Z O and Pillay Y (2017) The South African tuberculosis care cascade: estimated losses and methodological challenges. *The Journal of Infectious Diseases* 216(suppl_7): S702-S713.

Nakiyingi L, Nankabirwa H and Lamorde M (2013) Tuberculosis diagnosis in resource-limited settings: clinical use of GeneXpert in the diagnosis of smear-negative PTB: a case report. *African Health Sciences* 13(2): 522-524.

Namme L, Marie-Solange D, Hugo Bertrand M, Elvis T, Achu J and Christopher K (2013) Extrapulmonary tuberculosis and HIV coinfection in patients treated for tuberculosis at the Douala General Hospital in Cameroon. *Annals of Tropical Medicine and Public Health* 6(1): 100-104.

National, The (2010) 'Authorities respond to cholera on Daru', *The National*, 10 November 2010, viewed 25 October 2017, <http://www.thenational.com.pg/authorities-respond-to-cholera-on-daru/>.

National Department of Health (2010) *The 2009 STI, HIV and AIDS annual surveillance report*, National Department of Health: STI, HIV and AIDS Surveillance Unit, Port Moresby (Papua New Guinea), viewed 30 June 2018, <http://www.aidsdatahub.org/sites/default/files/documents/2009_STI_and_HIV_Annual_Surveillance_Report.pdf>.

National Department of Health (2012) *Standard treatment guidelines for common illness of adults in Papua New Guinea: a manual for nurses, health extension officers and doctors*, 6th edn, National Department of Health, Port Moresby (Papua New Guinea), viewed 14 July 2018, <http://www.adi.org.au/wp-content/uploads/2016/11/Standard-Treatment-Guidelines-for-Common-Illness-of-Adults-in-PNG.pdf>.

National Research Institute (2010) *Papua New Guinea: district and provincial profiles*, National Research Institute, Boroko (Papua New Guinea), viewed 15 July 2015, <https://spmt.files.wordpress.com/2010/09/png-distrist-and-provnical-profiles.pdf>.

National Statistical Office (2013) *Final figures - Papua New Guinea: national population & housing census 2011*, National Statistical Office, Port Moresby (Papua New Guinea), viewed 31 October 2017, <http://www.nso.gov.pg/index.php/document-library?view=download&fileId=65>.

National Statistical Office (2014) *2011 national population & housing census: ward population profile - southern region*, National Statistical Office, Port Moresby (Papua New Guinea), viewed 31 October 2017, <http://www.nso.gov.pg/index.php/document-library?view=download&fileId=64>.

National Statistical Office (2015) *Papua New Guinea: 2011 national report*, National Statistical Office, Waigani (Papua New Guinea), viewed 31 October 2017, <http://sdd.spc.int/en/resources/document-library?view=preview&format=raw&fileId=218>.

Ncayiyana J R, Bassett J, West N, Westreich D, Musenge E, Emch M, Pettifor A, Hanrahan C F, Schwartz S R, Sanne I and van Rie A (2016) Prevalence of latent tuberculosis infection and predictive factors in an urban informal settlement in Johannesburg, South Africa: a cross-sectional study. *BMC Infectious Diseases* 16(661).

Nebenzahl-Guimaraes H, Yimer S A, Holm-Hansen C, de Beer J, Brosch R and van Soolingen D (2016) Genomic characterization of *Mycobacterium tuberculosis* lineage 7 and a proposed name: 'Aethiops vetus'. *Microbial Genomics* 2(6).

Needham D M, Godfrey-Faussett P and Foster S D (1998) Barriers to tuberculosis control in urban Zambia: the economic impact and burden on patients prior to diagnosis. *The International Journal of Tuberculosis and Lung Disease* 2(10): 811-817.

Nerlich A G, Haas C J, Zink A, Szeimies U and Hagedorn H G (1997) Molecular evidence for tuberculosis in an ancient Egyptian mummy. *The Lancet* 350(9088): 1404.

Newell J N, Baral S C, Pande S B, Bam D S and Malla P (2006) Family-member DOTS and community DOTS for tuberculosis control in Nepal: cluster-randomised controlled trial. *The Lancet* 367(9514): 903-909.

Ngabonziza J C S, Ssengooba W, Mutua F, Torrea G, Dushime A, Gasana M, Andre E, Uwamungu S, Nyaruhirira A U, Mwaengo D and Muvunyi C M (2016) Diagnostic performance of smear microscopy and incremental yield of Xpert in detection of pulmonary tuberculosis in Rwanda. *BMC Infectious Diseases* 16: 660.

Nikolova M, Markova R, Drenska R, Muhtarova M, Todorova Y, Dimitrov V, Taskov H, Saltini C and Amicosante M (2013) Antigen-specific CD4- and CD8-positive signatures in different phases of *Mycobacterium tuberculosis* infection. *Diagnostic Microbiology and Infectious Disease* 75(3): 277-281.

Norbis L, Alagna R, Tortoli E, Codecasa L R, Migliori G B and Cirillo D M (2014) Challenges and perspectives in the diagnosis of extrapulmonary tuberculosis. *Expert Review of Anti-Infective Therapy* 12(5): 633-647.

Nyirenda T (2006) Epidemiology of tuberculosis in Malawi. *Malawi Medical Journal* 18(3): 147-159.

Obermeyer Z, Abbott-Klafter J and Murray C J L (2008) Has the DOTS strategy improved case finding or treatment success? An empirical assessment. *PLoS ONE* 3(3): e1721.

Okwaraji Y B, Cousens S, Berhane Y, Mulholland K and Edmond K (2012) Effect of geographical access to health facilities on child mortality in rural Ethiopia: a community based cross sectional study. *PLoS ONE* 7(3): e33564.

Olson S, English R, Claiborne A, Forum on Drug Discovery, Development, and Translation, Institute of Medicine and Russian Academy of Medical Sciences (2011) The second-line drug supply chain. In *The new profile of drug-resistant tuberculosis in Russia: a global and local perspective*. The National Academies Press, Washington, D.C. (USA), pp. 87-94, viewed 12 June 2015, <http://www.ncbi.nlm.nih.gov/books/NBK62469/>.

Omar S V, Peters R P H, Ismail N A, Jonkman K, Dreyer A W, Said H M, Gwala T, Ismail N and Fourie B (2016) Field evaluation of a novel preservation medium to transport sputum specimens for molecular detection of *Mycobacterium tuberculosis* in a rural African setting. *Tropical Medicine & International Health* 21(6): 776-782.

Ongugo K, Hall J and Attia J (2011) Implementing tuberculosis control in Papua New Guinea: a clash of culture and science? *Journal of Community Health* 36: 423-430.

Onifade D A, Bayer A M, Montoya R, Haro M, Alva J, Franco J, Sosa R, Valiente B, Valera E, Ford C M, Acosta C D and Evans C A (2010) Gender-related factors influencing tuberculosis control in shantytowns: a qualitative study. *BMC Public Health* 10: 381.

Opota O, Senn L, Prod'hom G, Mazza-Stalder J, Tissot F, Greub G and Jaton K (2016) Added value of molecular assay Xpert MTB/RIF compared to sputum smear microscopy to assess the risk of tuberculosis transmission in a low-prevalence country. *Clinical Microbiology and Infection* 22(7): 613-619.

Oppong J R, Mayer J and Oren E (2015) The global health threat of African urban slums: the example of urban tuberculosis. *African Geographical Review* 34(2): 182-195.

Orere B (2017) 'Balimo lacks basic govt services', *The Post Courier*, 23 January 2017, viewed 16 June 2018, <https://postcourier.com.pg/balimo-lacks-basic-govt-services/>.

Paediatric Society of Papua New Guinea (2016) *Standard treatment for common illnesses of children in Papua New Guinea: a manual for nurses, community health workers, health extension officers, and doctors*, 10th edn, Paediatric Society of Papua New Guinea, Boroko (Papua New Guinea), viewed 14 July 2018, <http://pngpaediatricsociety.org/wp-content/uploads/2016/11/PNG-Standard-Treatment-Book-10th-edition-2016.pdf>.

Pai M, Denkinger C M, Kik S V, Rangaka M X, Zwerling A, Oxlade O, Metcalfe J Z, Cattamanchi A, Dowdy D W, Dheda K and Banaei N (2014) Gamma interferon release assays for detection of *Mycobacterium tuberculosis* infection. *Clinical Microbiology Reviews* 27(1): 3-20.

Pai M, Behr M A, Dowdy D, Dheda K, Divangahi M, Boehme C C, Ginsberg A, Swaminathan S, Spigelman M, Getahun H, Menzies D and Raviglione M (2016) Tuberculosis. *Nature Reviews Disease Primers* 2: 16076.

Pan L, Jia H, Liu F, Sun H, Gao M, Du F, Xing A, Du B, Sun Q, Wei R, Gu S and Zhang Z (2015) Risk factors for false-negative T-SPOT.TB assay results in patients with pulmonary and extra-pulmonary TB. *The Journal of Infection* 70(4): 367-380.

Pandey S, Lavu E, Congdon J, Moke R, Bainomugisa A and Coulter C (2018) Characterization of *pncA* mutations in multi-drug and pyrazinamide resistant *Mycobacterium tuberculosis* isolates cultured from Queensland migrants and Papua New Guinea residents. *Tuberculosis* 111: 109-113.

Pandit S, Choudhury S, Das A, Das S K and Bhattacharya S (2014) Cervical lymphadenopathy — pitfalls of blind antitubercular treatment. *Journal of Health, Population and Nutrition* 32(1): 155-159.

Papua New Guinea Electoral Commission (2012) *Election commencing 23 June 2012: polling schedule for Middle Fly electorate,* Papua New Guinea Electoral Commission, Port Moresby (Papua New Guinea), viewed 4 March 2018, <https://garamut.files.wordpress.com/2012/06/western_middle-fly-schedule.pdf>.

Papua New Guinea Electoral Commission (2017) *Election commencing 20th April 2017: polling schedule for Middle Fly Open electorate,* Papua New Guinea Electoral Commission, Port Moresby (Papua New Guinea), viewed 4 March 2018, <http://www.pngec.gov.pg/docs/default-source/default-document-library/polling-schedule-v6.pdf?sfvrsn=2>.

Paramasivan C N, Narayana A S L, Prabhakar R, Rajagopal M S, Somasundaram P R and Tripathy S P (1983) Effect of storage of sputum specimens at room temperature on smear and culture results. *Tubercle* 64(2): 119-124.

Parija D, Patra T K, Kumar A M V, Swain B K, Satyanarayana S, Sreenivas A, Chadha V K, Moonan P K and Oeltmann J E (2014) Impact of awareness drives and community-based active tuberculosis case finding in Odisha, India. *The International Journal of Tuberculosis and Lung Disease* 18(9): 1105-1107.

Patel N R, Zhu J, Tachado S D, Zhang J, Wan Z, Saukkonen J and Koziel H (2007) HIV impairs TNF-α mediated macrophage apoptotic response to *Mycobacterium tuberculosis*. *The Journal of Immunology* 179(10): 6973-6980.

Pawlowski A, Jansson M, Sköld M, Rottenberg M E and Källenius G (2012) Tuberculosis and HIV co-infection. *PLoS Pathogens* 8(2): e1002464.

Pehme L, Hollo V, Rahu M and Altraja A (2005) Tuberculosis during fundamental societal changes in Estonia with special reference to extrapulmonary manifestations. *Chest* 127(4): 1289-1295.

Peirse M and Houston A (2017) Extrapulmonary tuberculosis. *Medicine* 45(12): 747-752.

Pérez-Padilla R, Pérez-Guzmán C, Báez-Saldaña R and Torres-Cruz A (2001) Cooking with biomass stoves and tuberculosis: a case control study. *The International Journal of Tuberculosis and Lung Disease* 5(5): 441-447.

Peto H M, Pratt R H, Harrington T A, LoBue P A and Armstrong L R (2009) Epidemiology of extrapulmonary tuberculosis in the United States, 1993-2006. *Clinical Infectious Diseases* 49(9): 1350-1357.

Petroff S A (1915) A new and rapid method for the isolation and cultivation of tubercle bacilli directly from the sputum and feces. *The Journal of Experimental Medicine* 21(1): 38.

Petruccioli E, Vanini V, Chiacchio T, Cuzzi G, Cirillo D M, Palmieri F, Ippolito G and Goletti D (2017) Analytical evaluation of QuantiFERON- Plus and QuantiFERON- Gold In-tube assays in subjects with or without tuberculosis. *Tuberculosis* 106: 38-43.

Pezzoli L, Gounder S, Tamani T, Daulako M R, Underwood F, Mainawalala S, Nawadra-Taylor V, Rafai E and Gillini L (2016) Tuberculosis, Fiji, 2002-2013. *Emerging Infectious Diseases* 22(3): 547-549.

Philipsen R H H M, Sánchez P, Maduskar J, Melendez J, Peters-Bax L, Peter J G, Dawson R, Theron G, Dheda K and van Ginneken B (2015) Automated chest-radiograhy as a triage for Xpert testing in resource-constrained settings: a prospective study of diagnostic accuracy and costs. *Scientific Reports* 5: 12215.

Phuanukoonnon S, Suarkia D L, Luke L N, Mueller I, Usurup J, Carter R, Gilpin C M, Coulter C, McCarthy J and Siba P M (2008) Evidence of primary MDR resistance among tuberculosis cases in Papua New Guinea. *The American Journal of Tropical Medicine and Hygiene* 79(6 Suppl): 207.

Pieterman E D, Liqui Lung F G, Verbon A, Bax H I, Ang C W, Berkhout J, Blaauw G, Brandenburg A, van Burgel N D, Claessen A, van Dijk K, Heron M, Hooghiemstra M, Leussenkamp-Hummelink R, van Lochem E, van Loo I H M, Mulder B, Ott A, Pontesilli O, Reuwer A, Rombouts P, Saegeman V, Scholing M, Vainio S and de Steenwinkel J E M (2018) A multicentre verification study of the QuantiFERON®-TB Gold Plus assay. *Tuberculosis* 108: 136-142.

Pinyopornpanish K, Chaiwarith R, Pantip C, Keawvichit R, Wongworapat K, Khamnoi P, Supparatpinyo K and Sirisanthana T (2015) Comparison of Xpert MTB/RIF assay and the conventional sputum microscopy in detecting *Mycobacterium tuberculosis* in northern Thailand. *Tuberc Res Treat* 2015(571782).

Pokhrel A K, Bates M N, Verma S C, Joshi H S, Sreeramareddy C T and Smith K R (2010) Tuberculosis and indoor biomass and kerosene use in Nepal: a case–control study. *Environmental Health Perspectives* 118(4): 558-564.

Pollett S, Banner P, O'Sullivan M V N and Ralph A P (2016) Epidemiology, diagnosis and management of extra-pulmonary tuberculosis in a low-prevalence country: a four year retrospective study in an Australian tertiary infectious diseases unit. *PLoS ONE* 11(3): e0149372.

Prakasha S R, Suresh G, D'sa I P, Shetty S S and Kumar S G (2013) Mapping the pattern and trends of extrapulmonary tuberculosis. *Journal of Global Infectious Diseases* 5(2): 54-59.

Prasanna T, Jeyashree K, Chinnakali P, Bahurupi Y, Vasudevan K and Das M (2018) Catastrophic costs of tuberculosis care: a mixed methods study from Puducherry, India. *Global Health Action* 11(1): 1477493.

Public Health Laboratory Network (2006) *Tuberculosis laboratory case definition (LCD),* Department of Health, Canberra (Australia), viewed 27 April 2018, <http://www.health.gov.au/internet/main/publishing.nsf/Content/health-central.htm>.

Purohit M and Mustafa T (2015) Laboratory diagnosis of extra-pulmonary tuberculosis (EPTB) in resource-constrained setting: state of the art, challenges and the need. *Journal of Clinical and Diagnostic Research* 9(4): EE01-EE06.

QIAGEN (2016), *QuantiFERON®-TB Gold Plus (QFT®-Plus) ELISA package insert, Doc No. 1083163 Rev. 04, February 2016*, QIAGEN, Hilden (Germany).

Qian X, Nguyen D T, Lyu J, Albers A E, Bi X and Graviss E A (2018) Risk factors for extrapulmonary dissemination of tuberculosis and associated mortality during treatment for extrapulmonary tuberculosis. *Emerging Microbes & Infections* 7(1): 102.

Quest Diagnostics™ (2014) *Clinical education center: QuantiFERON®-TB Gold, (draw site incubated),* Quest Diagnostics, Secaucus (USA), viewed 5 April 2018, <https://education.questdiagnostics.com/faq/QFT>.

Rahayu S R, Katsuyama H, Demura M, Katsuyama M, Ota Y, Tanii H, Higashi T, Semadi N P D and Saijoh K (2015) Factors associated with tuberculosis cases in Semarang District, Indonesia: case-control study performed in the area where case detection rate was extremely low. *Environmental Health and Preventive Medicine* 20(4): 253-261.

Ramaswamy S and Musser J M (1998) Molecular genetic basis of antimicrobial agent resistance in *Mycobacterium tuberulosis*: 1998 update. *Tubercle and Lung Disease* 79(1): 3-29.

Ramaswamy S V, Dou S-J, Rendon A, Yang Z, Cave M D and Graviss E A (2004) Genotypic analysis of multidrug-resistant *Mycobacterium tuberculosis* isolates from Monterrey, Mexico. *Journal of Medical Microbiology* 53(2): 107-113.

Rangaka M X, Cavalcante S C, Marais B J, Thim S, Martinson N A, Swaminathan S and Chaisson R E (2015) Controlling the seedbeds of tuberculosis: diagnosis and treatment of tuberculosis infection. *The Lancet* 386(10010): 2344-2353.

Riedel L, Segal I, Mohamed A E, Hale M and Mannell A (1989) The prolonged course of gastrointestinal tuberculosis. *Journal of Clinical Gastroenterology* 11(6): 671-674.

Riley I (2009) Demography and the epidemiology of disease in Papua New Guinea. *Papua New Guinea Medical Journal* 52(3-4): 83-95.

Robel Y, Lemessa F, Hirpa S, Abraham A and Klinkenberg E (2017) Determinants of delayed care seeking for TB suggestive symptoms in Seru district, Oromiya region, Ethiopia: a community based unmatched case-control study. *BMC Infectious Diseases* 17: 292.

Roberts T, Beyers N, Aguirre A and Walzl G (2007) Immunosuppression during active tuberculosis is characterized by decreased interferon-γ production and CD25 expression with elevated forkhead box P3, transforming growth factor-β, and interleukin-4 mRNA levels. *The Journal of Infectious Diseases* 195(6): 870-878.

Rock R B, Sutherland W M, Baker C and Williams D N (2006) Extrapulmonary tuberculosis among Somalis in Minnesota. *Emerging Infectious Diseases* 12(9): 1434-1436.

Rodriguez-Campos S, Smith N H, Boniotti M B and Aranaz A (2014) Overview and phylogeny of *Mycobacterium tuberculosis* complex organisms: implications for diagnostics and legislation of bovine tuberculosis. *Research in Veterinary Science* 97(Supplement): S5-S19.

Roy A, Eisenhut M, Harris R J, Rodrigues L C, Sridhar S, Habermann S, Snell L, Mangtani P, Adetifa I, Lalvani A and Abubakar I (2014) Effect of BCG vaccination against *Mycobacterium tuberculosis* infection in children: systematic review and meta-analysis. *BMJ* 349(g4643).

Rozot V, Vigano S, Mazza-Stalder J, Idrizi E, Day C L, Perreau M, Lazor-Blanchet C, Petruccioli E, Hanekom W, Goletti D, Bart P A, Nicod L, Pantaleo G and Harari A (2013) *Mycobacterium tuberculosis*-specific CD8+ T cells are functionally and phenotypically different between latent infection and active disease. *European Journal of Immunology* 43(6): 1568-1577.

Rumende C M, Hadi E J, Tanjung G, Saputri I N and Sasongko R (2018) The benefit of interferon-gamma release assay for diagnosis of extrapulmonary tuberculosis. *The Indonesian Journal of Internal Medicine* 50(2): 138-143.

Rusen I D, Harries A D, Heldal E and Macé C (2010) Drug supply shortages in 2010: the inexcusable failure of global tuberculosis control [Editorial]. *The International Journal of Tuberculosis and Lung Disease* 14(3): 253-254.

Ryu Y J (2015) Diagnosis of pulmonary tuberculosis: recent advances and diagnostic algorithms. *Tuberculosis and Respiratory Diseases* 78(2): 64-71.

Sagbakken M, Frich J C and Bjune G (2008) Barriers and enablers in the management of tuberculosis treatment in Addis Ababa, Ethiopia: a qualitative study. *BMC Public Health* 8: 11.

Sagbakken M, Frich J C, Bjune G A and Porter J D H (2013) Ethical aspects of directly observed treatment for tuberculosis: a cross-cultural comparison. *BMC Medical Ethics* 14: 25.

Salim M A H, Uplekar M, Daru P, Aung M, Declercq E and Lönnroth K (2006) Turning liabilities into resources: informal village doctors and tuberculosis control in Bangladesh. *Bulletin of the World Health Organization* 84(6): 479-484.

Samiak L and Emeto T I (2017) Vaccination and nutritional status of children in Karawari, East Sepik Province, Papua New Guinea. *PLoS ONE* 12(11): e0187796.

Sandgren A, Strong M, Muthukrishnan P, Weiner B K, Church G M and Murray M B (2009) Tuberculosis drug resistance mutation database. *PLoS Medicine* 6(2): e1000002.

Sandgren A, Hollo V and van der Werf M J (2013) Extrapulmonary tuberculosis in the European Union and European Economic Area, 2002 to 2011. *Eurosurveillance* 18(12): 20431.

Sarita S N, Auld S C, Brust James C M, Mathema B, Ismail N, Moodley P, Mlisana K, Salim A, Campbell A, Mthiyane T, Morris N, Primrose M, van der Meulen H, Omar S V, Brown T S, Narechania A, Shaskina E, Kapwata T, Kreiswirth B and Gandhi N R (2017) Transmission of extensively drug-resistant tuberculosis in South Africa. *The New England Journal of Medicine* 376(3): 243-253.

Saweri O P M, Hetzel M W, Mueller I, Siba P M and Pulford J (2017) The treatment of non-malarial febrile illness in Papua New Guinea: findings from cross sectional and longitudinal studies of health worker practice. *BMC Health Services Research* 17: 10.

Scarparo C, Ricordi P, Ruggiero G and Piccoli P (2004) Evaluation of the fully automated BACTEC MGIT 960 system for testing susceptibility of *Mycobacterium tuberculosis* to pyrazinamide, streptomycin, isoniazid, rifampin, and ethambutol and comparison with the radiometric BACTEC 460TB method. *Journal of Clinical Microbiology* 42(3): 1109-1114.

Schwartz A (2006) *Diagnostic test calculator,* University of Illinois, Chicago (USA), viewed 25 April 2018, <http://araw.mede.uic.edu/cgi-bin/testcalc.pl>.

Schwartzman K and Menzies D (2000) How long are TB patients infectious? *Canadian Medical Association Journal* 163(2): 157-158.

Seddon J A, Jenkins H E, Liu L, Cohen T, Black R E, Vos T, Becerra M C, Graham S M, Sismanidis C and Dodd P J (2015) Counting children with tuberculosis: why numbers matter. *The International Journal of Tuberculosis and Lung Disease* 19(12): S9-S16.

Segagni Lusignani L, Quaglio G, Atzori A, Nsuka J, Grainger R, Da Conceiçao Palma M, Putoto G and Manenti F (2013) Factors associated with patient and health care system delay in diagnosis for tuberculosis in the province of Luanda, Angola. *BMC Infectious Diseases* 13: 168.

Sehgal M, Rizwan S A and Krishnan A (2014) Disease burden due to biomass cooking-fuel-related household air pollution among women in India. *Global Health Action* 7: 25326.

Seifert M, Catanzaro D, Catanzaro A and Rodwell T C (2015) Genetic mutations associated with isoniazid resistance in *Mycobacterium tuberculosis*: a systematic review. *PLoS ONE* 10(3): e0119628.

Sergeant E S G (2017) *Epitools epidemological calculators,* Ausvet Pty Ltd, Bruce (Australia), viewed 12 June 2017, <http://epitools.ausvet.com.au/>.

Shah P A, Coj M and Rohloff P (2017) Delays in diagnosis and treatment of extrapulmonary tuberculosis in Guatemala. *BMJ Case Reports* 2017.

Shann F, Biddulph J and Vince J (2003) *Paediatrics for doctors in Papua New Guinea: a guide for doctors providing health services for children*, 2nd edn, Papua New Guinea Department of Health, Port Moresby (Papua New Guinea), viewed 14 July 2018, <http://pngpaediatricsociety.org/wp-content/uploads/2013/05/Paediatrics-for-Doctors-in-Papua-New-Guinea.pdf>.

Shargie E and Lindtjorn B (2005) DOTS improves treatment outcomes and service coverage for tuberculosis in South Ethiopia: a retrospective trend analysis. *BMC Public Health* 5: 62.

Sharma S K, Kohli M, Raj Narayan Y, Chaubey J, Bhasin D, Sreenivas V, Sharma R and Singh B K (2015) Evaluating the diagnostic accuracy of Xpert MTB/RIF assay in pulmonary tuberculosis. *PLoS ONE* 10(10): e0141011.

Shield J M and Kow F (2013) A comparative study of intestinal helminths in pre-school-age urban and rural children in Morobe Province, Papua New Guinea. *Papua New Guinea Medical Journal* 56(1-2): 14-31.

Shin J A, Chang Y S, Kim H J, Ahn C M and Byun M K (2015) Diagnostic utility of interferon-gamma release assay in extrapulmonary tuberculosis. *Diagnostic Microbiology and Infectious Disease* 82(1): 44-48.

Simpson G (2011) Multidrug-resistant tuberculosis on Australia's northern border. *Internal Medicine Journal* 41: 759-761.

Simpson G, Coulter C, Weston J, Knight T, Carter R, Vincent S, Robertus L and Konstantinos A (2011) Resistance patterns of multidrug-resistant tuberculosis in Western Province, Papua New Guinea. *The International Journal of Tuberculosis and Lung Disease* 15(4): 551-552.

Skinner D and Claassens M (2016) Its complicated: why do tuberculosis patients not initiate or stay adherent to treatment? A qualitative study from South Africa. *BMC Infectious Diseases* 16: 712.

Solovic I, Jonsson J, Korzeniewska- Koseła M, Chiotan D I, Pace-Asciak A, Slump E, Rumetshofer R, Abubakar I, Kos S, Svetina-Sorli P, Haas W, Bauer T, Sandgren A and van der Werf M J (2013) Challenges in diagnosing extrapulmonary tuberculosis in the European Union, 2011. *Eurosurveillance* 18(12): 20432.

Song K-H, Jeon J H, Park W B, Kim S-H, Park K U, Kim N J, Oh M-d, Kim H B and Choe K W (2009) Usefulness of the whole-blood interferon-gamma release assay for diagnosis of extrapulmonary tuberculosis. *Diagnostic Microbiology and Infectious Disease* 63(2): 182-187.

Squire S B, Belaye A K, Kashoti A, Salaniponi F M L, Mundy C J F, Theobald S and Kemp J (2005) 'Lost' smear-positive pulmonary tuberculosis cases: where are they and why did we lose them? *The International Journal of Tuberculosis and Lung Disease* 9(1): 25-31.

Steingart K R, Henry M, Ng V, Hopewell P C, Ramsay A, Cunningham J, Urbanczik R, Perkins M, Aziz M A and Pai M (2006a) Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. *The Lancet Infectious Diseases* 6(9): 570-581.

Steingart K R, Ng V, Henry M, Hopewell P C, Ramsay A, Cunningham J, Urbanczik R, Perkins M D, Aziz M A and Pai M (2006b) Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. *The Lancet Infectious Diseases* 6(10): 664-674.

Stop Stockouts (2016) *2015 stock outs national survey: third annual report - South Africa*, Stop Stockouts, Johannesburg (South Africa), viewed 22 October 2018, <http://stockouts.org/Download/2015_stock_outs_national_survey.pdf>.

Stumpf B P, Carneiro-Proietti A B, Proietti F A and Rocha F L (2008) Higher rate of major depression among blood donor candidates infected with human T-cell lymphotropic virus type 1. *The International Journal of Psychiatry in Medicine* 38(3): 345-355.

Subramani R, Radhakrishna S, Frieden T R, Kolappan C, Gopi P G, Santha T, Wares F, Selvakumar N and Narayanan P R (2008) Rapid decline in prevalence of pulmonary tuberculosis after DOTS implementation in a rural area of South India. *The International Journal of Tuberculosis and Lung Disease* 12(8): 916-920.

Šula L, Sundaresan T K and Langerová M (1960) Effects of storage and transport on the cultivability of mycobacteria. *Bulletin of the World Health Organization* 23: 635-651.

Summer Institute of Linguistics: Papua New Guinea (2004) *PNG languages: language distribution maps,* SIL International, Ukarumpa (Papua New Guinea), viewed 12 Jan 2017, <http://www-01.sil.org/pacific/png/show_maps.asp?map=Western>.

Sunnetcioglu A, Sunnetcioglu M, Binici I, Baran A I, Karahocagil M K and Saydan M R (2015) Comparative analysis of pulmonary and extrapulmonary tuberculosis of 411 cases. *Annals of Clinical Microbiology and Antimicrobials* 14: 34.

Suzana S, Ninan M M, Gowri M, Venkatesh K, Rupali P and Michael J S (2016) Xpert MTB/Rif for the diagnosis of extrapulmonary tuberculosis--an experience from a tertiary care centre in South India. *Tropical Medicine & International Health* 21(3): 385-392.

Tadesse T, Demissie M, Berhane Y, Kebede Y and Abebe M (2013) Long distance travelling and financial burdens discourage tuberculosis DOTs treatment initiation and compliance in Ethiopia: a qualitative study. *BMC Public Health* 13: 424.

Takasaki J, Manabe T, Morino E, Muto Y, Hashimoto M, Iikura M, Izumi S, Sugiyama H and Kudo K (2018) Sensitivity and specificity of QuantiFERON-TB Gold Plus compared with QuantiFERON-TB Gold In-Tube and T-SPOT.TB on active tuberculosis in Japan. *Journal of Infection and Chemotherapy* 24(3): 188-192.

Telisinghe L, Amofa-Sekyi M, Maluzi K, Kaluba-Milimo D, Cheeba-Lengwe M, Chiwele K, Kosloff B, Floyd S, Bailey S L and Ayles H (2017) The sensitivity of the QuantiFERON®-TB Gold Plus assay in Zambian adults with active tuberculosis. *The International Journal of Tuberculosis and Lung Disease* 21(6): 690-696.

Tetteh E (2009) Creating reliable pharmaceutical distribution networks and supply chains in African countries: implications for access to medicines. *Research in Social and Administrative Pharmacy* 5(3): 286-297.

Thakkar K, Ghaisas S M and Singh M (2016) Lymphadenopathy: differentiation between tuberculosis and other non-tuberculosis causes like follicular lymphoma. *Frontiers in Public Health* 4(31).

Theron G, Zijenah L, Chanda D, Clowes P, Rachow A, Lesosky M, Bara W, Mungofa S, Pai M, Hoelscher M, Dowdy D, Pym A, Mwaba P, Mason P, Peter J, Dheda K and for the TB-NEAT team (2014) Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial. *The Lancet* 383(9915): 424-435.

Thet Lwin Z M, Sahu S K, Owiti P, Chinnakali P and Majumdar S S (2017) Public-private mix for tuberculosis care and control in Myanmar: a strategy to scale up? *Public Health Action* 7(1): 15-20.

Thirouvengadame S, Muthusamy S, Balaji V K and Easow J M (2017) Unfolding of a clinically suspected case of pulmonary tuberculosis. *Journal of Clinical and Diagnostic Research* 11(8): DD01-DD03.

Thomas T A, Mondal D, Noor Z, Liu L, Alam M, Haque R, Banu S, Sun H and Peterson K M (2010) Malnutrition and helminth infection affect performance of an interferon gamma-release assay. *Pediatrics* 126(6): e1522.

Thornsberry C, Hill B C, Swenson J M and McDougal L K (1983) Rifampin: spectrum of antibacterial activity. *Reviews of Infectious Diseases* 5(Suppl 3): S412-S417.

Thwaites G (2017) Tuberculous meningitis. *Medicine* 45(11): 670-673.

Toms C, Stapledon R, Coulter C, Douglas P, National Tuberculosis Advisory Committee, Communicable Diseases Network Australia and Australian Mycobacterium Reference Laboratory Network (2017) Tuberculosis notifications in Australia, 2014. *Communicable Diseases Intelligence* 41(3): E247-E263.

Torres-Gonzalez P, Cervera-Hernandez M E, Martinez-Gamboa A, Garcia-Garcia L, Cruz-Hervert L P, Bobadilla-del Valle M, Ponce-de Leon A and Sifuentes-Osornio J (2016) Human tuberculosis caused by *Mycobacterium bovis*: a retrospective comparison with *Mycobacterium tuberculosis* in a Mexican tertiary care centre, 2000–2015. *BMC Infectious Diseases* 16: 657.

Tostmann A, Kik S V, Kalisvaart N A, Sebek M M, Verver S, Boeree M J and van Soolingen D (2008) Tuberculosis transmission by patients with smear-negative pulmonary tuberculosis in a large cohort in the Netherlands. *Clinical Infectious Diseases* 47(9): 1135-1142.

Traore H, Fissette K, Bastian I, Devleeschouwer M and Portaels F (2000) Detection of rifampicin resistance in *Mycobacterium tuberculosis* isolates from diverse countries by a commercial line probe assay as an initial indicator of multidrug resistance. *The International Journal of Tuberculosis and Lung Disease* 4(5): 481-484.

Traore H, van Deun A, Shamputa I C, Rigouts L and Portaels F (2006) Direct detection of *Mycobacterium tuberculosis* complex DNA and rifampin resistance in clinical specimens from tuberculosis patients by line probe assay. *Journal of Clinical Microbiology* 44(12): 4384-4388.

Trauer J M, Moyo N, Tay E L, Dale K, Ragonnet R, McBryde E S and Denholm J T (2016) Risk of active tuberculosis in the five years following infection . . . 15%? *Chest* 149(2): 516-525.

Trunz B B, Fine P E M and Dye C (2006) Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. *The Lancet* 367(9517): 1173-1180.

Udwadia Z F, Amale R A, Ajbani K K and Rodrigues C (2012) Totally drug-resistant tuberculosis in India. *Clinical Infectious Diseases* 54(4): 579-581.

Ukwaja K N, Alobu I, lgwenyi C and Hopewell P C (2013a) The high cost of free tuberculosis services: patient and household costs associated with tuberculosis care in Ebonyi State, Nigeria. *PLoS ONE* 8(8): e73134.

Ukwaja K N, Alobu I, Nweke C O and Onyenwe E C (2013b) Healthcare-seeking behavior, treatment delays and its determinants among pulmonary tuberculosis patients in rural Nigeria: a cross-sectional study. *BMC Health Services Research* 13: 25.

Uluk T, Allison W E, Vince J, Wand H, Tefuarani N, Causer L M, Ripa P, Kariko M, Kaminiel O, Cunningham P, Graham S M and Kaldor J M (2013) Evaluation of an interferon-gamma release assay in children with suspected tuberculosis in Papua New Guinea. *The Pediatric Infectious Disease Journal* 32(2): 187.

UNAIDS (2018) *Country factsheets: Papua New Guinea - 2017*, viewed 22 October 2018, <http://www.unaids.org/en/regionscountries/countries/papuanewguinea>.

United Nations (n.d.-a) *The sustainable development agenda,* United Nations, New York (USA), viewed 24 May 2018, <https://www.un.org/sustainabledevelopment/development-agenda/>.

United Nations (n.d.-b) *Goal 3: ensure healthy lives and promote well-being for all at all ages,* United Nations, New York (USA), viewed 24 May 2018, <https://www.un.org/sustainabledevelopment/health/>.

United Nations Development Programme (2016) *Human development report 2016: human development for everyone*, United National Development Programme, New York (USA), viewed 6 June 2018, <http://hdr.undp.org/sites/default/files/2016_human_development_report.pdf>.

Uplekar M, Weil D, Lonnroth K, Jaramillo E, Lienhardt C, Dias H M, Falzon D, Floyd K, Gargioni G, Getahun H, Gilpin C, Glaziou P, Grzemska M, Mirzayev F, Nakatani H and Raviglione M (2015) WHO's new End TB Strategy. *The Lancet* 385(9979): 1799-1801.

Van Der Zanden A G, Te Koppele-Vije E M, Vijaya Bhanu N, Van Soolingen D and Schouls L M (2003) Use of DNA extracts from Ziehl-Neelsen-stained slides for molecular detection of rifampin resistance and spoligotyping of *Mycobacterium tuberculosis*. *Journal of Clinical Microbiology* 41(3): 1101-1108.

Velayati A A, Masjedi M R, Farnia P, Tabarsi P, Ghanavi J, ZiaZarifi A H and Hoffner S E (2009) Emergence of new forms of totally drug-resistant tuberculosis bacilli: super extensively drug-resistant tuberculosis or totally drug-resistant strains in Iran. *Chest* 136(2): 420-425.

Verkuijl S and Middelkoop K (2016) Protecting our front-liners: occupational tuberculosis prevention through infection control strategies. *Clinical Infectious Diseases* 62(Suppl 3): S231-S237.

Vilchèze C and Jacobs Jr W R (2014) Resistance to isoniazid and ethionamide in *Mycobacterium tuberculosis*: genes, mutations, and causalities. *Microbiology Spectrum* 2(4): MGM2-0014-2013.

Virenfeldt J, Rudolf F, Camara C, Furtado A, Gomes V, Aaby P, Petersen E and Wejse C (2014) Treatment delay affects clinical severity of tuberculosis: a longitudinal cohort study. *BMJ Open* 4(6): e004818.

Wand H, Lote N, Semos I and Siba P (2012) Investigating the spatial variations of high prevalences of severe malnutrition among children in Papua New Guinea: results from geoadditive models. *BMC Research Notes* 5: 228.

Wang X, Yang Z, Fu Y, Zhang G, Wang X, Zhang Y and Wang X (2014) Insight to the epidemiology and risk factors of extrapulmonary tuberculosis in Tianjin, China during 2006-2011. *PLoS ONE* 9(12): e112213.

Wanyeki I, Olson S, Brassard P, Menzies D, Ross N, Behr M and Schwartzman K (2006) Dwellings, crowding, and tuberculosis in Montreal. *Social Science & Medicine* 63(2): 501-511.

Warner J M, Pelowa D B, Currie B J and Hirst R G (2007) Melioidosis in a rural community of Western Province, Papua New Guinea. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 101: 809-813.

Warner J M, Pelowa D B, Gal D, Rai G, Mayo M, Currie B J, Govan B, Skerratt L F and Hirst R G (2008) The epidemiology of melioidosis in the Balimo region of Papua New Guinea. *Epidemiology & Infection* 136(7): 965-971.

Warner J M, Pelowa D B and Currie B J (2010) Melioidosis - an uncommon but also under-recognized cause of pneumonia in Papua New Guinea. *Papua New Guinea Medical Journal* 53(3-4): 176-179.

Watch V, Aipit J, Kote-Yarong T, Rero A, Bolnga J W, Lufele E and Laman M (2017) The burden of presumed tuberculosis in hospitalized children in a resource-limited setting in Papua New Guinea: a prospective observational study. *International Health* 9(6): 374-378.

Watkins R E and Plant A J (2006) Does smoking explain sex differences in the global tuberculosis epidemic? *Epidemiology & Infection* 134(2): 333-339.

Wei X, Walley J D, Liang X, Liu F, Zhang X and Li R (2008) Adapting a generic tuberculosis control operational guideline and scaling it up in China: a qualitative case study. *BMC Public Health* 8: 260.

Wei X, Chen J, Chen P, Newell J N, Li H, Sun C, Mei J and Walley J D (2009) Barriers to TB care for rural-to-urban migrant TB patients in Shanghai: a qualitative study. *Tropical Medicine & International Health* 14(7): 754-760.

Whittaker M, Piliwas L, Agale J and Yaipupu J (2009) Beyond the numbers: Papua New Guinean perspectives on the major health conditions and programs of the country. *Papua New Guinea Medical Journal* 52(3-4): 96-113.

Wigley S C (n.d.) *Collection MS 1182 - papers on tuberculosis, other health matters and conservation in Papua New Guinea,* Pacific Manuscripts Bureau, Canberra (Australia), viewed 6 June 2018, <http://asiapacific.anu.edu.au/pambu/catalogue/index.php/papers-on-tuberculosis-other-health-matters-and-conservation-in-papua-new-guinea>.

Wilkinson D and Moore D A J (1996) HIV-related tuberculosis in South Africa - clinical features and outcome. *South African Medical Journal* 86(1): 64-67.

Willems L, Hasegawa H, Accolla R, Bangham C, Bazarbachi A, Bertazzoni U, Carneiro-Proietti A B d F, Cheng H, Chieco-Bianchi L, Ciminale V, Coelho-dos-Reis J, Esparza J, Gallo R C, Gessain A, Gotuzzo E, Hall W, Harford J, Hermine O, Jacobson S, Macchi B, Macpherson C, Mahieux R, Matsuoka M, Murphy E, Peloponese J-M, Simon V, Tagaya Y, Taylor G P, Watanabe T and Yamano Y (2017) Reducing the global burden of HTLV-1 infection: An agenda for research and action. *Antiviral Research* 137: 41-48.

Wirth T, Hildebrand F, Allix-Béguec C, Wölbeling F, Kubica T, Kremer K, van Soolingen D, Rüsch-Gerdes S, Locht C, Brisse S, Meyer A, Supply P and Niemann S (2008) Origin, spread and demography of the *Mycobacterium tuberculosis* complex. *PLoS Pathogens* 4(9): e1000160.

Wood R, Liang H, Wu H, Middelkoop K, Oni T, Rangaka M X, Wilkinson R J, Bekker L-G and Lawn S D (2010) Changing prevalence of tuberculosis infection with increasing age in high-burden townships in South Africa. *The International Journal of Tuberculosis and Lung Disease* 14(4): 406-412.

World Bank Group, The (n.d.) *World Bank country and lending groups,* The World Bank, Washington, D.C. (USA), viewed 6 June 2018, <https://datahelpdesk.worldbank.org/knowledgebase/articles/906519>.

World Health Organization (1999) *What is DOTS? A guide to understanding the WHO-recommended TB control strategy known as DOTS*, World Health Organization, Geneva (Switzerland), viewed 7 June 2018, <http://apps.who.int/iris/bitstream/handle/10665/65979/WHO_CDS_CPC_TB_99.270.pdf;jsessionid=FFE07BAC6BC0D4191C56F95B223E493D?sequence=1>.

World Health Organization (2002) *An expanded DOTS framework for effective tuberculosis control*, World Health Organization, Geneva (Switzerland), viewed 19 February 2015, <http://whqlibdoc.who.int/hq/2002/WHO_CDS_TB_2002.297.pdf?ua=1>.

World Health Organization (2006) *The stop TB strategy: building on and enhancing DOTS to meet the TB-related Millennium Development Goals*, World Health Organization, Geneva (Switzerland), viewed 19 February 2015, <http://whqlibdoc.who.int/hq/2006/WHO_HTM_STB_2006.368_eng.pdf?ua=1>.

World Health Organization (2010) *Treatment of tuberculosis: guidelines - 4th edition*, World Health Organization, Geneva (Switzerland), viewed 5 December 2017, <http://www.who.int/tb/publications/2010/9789241547833/en/>.

World Health Organization (2012) *Tuberculosis laboratory biosafety manual*, World Health Organization, Geneva (Switzerland), viewed 18 May 2018, <http://www.who.int/tb/publications/2012/tb_biosafety/en/>.

World Health Organization and National Department of Health (2012) *Health service delivery profile: Papua New Guinea 2012*, World Health Organization Regional Office for the Western Pacific, Manila (Philippines), viewed 22 April 2015, <http://www.wpro.who.int/health_services/service_delivery_profile_papua_new_guinea.pdf?ua=1>.

World Health Organization (2013a) *Definitions and reporting framework for tuberculosis - 2013 revision (updated December 2014)*, World Health Organization, Geneva (Switzerland), viewed 18 January 2018, <http://apps.who.int/iris/bitstream/10665/79199/1/9789241505345_eng.pdf?ua=1>.

World Health Organization (2013b) *The use of bedaquiline in the treatment of multidrug-resistant tuberculosis: interim policy guidance*, World Health Organization, Geneva (Switzerland), viewed 3 August 2018, <http://apps.who.int/iris/bitstream/handle/10665/84879/9789241505482_eng.pdf?sequence=1>.

World Health Organization (2013c) *Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children - policy update*, World Health Organization, Geneva (Switzerland), viewed 10 October 2017, <http://apps.who.int/iris/bitstream/10665/112472/1/9789241506335_eng.pdf>.

World Health Organization (2014a) *The use of delamanid in the treatment of multidrug-resistant tuberculosis*, World Health Organization, Geneva (Switzerland), viewed 3 August 2018, <http://apps.who.int/iris/bitstream/handle/10665/137334/WHO_HTM_TB_2014.23_eng.pdf?sequence=1>.

World Health Organization (2014b) *Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis*, World Health Organization, Geneva (Switzerland), viewed 3 August 2018, <http://apps.who.int/iris/bitstream/handle/10665/130918/9789241548809_eng.pdf?sequence=1>.

World Health Organization (2014c) *HIV-associated tuberculosis,* World Health Organization, Geneva (Switzerland), viewed 8 April 2015, <http://who.int/tb/challenges/hiv/tbhiv_factsheet_2014.pdf?ua=1>.

World Health Organization (2015a) *Papua New Guinea: WHO statistical profile,* World Health Organization, Geneva (Switzerland), viewed 5 January 2017, <http://www.who.int/gho/countries/png.pdf?ua=1>.

World Health Organization (2015b) *Implementing tuberculosis diagnostics: policy framework*, World Health Organization, Geneva (Switzerland), viewed 18 May 2018, <http://www.who.int/tb/publications/implementing_TB_diagnostics/en/>.

World Health Organization (2015c) *Implementing the end TB strategy: the essentials*, World Health Organization, Geneva (Switzerland), viewed 7 June 2018, <http://www.who.int/tb/publications/2015/end_tb_essential.pdf>.

World Health Organization (2015d) *Papua New Guinea: WHO statistical profile,* World Health Organization, Geneva (Switzerland), viewed 7 June 2018, <http://www.who.int/gho/countries/png.pdf>.

World Health Organization (2015e) *The end TB strategy*, World Health Organization, Geneva (Switzerland), viewed 7 June 2018, <http://www.who.int/tb/End_TB_brochure.pdf>.

World Health Organization (2016) *WHO treatment guidelines for drug-resistant tuberculosis: 2016 update - October 2016 revision*, World Health Organization, Geneva (Switzerland), viewed 23 January 2018, viewed 23 January 2018, <http://apps.who.int/iris/bitstream/10665/250125/1/9789241549639-eng.pdf?ua=1>.

World Health Organization (2017a) *Global tuberculosis report 2017*, World Health Organization, Geneva (Switzerland), viewed 16 January 2018, <http://apps.who.int/iris/bitstream/10665/259366/1/9789241565516-eng.pdf?ua=1>.

World Health Organization (2017b) *Treatment of tuberculosis: guidelines for treatment of drug-susceptible tuberculosis and patient care - 2017 update*, World Health Organization, Geneva (Switzerland), viewed 7 June 2018, <http://www.who.int/tb/publications/2017/dstb_guidance_2017/en/>.

World Health Organization (2017c) *WHO report on the global tobacco epidemic, 2017 - country profile: Papua New Guinea*, World Health Organization, Geneva (Switzerland), viewed 18 May 2018, <http://www.who.int/tobacco/surveillance/policy/country_profile/png.pdf?ua=1>.

World Health Organization (2017d) *WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF*, World Health Organization, Geneva (Switzerland), viewed 12 June 2018, <http://apps.who.int/iris/bitstream/10665/254792/1/WHO-HTM-TB-2017.04-eng.pdf>.

World Health Organization (2018a) *TB_notifications_2018-01-17.csv,* World Health Organization, Geneva (Switzerland), viewed 18 January 2018, <http://www.who.int/tb/country/data/download/en/>.

World Health Organization (2018b) *Global Health Observatory data repository: prevalence of HIV among adults aged 15 to 49 - estimates by country (last updated: 2018-07-17),* World Health Organization, Geneva (Switzerland), viewed 31 July 2018, <http://apps.who.int/gho/data/view.main.22500?lang=en>.

World Health Organization (2018c) *Australia: tuberculosis profile,* World Health Organization, Geneva (Switzerland), viewed 6 June 2018, <https://extranet.who.int/sree/Reports?op=Replet&name=/WHO_HQ_Reports/G2/PROD/EXT/TBCountryProfile&ISO2=AU&outtype=html>.

World Health Organization (2018d) *Technical report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis*, World Health Organization, Geneva (Switzerland), viewed 18 June 2018, <http://www.who.int/tb/publications/2018/WHO_technical_report_concentrations_TB_drug_susceptibility/en/>.

World Health Organization (2018e) *Drug-resistant TB: totally drug-resistant TB FAQ,* World Health Organization, Geneva (Switzerland), viewed 5 January 2017, <http://who.int/tb/areas-of-work/drug-resistant-tb/totally-drug-resistant-tb-faq/en/>.

World Health Organization (2018f) *World Health Organization model list of essential in vitro diagnostics*, 1st edn, World Health Organization, Geneva (Switzerland), viewed 7 June 2018, <http://www.who.int/medical_devices/diagnostics/WHO_EDL_2018.pdf>.

World Health Organization Regional Office for the Western Pacific (2017a) *Australia country profile (CHIPS 2015-2016),* World Health Organization Regional Office for the Western Pacific, Manila (Philippines), viewed 7 June 2018, <http://hiip.wpro.who.int/portal/portals/0/CountryProfiles/PDF/AUS_Australia2016.pdf>.

World Health Organization Regional Office for the Western Pacific (2017b) *Papua New Guinea country profile (CHIPS 2015-2016),* World Health Organization Regional Office for the Western Pacific, Manila (Philippines), viewed 6 June 2018, <http://hiip.wpro.who.int/portal/portals/0/CountryProfiles/PDF/PNG_PapuaNewGuinea2015.pdf>.

World Health Organization Representative Office: Papua New Guinea (2016) *Update on the situation of drug-resistant tuberculosis in Papua New Guinea, with special emphasis on Daru Island,* The World Health Organization Regional Office for the Western Pacific, Manila (Philippines), viewed 17 July 2018, <http://www.wpro.who.int/papuanewguinea/areas/tb_leprosy/daru_update/en/>.

World Vision TB DOTS Project (2013) *TB Owera - Quarterly News: April - June 2013, Issue #2*. World Vision TB DOTS Project, Boroko (Papua New Guinea).

Xia H, Wang X, Li F, Longuet C, Vernet G, Goletti D, Zhao Y and Lagrange P H (2015) Diagnostic values of the QuantiFERON-TB Gold In-Tube assay carried out in China for diagnosing pulmonary tuberculosis. *PLoS ONE* 10(4): e0121021.

Xie Y L, Cronin W A, Proschan M, Oatis R, Cohn S, Curry S R, Golub J E, Barry Iii C E and Dorman S E (2018) Transmission of *Mycobacterium tuberculosis* from patients who are nucleic acid amplification test- negative. *Clinical Infectious Diseases*: ciy365-ciy365.

Xu L, Gai R, Wang X, Liu Z, Cheng J, Zhou C, Liu J, Zhang H, Li H and Tang W (2010) Socio-economic factors affecting the success of tuberculosis treatment in six counties of Shandong Province, China. *The International Journal of Tuberculosis and Lung Disease* 14(4): 440-446.

Yanagihara R, Garruto R M, Miller M A, Leon-Monzon M, Liberski P P, Gajdusek D C, Jenkins C L, Sanders R C and Alpers M P (1990a) Isolation of HTLV-I from members of a remote tribe in New Guinea. *The New England Journal of Medicine* 323: 993-994.

Yanagihara R, Jenkins C L, Alexander S S, Mora C A and Garruto R M (1990b) Human T lymphotropic virus type I infection in Papua New Guinea: high prevalence among the Hagahai confirmed by western analysis. *The Journal of Infectious Diseases* 162(3): 649-654.

Yang Z, Kong Y, Wilson F, Foxman B, Fowler A H, Marrs C F, Cave M D and Bates J H (2004) Identification of risk factors for extrapulmonary tuberculosis. *Clinical Infectious Diseases* 38(2): 199-205.

Yassin M A, Datiko D G, Tulloch O, Markos P, Aschalew M, Shargie E B, Dangisso M H, Komatsu R, Sahu S, Blok L, Cuevas L E and Theobald S (2013) Innovative community-based approaches doubled tuberculosis case notification and improve treatment outcome in southern Ethiopia. *PLoS ONE* 8(5): e63174.

Yates T A, Khan P Y, Knight G M, Taylor J G, McHugh T D, Lipman M, White R G, Cohen T, Cobelens F G, Wood R, Moore D A J and Abubakar I (2016) The transmission of *Mycobacterium tuberculosis* in high burden settings. *The Lancet Infectious Diseases* 16(2): 227-238.

Yi L, Sasaki Y, Nagai H, Ishikawa S, Takamori M, Sakashita K, Saito T, Fukushima K, Igarashi Y, Aono A, Chikamatsu K, Yamada H, Takaki A, Mori T and Mitarai S (2016) Evaluation of QuantiFERON-TB Gold Plus for detection of *Mycobacterium tuberculosis* infection in Japan. *Scientific Reports* 6: 30617.

Yoon J-H, Nam J-S, Kim K-J, Choi Y, Lee H, Cho S-N and Ro Y-T (2012) Molecular characterization of drug-resistant and -susceptible *Mycobacterium tuberculosis* isolated from patients with tuberculosis in Korea. *Diagnostic Microbiology and Infectious Disease* 72(1): 52-61.

Zeka A N, Tasbakan S and Cavusoglu C (2011) Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extrapulmonary specimens. *Journal of Clinical Microbiology* 49(12): 4138-4141.

Zelnick J R, Gibbs A, Loveday M, Padayatchi N and O'Donnell M R (2013) Health-care workers' perspectives on workplace safety, infection control, and drug-resistant tuberculosis in a high-burden HIV setting. *Journal of Public Health Policy* 34(3): 388-402.

Zhao L-l, Liang Y-p, Huang M-x, Tan Y-h, Jiang Y, Chen Y, Liu Z, Gao M, Wei S, Chen Z, Wu J, Jiang Y and Wan K-l (2013) Multicenter research on the BACTEC MGIT 960 system for the second-line drugs susceptibility testing of *Mycobacterium tuberculosis* in China. *Diagnostic Microbiology and Infectious Disease* 770: 330-334.

Zhao Y, Xu S, Wang L, Chin D P, Wang S, Jiang G, Xia H, Zhou Y, Li Q, Ou X, Pang Y, Song Y, Zhao B, Zhang H, He G, Guo J and Wang Y (2012) National survey of drug-resistant tuberculosis in China. *The New England Journal of Medicine* 366(23): 2161-2170.

Zhu M, Han G, Takiff H E, Wang J, Ma J, Zhang M and Liu S (2018) Times series analysis of age-specific tuberculosis at a rapid developing region in China, 2011–2016. *Scientific Reports* 8: 8727.

Zimmerman M R (1979) Pulmonary and osseous tuberculosis in an Egyptian mummy. *Bulletin of the New York Academy of Medicine* 55(6): 604-608.

Zink A R, Grabner W, Reischl U, Wolf H and Nerlich A G (2003) Molecular study on human tuberculosis in three geographically distinct and time delineated populations from ancient Egypt. *Epidemiology & Infection* 130(2): 239-249.

Zumla A, Nahid P and Cole S T (2013) Advances in the development of new tuberculosis drugs and treatment regimens. *Nature Reviews Drug Discovery* 12: 388-404.

APPENDIX 1

EXTRAPULMONARY TUBERCULOSIS DATA

This appendix details extrapulmonary TB proportions from studies identified throughout the course of this research. The included data is neither systematic nor exhaustive, but simply reflects studies that were identified throughout this PhD.

Appendix 1 - Table 1: Geographic locations, diagnostic methods, and proportions of extrapulmonary TB identified in setting-specific research.

WHO region	Country	EPTB notification proportion[@]	Income group*	Setting	Years	Diagnosis methods	EPTB proportion^	Reference
Africa	Benin	10%	Low	Nationwide	2011	Laboratory and clinical	9%	(Ade et al. 2014)
	Cameroon	17%	Lower-Middle	Douala	2007-2011	Laboratory and clinical	43%	(Namme et al. 2013)
	Ethiopia	32%	Low	Sidama Zone	2003-2012	Laboratory and clinical	17%	(Dangisso et al. 2014)
	Ethiopia	32%	Low	Dilla Town, Gedeo Zone	Nov 2012-May 2013	Laboratory and clinical	12%	(Gebrezgabiher et al. 2016)
	Malawi	26%	Low	Nationwide	1994-2003	Not stated	23%	(Nyirenda 2006)
	South Africa	10%	Upper-Middle	Durban	Jan-Mar 2016	Laboratory and clinical	43%[#]	(Gounden et al. 2018)
Americas	Brazil	13%	Upper-Middle	Nationwide	2007-2011	Not stated	13%	(Gomes et al. 2014)
	USA	21%	High	Houston, Texas	1995-1999	Laboratory and clinical	29%[#]	(Gonzalez et al. 2003)

	USA	21%	High	Minnesota (Somali-born)	1993-2003	Not stated	49%	(Rock et al. 2006)
	USA	21%	High	North Carolina	1993-2006	Laboratory and clinical	22%^	(Kipp et al. 2008)
	USA	21%	High	Nationwide	1993-2006	Not stated	20%[&]	(Peto et al. 2009)
	USA	21%	High	Texas	2009-2015	Not stated	24%[#]	(Qian et al. 2018)
Eastern Mediterranean	Saudi Arabia	26%	High	Nationwide	2010-2011	Not stated	31%	(Memish et al. 2014)
Europe	Austria	24%	High	Nationwide	2011	Not stated	20%	(Solovic et al. 2013)
	Czech Republic	14%	High	Nationwide	2011	Laboratory and clinical	13%	(Solovic et al. 2013)
	Estonia	7%	High	Nationwide	1991-2000	Laboratory and clinical	12%	(Pehme et al. 2005)
	Germany	25%	High	Nationwide	1996-2000	Laboratory and clinical	22%	(Forssbohm et al. 2008)
	Germany	25%	High	Nationwide	2011	Not stated	21%	(Solovic et al. 2013)
	Malta	26%	High	Nationwide	2011	Laboratory and clinical	33%	(Solovic et al. 2013)
	Netherlands	46%	High	Nationwide	2011	Laboratory and clinical	44%	(Solovic et al. 2013)
	Poland	5%	High	Nationwide	2011	Not stated	7%	(Solovic et al. 2013)
	Romania	16%	Upper-Middle	Nationwide	2011	Not stated	14%	(Solovic et al. 2013)
	Slovakia	15%	High	Nationwide	2011	Laboratory and clinical	16%	(Solovic et al. 2013)
	Slovenia	13%	High	Nationwide	2011	Guidelines require positive culture, but some not confirmed	14%	(Solovic et al. 2013)
	Spain	28%	High	La Coruña, Galicia	1991-2008	Laboratory and clinical	38%[&]	(García-Rodríguez et al. 2011)

	Sweden	34%	High	Nationwide	2011	Laboratory and clinical	39%	(Solovic et al. 2013)
	Turkey	34%	Upper-Middle	Kahramanmaras	2007-2012	Laboratory and clinical	45%^	(Ates Guler et al. 2015)
	Turkey	34%	Upper-Middle	Kocaeli, Marmara	1996-2000	Laboratory and clinical	31%^	(Ilgazli et al. 2004)
	UK (England and Wales)	46%	High	Nationwide	1999-2006	Laboratory and clinical	41%	(Kruijshaar & Abubakar 2009)
	UK	46%	High	Nationwide	2011	Not stated	48%	(Solovic et al. 2013)
South-East Asia	Bhutan	49%	Lower-Middle	Nationwide	2001-2010	Laboratory and clinical	26-42% per year	(Jamtsho et al. 2013)
	India	16%	Lower-Middle	Delhi	1996-2003	Laboratory and clinical	20%	(Arora & Gupta 2006)
	India	16%	Lower-Middle	Deralakatte, Karnataka	2005-2011	Laboratory and clinical	42%	(Prakasha et al. 2013)
	India	16%	Lower-Middle	Kanpur, Kerala	2013-2015	Unknown	52%	(Mavila et al. 2015)
	India	16%	Lower-Middle	Mumbai	2000-2002	Laboratory	22%	(Gothi & Joshi 2004)
	Myanmar	11%	Lower-Middle	Nationwide, Myanmar Medical Association public-private mix clinics only	2013	Unknown	9%	(Thet Lwin et al. 2017)
Western Pacific	China	5%	Upper-Middle	Tianjin	2006-2011	Laboratory	10%	(Wang et al. 2014)
	China	5%	Upper-Middle	Nanshan district, Shenzhen	2001-2016	Unknown	11%	(Zhu et al. 2018)
	Fiji	29%	Upper-Middle	Nationwide	2002-2013	Laboratory and clinical	25%	(Pezzoli et al. 2016)

	Korea (Republic)	19%	High	Nationwide	2013	Unknown	20%	(Lee 2015)

EPTB: extrapulmonary tuberculosis; UK: United Kingdom; USA: United States of America

*Income groups are based on The World Bank list of economies for fiscal year 2018 (World Bank Group n.d.)

@Based on the 2017 Global TB Report published by the World Health Organization (World Health Organization 2017a), with extrapulmonary TB proportion estimated to be 100% less the % pulmonary

^Excludes patients with concurrent pulmonary and extrapulmonary infection, unless otherwise noted, and where these groups were defined in the study

#Includes concomitant pulmonary and extrapulmonary infection

&Includes disseminated infection, but not concomitant pulmonary and extrapulmonary infection.

APPENDIX 2

DETAILS OF PERIPHERAL HEALTH CENTRES IN THE BALIMO REGION

Appendix 2 - Table 1: Peripheral health centre details for sites where TB can be diagnosed and/or treated in the Gogodala and Bamu regions. The details of these sites were obtained from Sister Bisato Gula.

Location	Type	LLG	Affiliation	TB staff	TB register	Treatment	Other staff and facilities
Adiba	Sub-HC	Gogodala Rural	ECPNG	1 x CHW	No (record only)	No (send to Balimo)	
Ali	Aid post	Gogodala Rural	Government	1 x CHW	Yes	Yes	
Awaba	HC	Gogodala Rural	ECPNG	2 x nurse	Yes	Yes	TB ward ZN staining
Bamio	HC	Bamu Rural	Catholic	1 x nurse	Yes	Yes	
Emeti	HC	Bamu Rural	Government	2 x CHW	Yes	Yes	1 x nurse
Kamusie	Clinic	Bamu Rural	Logging company	1 x doctor 1 x CHW	Yes	Yes	1 x CHW
Mapodo	HC	Gogodala Rural	ECPNG	2 x CHW	Yes	Yes	1 x nurse 1 x CHW
Pikiwa	Treatment booth	Gogodala Rural	Logging company	1 x tx supporter	Yes	No (send to Awaba)	
Sisiami	Aid post	Bamu Rural	Government	1 x CHW	Yes	No (send to Bamio or Balimo)	
Tapila	HC	Gogodala Rural	Government	1 x HEO	Yes	Yes	1 x nurse 3 x CHW

Wasua	HC	Gogodala Rural	ECPNG	1 x nurse 1 x CHW	Yes	Yes	3 x CHW
Wawoi Falls	HC	Bamu Rural	ECPNG	1 x nurse	Yes	Yes (sputum not sent to Balimo)	2 x CHW

CHW: community health worker; ECPNG: Evangelical Church of Papua New Guinea; HC: health centre; HEO: health extension officer; LLG: local level government; TB: tuberculosis; tx: treatment; ZN: Ziehl-Neelsen

APPENDIX 3

ANALYSIS OF BIAS IN REPORTING OF AGE

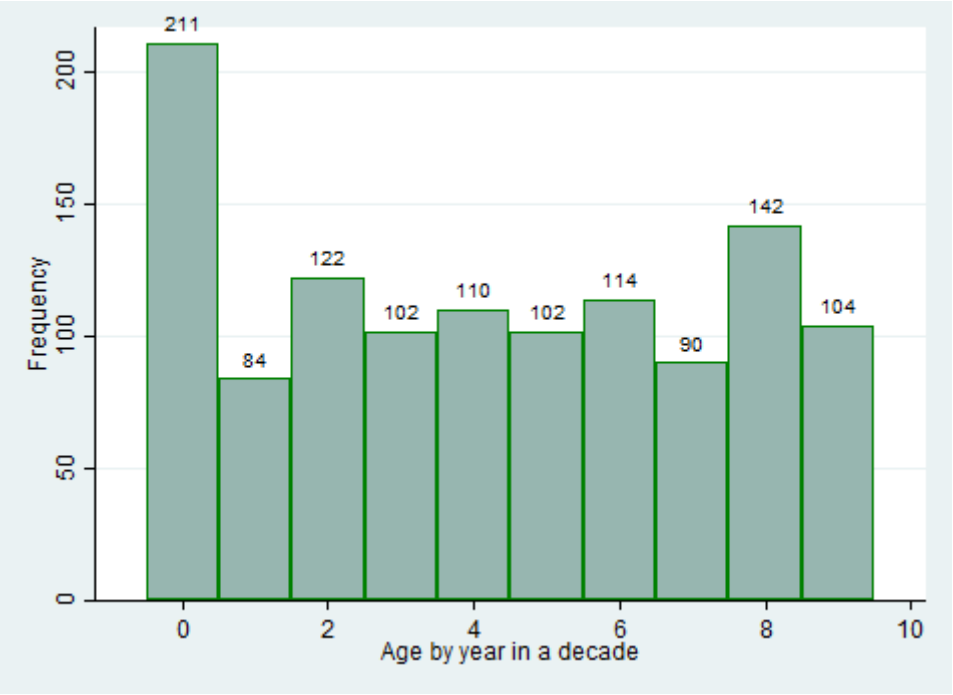
Method:

Potential bias in age was examined to determine whether reported ages were likely to be accurate. To do this, reported ages were recoded into each year of a decade. For example, all patients who reported their age as 10, 20, 30, 40, 50, 60, or 70 were recoded as 0; all patients who reported their age as 11, 21, 31, 41, 51, 61, or 71 were recoded as 1; and so on. Patients under the age of 10 were excluded from the analysis, as it was thought that age would be more likely to be correct in the early years of life. Age reporting was then examined using the Chi-square goodness of fit test, to determine if there was a significant difference between any of the reported years.

Results:

Evidence of bias was identified in the reporting of age. Graphing of the recoded age groups showed '0' and even-numbered age groups to be more frequently reported (Appendix 3 - Figure 1), and this result was statistically significant ($X^2 = 101.35$, $p < 0.01$).

The significant association continued when the '0' age group was removed, with observations in the odd-numbered groups below the expected frequency, and observations in the even-numbered groups above the expected frequency ($X^2 = 22.08$, $p < 0.01$). As a result, all further analyses involving age were based on the WHO age category groupings only, and no analyses were undertaken based on age as a continuous variable.



Appendix 3 - Figure 1: Graph of age group recoding, showing frequency of reported age in each year of a decade.

APPENDIX 4

DETAILS OF RESIDENTIAL LOCATIONS AND LANGUAGE GROUPS

Residential locations and derived language groups for patients recorded in the TB patient register.

Appendix 4 - Table 1: Residential locations reported by TB patients (based on the first recorded address only). To ensure privacy only towns and villages with at least 10 TB cases are shown here. All locations with fewer cases are grouped under 'Other villages'.

Residential address	n	%
Aba-Bamustu	36	2.23
Adiba	59	3.66
Aketa	47	2.91
Ali	10	0.62
Balimo	244	15.12
Bimaramio	15	0.93
Bunigi	28	1.73
Dogono	18	1.12
Isago	33	2.04
Kaenewa	18	1.12
Kamusie	23	1.43
Kaniya	23	1.43
Kawiyapo	16	0.99
Kebane	10	0.62
Kewa	37	2.29
Kimama	162	10.04
Kini	20	1.24
Kotale	38	2.35
Madila	10	0.62
Makapa	35	2.17
Oropai	13	0.81
Panakawa	14	0.87
Parieme	11	0.68
Pikiwa	23	1.43
Pisi	73	4.52
Saiwase	17	1.05
Saweta	25	1.55
Sisiami (1 and 2)	37	2.29

Tai	32	1.98
Togowa	33	2.04
Ugu	31	1.92
Uladu	33	2.04
Upati	20	1.24
Waligi	31	1.92
Wasapeya	14	0.87
Widama	48	2.97
Yau	25	1.55
Other villages	219	13.57
Unknown	33	2.04
Total	**1614**	**100.00**

Appendix 4 - Table 2: Language groups spoken in residential locations reported by TB patients, based on the first residential address recorded.

Language group	n	%
Agob	1	0.06
Aimele	1	0.06
Arammba	1	0.06
Ari	10	0.62
Bamu	213	13.20
Baramu	1	0.06
Dibiyaso	95	5.89
Foia Foia	7	0.43
Gizrra	2	0.12
Gogodala	1102	68.28
Idi	1	0.06
Kamula	18	1.12
Makayam	6	0.37
Mubami	35	2.17
Southern Kiwai	21	1.30
Suki	4	0.25
Tabo	17	1.05
Waboda	4	0.25
Were	3	0.19
Unknown	72	4.46
Total	**1614**	**100.00**

APPENDIX 5

DETAILS OF GEOGRAPHIC DATA

Appendix 5 - Table 1: Geographic data and sources used for locations not listed in the census unit population data.

Village	Longitude	Latitude	Source
Diwame	143.162994	-7.746011	http://placesmap.net/PG/Diwame-3213496/
Kaniya	142.579102	-7.97672	http://placesmap.net/PG/Kaniya-9568/
Kubeai	143.044098	-7.306943	http://placesmap.net/PG/Kubeai-3160823/
Lake Campbell / Kembi Lake	142.609	-6.74903	ArcGIS Online
Madila	143.159	-7.99567	ArcGIS Online
Panakawa	143.133	-7.68503	ArcGIS Online
Parieme	142.973	-7.65253	ArcGIS Online
Sasareme	142.867	-7.6167	http://www.aic.gov.pg/pdf/FinRpts/2012/AIC%2012-1005%20P2-MCZ%20Final%20Report.pdf
Saweta	142.655	-8.01297	ArcGIS Online
Sogai	142.979	-7.60316	ArcGIS Online
Waliyama	143.157	-8.28449	ArcGIS Online

APPENDIX 6

COPY OF MANUSCRIPT PUBLISHED IN TROPICAL MEDICINE & INTERNATIONAL HEALTH AND PERMISSION FOR USE

The majority of Chapter 3 of this thesis has been published in the journal of *Tropical Medicine & International Health*, with publication details as follows:

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VOLUME 00 NO 00

The epidemiology of tuberculosis in the rural Balimo region of Papua New Guinea

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Abstract

OBJECTIVE Papua New Guinea (PNG) has an emerging tuberculosis (TB) epidemic which has become a national public health priority. In Western Province, there are few data about TB outside Daru and the South Fly District. This study describes the epidemiology of TB diagnosed at Balimo District Hospital (BDH) in the Middle Fly District of Western Province, PNG.

METHODS All patients (n = 1614) diagnosed with TB at BDH from April 2013 to February 2017 were recorded. Incidence of reported new cases was calculated for the combined Balimo Urban and Gogodala Rural local level government areas. Analyses investigated patient demographic and clinical information, differences between pulmonary and extrapulmonary TB patients, and predictors of treatment failure.

RESULTS The average case notification rate (2014–2016) was 727 TB cases per 100 000 people per year. One-quarter of TB cases were in children, and 77.1% of all cases had an extrapulmonary TB diagnosis. There was a 1:1.1 ratio of female to male TB cases. When comparing pulmonary and extrapulmonary TB patients, extrapulmonary TB was more likely in those aged up to 14 years and over 54 years. Extrapulmonary TB was more likely in new patients, and pulmonary TB more likely in previously treated patients. Residence in rural regions was associated with treatment failure.

CONCLUSION There is a high burden of TB in the Balimo region, including a very high proportion of extrapulmonary TB. These factors emphasise the importance of BDH as the primary hospital for TB cases in the Balimo region and the Middle Fly District, and the need for resources and staff to manage both drug-susceptible and drug-resistant TB cases.

keywords pulmonary tuberculosis, extrapulmonary tuberculosis, epidemiology, Papua New Guinea, drug resistance

Introduction

Tuberculosis (TB) is an urgent health concern in Papua New Guinea (PNG). In 2016, incidence was estimated at 432 cases per 100 000 people per year, equating to almost 35 000 people diagnosed with TB each year [1]. The burden of TB cases resistant to standard treatment is also substantial, with drug resistance estimated to occur in 3.4% of new cases, and 26% of retreatment cases [1].

Western Province is the largest province in PNG by area. The province has three districts – North Fly, Middle Hy and South Fly (Figure 1). Middle Fly District, the most populous of the three, includes five local level government (LLG) areas. These are Balimo Urban, Gogodala Rural, Bamu Rural, Lake Murray Rural and Nomad Rural (Figure 1). Balimo District Hospital (BDH) primarily serves the Balimo Urban and Gogodala Rural LLGs, and to a lesser extent the Bamu Rural LLG. In this study, the area served by BDH is generally referred to as the Balimo region, while the term 'Gogodala region' refers to the combined Balimo Urban and Gogodala Rural LLGs only.

In Western Province, TB incidence has been estimated at 549 cases per 100 000 people per year, and drug resistance has been seen in 25% of Western Province-based TB patients presenting at Australian health clinics in the Torres Strait [2–4]. More recently, a multi-site study that included two sites in Western Province found MDR-TB in 34.2% of Daru Hospital TB cases [5]. Cases with rifampicin mono-resistance were also identified, including

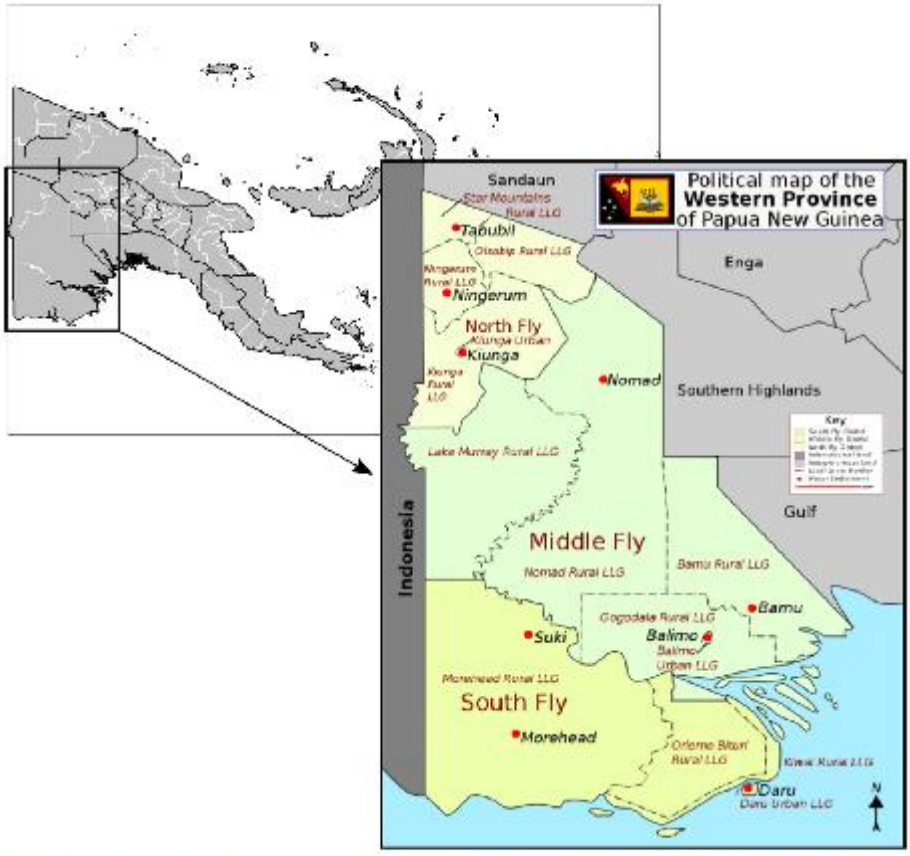

Figure 1 Western Province of Papua New Guinea (PNG), showing Balimo in the south-east of the Middle Fly District, and Daru on an island to the south of mainland PNG. (Image source: Wikimedia Commons).

7.3% of those from Daru, and 5.6% of those from Tabubil in the North Fly District [5] (Figure 1). However, data about the burden of TB in other areas of the province are scarce, particularly outside the South Fly District. Only one study examined TB in the province more broadly, an evaluation study which assessed the risks of TB across the province and concluded that the provincial burdens of TB and MDR-TB were much higher than official estimates, and that improvements within the TB control programme were essential to limit the spread of drug resistance [4].

A small number of health facilities across Western Province are able to provide TB diagnosis and treatment. However, the lack of roads and cost of travel makes access difficult [6]. Furthermore, TB patients with evidence of drug resistance, based on a lack of response to first-line treatment, or persistent positive smear microscopy results, must travel to the provincial capital on the island of Daru to access second-line treatment. Thus, Daru risks becoming a bottleneck for severe TB cases, with high numbers of drug-resistant cases concentrated on a small and densely populated island that has already

suffered from other health crises, including cholera [7, 8].

The known high rate of DR-TB cases in Daru, combined with the lack of research describing TB across the province more broadly, means there is an urgent need for epidemiological data describing TB patients from other areas of Western Province. This study describes the epidemiology of TB in the patient cohort of BDH located in the Middle Fly District of Western Province, PNG (Figure 1).

Methods

Balimo, located on the Aramia River floodplain in the Middle Fly District of Western Province, is a town of approximately 4400 people, and the urban centre of the Gogodala Rural LLG area [9]. Numerous small villages in the Gogodala Rural LLG area have a combined population of approximately 33 000 people [9]. There are very few roads outside of Balimo town, and travel is predominantly by foot, boat or canoe.

BDH is the primary health facility in Middle Fly District capable of diagnosing TB and initiating treatment. As a district hospital, BDH is classified as a level four facility, based on the National Health Service Standards [10]. These are a comprehensive set of standards that define objectives relevant to patient care, leadership and management, human resources management, information systems, the environment and improving performance [11]. A number of smaller health facilities in the region, including seven in the Gogodala Rural LLG and five in the Bamu Rural LLG, are able to commence patients on TB treatment. However, these facilities can only diagnose TB clinically, and sputum or other clinical specimens should be sent to Balimo for laboratory confirmation. At BDH, TB diagnoses are primarily passive, based on symptomatic patients seeking care at the hospital through self-referral, or onwards referral from health workers at a peripheral health centre or aid post.

BDH has not had the services of a resident medical officer since the 1990s. The only method available to confirm TB diagnoses is Ziehl-Neelsen (ZN)-stained smear microscopy, and X-ray was not available at the time of this study. As such, the majority of TB patients recorded in this study have received clinical diagnoses led by resident health extension officers (HEOs) trained in the management of drug-susceptible TB cases, and without laboratory confirmation of infection. In this region, the criteria for TB diagnoses and treatment outcomes are based on the PNG National Tuberculosis Management Protocol and the WHO definitions [12, 13]. Based on these definitions, all people given TB treatment are recorded as a TB case [12]. This occurs regardless of laboratory confirmation of infection, or whether the diagnosis is pulmonary or extrapulmonary TB. A pulmonary TB diagnosis may be indicated by symptoms such as prolonged cough (more than 2–3 weeks), fever, weight loss and night sweats; or in extrapulmonary TB by signs and symptoms including lymphadenitis, loss of function in the lower limbs, headache or mental confusion, as per the PNG National Tuberculosis Management Protocol [12].

Standardised TB treatment in the region is available only for drug-susceptible TB cases. Six months of treatment is provided using fixed dose combinations. Treatment is comprised of a 2-month intensive phase with rifampicin, isoniazid, ethambutol and pyrazinamide; followed by a 4-month continuation phase with rifampicin and isoniazid only [14]. Drug resistance is the presence of resistance to any of the anti-TB drugs. In Balimo medications for drug-resistant TB are not available, and patients who may have drug resistance must travel to Daru for diagnosis and treatment.

Treatment outcomes are defined in Table 1, based on the PNG National Tuberculosis Management Protocol [12]. Furthermore, in this study, binary categorisation of treatment success or treatment failure groups has been used (Table 1).

Patient data were primarily obtained from the TB patient register (hereafter called the TB register) held at BDH, including demographic, clinical and laboratory details of all patients diagnosed with TB and commenced on treatment at the hospital during the period 26 April

Table 1 Definitions used for treatment outcomes, based on the Papua New Guinea National Tuberculosis Management Protocol [12]

Group (this study)	Treatment outcome	Definition
Treatment success	Cured	Initially sputum smear-positive, but smear-negative in last month of treatment, and on at least one previous follow-up occasion
	Treatment complete	Completed treatment, but not classified as cure or treatment failure
Treatment failure	Treatment failure	Smear-positive at 5 months or later, or at 2 months if initially smear-negative
	Died	Death for any reason during treatment
	Default	Treatment interruption for two or more consecutive months

2013–25 February 2017. All pages of the register books available at the time of two study visits (April 2016 and February 2017) were transcribed into the TB study spreadsheet in Microsoft Excel. At the second study visit, the TB register was studied again, to update treatment outcomes added since the first visit.

In addition to the TB register, data were obtained from a secondary source – the BDH TB laboratory database. The BDH TB laboratory database is a register of all clinical samples investigated for TB at BDH using ZN-stained smear microscopy, and the results of these investigations.

The TB register records were matched with patients recorded in the BDH laboratory database, based on name and demographic data, and any additional demographic or laboratory data were added to the TB study spreadsheet. For minor discrepancies (e.g. similar but not exact age), the TB register was assumed to be correct for demographic information. However, the majority of patients (1294/1614, 80.2%) could not be matched to the laboratory database due to both a high proportion of clinical diagnoses and small overlap of the data sources.

In this region, people are often uncertain of their age, and there was evident bias towards reporting of even-numbered ages. For this reason, age range categories were used, and where multiple different ages were recorded (e.g. in the TB register and the laboratory database) people were placed in the age category that matched the average of the reported ages.

Statistical analyses were undertaken for 1518 of the 1614 patients recorded in the TB patient register. The 96 excluded patients were diagnosed through non-routine active case detection activities in the Bamu region and were thus not considered to be representative of the usual patient cohort seen at BDH.

Incidence of reported cases was estimated for the Gogodala region only, which consists of Balimo Urban LLG and Gogodala Rural LLG. Incidence was based on case numbers for new patients commenced on treatment in all full months and years of the study period. The total population for the two Gogodala LLG regions was used as the denominator, based on 2011 census data [9]. The most recent estimate of population growth in the province (2.5% per annum for the 2000–2011 period) was used to estimate population for all years subsequent to 2011 [15]. The monthly trend of reported TB cases was based on the month of treatment commencement.

Demographic and clinical categories were tabulated. Chi-square analyses were used to assess differences between patient groups based on TB presentation (pulmonary or extrapulmonary TB, with concurrent or unknown presentations excluded).

Predictors of treatment failure outcomes were analysed using univariate and multivariate logistic regression, based on binary categorisation of treatment success (cured and treatment complete outcomes) or treatment failure (treatment default, death and treatment failure outcomes). Excluded patients included those with a 'transfer out' status (n = 127) or unknown outcome (n = 293); as well as those patients commenced on treatment in the last six full months of the study period (August 2016–February 2017) (n = 368), as insufficient time had elapsed for these patients to have a treatment outcome recorded. All variables included in the univariate model were also included in the multivariate model.

Tabulations, charts and statistical analyses were performed using Stata/IC, version 14. Confidence intervals were calculated using the AusVet 'Confidence limits for a proportion' calculator [16]. Specific and direct standardised incidence rates were calculated using the Epi_Tools spreadsheet [17]. The pie chart and line graph were created using GraphPad Prism 7.

The study was approved by the James Cook University Human Research Ethics Committee under approval number H6432. The Middle Fly District Health Service and the Evangelical Church of PNG Health Service gave permission and support for the project. The PNG Medical Research Advisory Committee approved the study under MRAC No. 17.02.

Results

TB case numbers and the incidence of reported cases for patients commenced on treatment across the study period are shown in Figure 2. The average yearly reported incidence for the three complete years of the study period was 727 cases per 100 000 people per year (Table 2).

The distributions of patient demographic and clinical data are shown in Table 3. Of note is the large proportion of cases in children (25.0%), and that 77.1% of all cases had a diagnosis of extrapulmonary TB. The site of extrapulmonary infection was reported for 275 TB patients. The distribution of sites is shown in Figure 3, with the majority localised to the glands or lymph nodes (n = 108, 37.6%, 95% CI 32.2–43.4); or the spine (n = 88, 30.7%, 95% CI 25.6–36.2).

The 2014–2016 average specific and direct standardised rates per 100 000 people per year for sex are shown in Table 3, according to the national population proportion for PNG from the 2011 census [18]. Based on the standard population proportion, the number of cases in females was smaller than the reported incidence based on their proportion of the population, while the number of

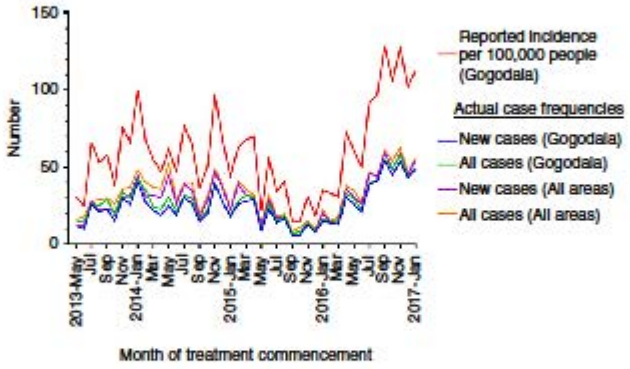

Figure 2 Case frequencies and incidence of reported new cases (per 100 000 people) per month for tuberculosis (TB) across the study period. Cases in the Gogodala region include the Balimo Urban and Gogodala Rural local level government (LLG) areas only.

Table 2 Case numbers and incidence of reported cases (per 100 000 people) per year for new tuberculosis (TB) patients in the Gogodala region

Year	*n*	Calculated population	Reported incidence (per 100 000)
2014	310	40 305	769
2015	197	41 313	477
2016	396	42 346	935
2014–2016	903		727 (Average)

cases in males was higher than the reported incidence based on their proportion of the population.

Pre-treatment ZN smear microscopy results were recorded for 359 of the 1518 TB patients (23.6%, 95% CI 21.6–25.9), including 101/1171 (8.6%, 95% CI 7.2–10.4) patients diagnosed with extrapulmonary TB, 250/321 (77.9%, 95% CI 73.0–82.1) with pulmonary TB, 4/6 (66.7%, 95% CI 30.0–90.3) with concurrent infection and 4/20 (20.0%, 95% CI 8.1–41.6) with unknown infection. Although the majority of smear results were obtained from sputum samples, a small proportion of extrapulmonary smear results were from other clinical specimens (e.g. discharge). For pulmonary TB patients, 65.7% ($n = 211$) were smear-positive, and 12.1% ($n = 39$) were smear-negative; no smear result was recorded for the remaining pulmonary TB patients. The microscopy results for each of the patient groups are shown in Table 4. Where more than one smear result was recorded, the highest smear grade is shown here.

Analysis of pulmonary and extrapulmonary TB patient groups

The analysis comparing pulmonary and extrapulmonary patient groups is shown in Table 5. There was a greater proportion of extrapulmonary TB cases in the 0–14 years group ($X^2 = 117.45$, $P < 0.01$) and those aged 55 and over ($X^2 = 11.45$, $P < 0.01$); and a greater proportion of pulmonary cases in the 15–54 years age groups compared to both the 0–14 years and 55+ years age groups (Table 5). New patients were more likely to be diagnosed with extrapulmonary than pulmonary TB when compared to previously treated patients ($X^2 = 6.15$, $P = 0.01$).

Analysis of treatment outcomes

Of the 1150 patients who had completed the 6-month treatment period, outcomes were known for 857 (Table 6). A complete summary of all registered patients and treatment outcomes is shown in Figure 4.

The logistic regression results for the analysis of treatment outcomes are shown in Table 7. In the univariate analysis, residence in the Bamu Rural LLG was a predictor of treatment failure (OR = 3.00, $P = 0.01$), while in the multivariate analysis, residence in either the Gogodala Rural LLG or Bamu Rural LLG was a predictor of treatment failure (Gogodala Rural, OR = 2.49, $P = 0.02$; Bamu Rural, OR = 4.11, $P < 0.01$).

Co-infection of HIV and TB

Of the 1614 patients recorded in the TB register, 74 (4.6%, 95% CI 3.7–5.7) had a HIV test outcome

Table 3 Patient demographic and clinical data for sex, age category, local level government (LLG) area, tuberculosis (TB) type and patient status

Variable	Frequency	% (95% CI)	Specific and direct standardised incidence rates per 100 000 (2014–2016 average)
Sex			
Female	722	47.6 (45.1–50.1)	709 (specific)
Male	793	52.2 (49.7–54.7)	742 (specific)
Unknown	3	0.2 (0.1–0.6)	726 (standardised)
Age category			
0–14 years	379	25.0 (22.9–27.2)	
15–24 years	254	16.7 (14.9–18.7)	
25–34 years	259	17.1 (15.3–19.0)	
35–44 years	211	13.9 (12.3–15.7)	
45–54 years	192	12.6 (11.1–14.4)	
55–64 years	138	9.1 (7.7–10.6)	
65+ years	31	2.0 (1.4–2.9)	
Unknown	54	3.6 (2.7–4.6)	
LLG area			
Balimo Urban	252	16.6 (14.8–18.6)	
Gogodala Rural	1010	66.5 (64.1–68.9)	
Bamu Rural	199	13.1 (11.5–14.9)	
Kiwai Rural	6	0.4 (0.2–0.9)	
Morehead Rural	4	0.3 (0.1–0.7)	
Nomad Rural	1	0.1 (0.1–0.4)	
Unknown	46	3.0 (2.3–4.0)	
TB type			
Pulmonary	321	21.1 (19.2–23.3)	
Extrapulmonary	1171	77.1 (75.0–79.2)	
Both	6	0.4 (0.2–0.9)	
Unknown	20	1.3 (0.9–2.0)	
Patient status			
New	1375	90.6 (89.0–91.9)	
Treatment after relapse	26	1.7 (1.2–2.5)	
Treatment after failure	11	0.7 (0.4–1.3)	
Treatment after default	77	5.1 (4.1–6.3)	
Transfer in	6	0.4 (0.2–0.9)	
Other	2	0.1 (0.0–0.5)	
Unknown	21	1.4 (0.9–2.1)	

CI, confidence interval; LLG, local level government; TB, tuberculosis.

recorded. Of these, four were positive (5.4%, 95% CI 2.1–13.1), one had no result recorded and the remainder were negative.

Discussion

This study describes the high burden of TB in the Balimo region of PNG and highlights a very high proportion of clinically diagnosed extrapulmonary TB patients. In PNG TB may be diagnosed clinically based on the PNG National Tuberculosis Management Protocol and the WHO definitions [12, 13]. Misdiagnosis due to limited access to laboratory confirmation is possible, and indeed, it is a feature of TB epidemics in these resource-limited settings. Therefore, this study also highlights the need to increase the capacity of laboratory and medical imaging-based diagnostics to aid in the accuracy of diagnosis, which will lead to more directed and evidence-based therapeutic interventions.

There are a number of limitations that may have influenced patient data analysis. Patient data are recorded in handwritten registers by a number of different health workers. Data entry mistakes may occur, and some patient data may go unrecorded. Record-keeping in this resource-limited region is undoubtedly difficult, especially given the lack of digital records. There are also inherent challenges resulting from the use of records in separate

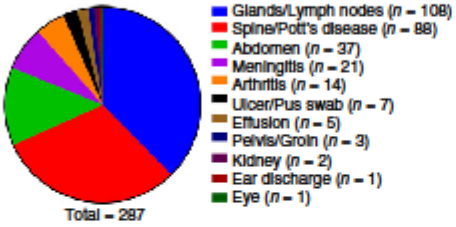

Figure 3 Distribution of extrapulmonary tuberculosis (TB) site of infection. The site was known for 275 TB patients, with a total of 287 sites recorded as some patients had more than one site recorded.

patient and laboratory registers, particularly in locating and updating historical records. Despite this, the TB register was assumed to be correct in the classification of patients, even if it was unclear how those decisions were reached, and given that the TB register represents the official TB record. For example, if a patient was classified as cured, but only one negative smear was recorded in the register, the cured classification was still used in this study. Furthermore, any missing treatment commencement dates were assumed to be the same as the patient registration date. As already noted, there are inherent problems with some data, including age inaccuracies. These problems are unavoidable in a data set such as this, but are mentioned here due to the need to interpret the results with caution. Given the challenges of travel throughout the South Fly and Middle Fly Districts of the province, it is possible that some Gogodala- and Bamu-LLG area patients present directly at Daru or other peripheral health facilities instead of at Balimo. Thus, the TB data presented here are not intended to be an exhaustive record of TB patients for the Balimo region, but a description of the TB cases presenting at BDH, the only hospital in the Middle Fly District.

The overall incidence of reported new cases for TB in the Balimo Urban and Gogodala Rural LLG areas, estimated here to range from 477 to 935 cases per 100 000 people per year, is substantially higher than the PNG-wide WHO estimates. In 2016, 27 294 new TB cases were notified in a population of 8.1 million people, equating to 337 case notifications per 100 000 people per year [19]. By further comparison, the Western Pacific Region reported 1 305 408 new cases in a population of 1.9 billion people, equating to 69 case notifications per 100 000 people per year [19].

The extremely high number of TB diagnoses emphasises the heavy burden of disease that TB causes in this region. Our estimate for the Balimo region is higher than the previous Western Province region incidence estimate of 549 cases per 100 000 people per year [4]. The South Fly District and the provincial capital of Daru have previously been noted for having a high burden of TB, and particularly DR-TB [2–5, 20–22]. A heavy burden of TB cases in the North Fly District has previously been observed, while more recent research described 18 cases of TB from Tabubil, with one of these identified as rifampicin resistant [4, 5]. The data from the North Fly District, in combination with the Middle Fly District data described in our study, highlight the broad reach of TB across Western Province. Well-resourced health services across all three districts are essential to respond to TB across the province, and until that time, the burden on the urban capital Daru will continue.

In this study of the Balimo region, extrapulmonary TB accounted for more than 75% of TB diagnoses. Globally, extrapulmonary TB usually accounts for about 15% of TB cases, although in the WHO Western Pacific Region, extrapulmonary TB cases are reported to be as low as 8% [1]. The most recent nation-wide data for TB in PNG reported that 43% of cases were extrapulmonary;

Table 4 Pre-treatment Ziehl-Neelsen (ZN) smear microscopy results for patients diagnosed with tuberculosis (TB) at Balimo District Hospital (BDH), based on the highest smear grade recorded for each patient

	Extrapulmonary, *n* (%)	Pulmonary, *n* (%)	Concurrent, *n* (%)	Unknown, *n* (%)
3+	4 (0.3)	100 (31.2)	0 (0)	1 (5.0)
2+	2 (0.2)	58 (18.1)	1 (16.7)	0 (0)
1+	4 (0.3)	32 (10.0)	0 (0)	1 (5.0)
Scanty	0 (0)	19 (5.9)	0 (0)	0 (0)
Positive*	0 (0)	2 (0.6)	0 (0)	0 (0)
NAFB	91 (7.8)	39 (12.1)	3 (50.0)	2 (10.0)
Unsatisfactory	1 (0.1)	0 (0)	0 (0)	0 (0)
Unknown	1 (0.1)	0 (0)	0 (0)	0 (0)
No smear	1068 (91.2)	71 (22.1)	2 (33.3)	16 (80.0)
Total	1171 (100.0)	321 (100.0)	6 (100.0)	20 (100.0)

*Grade unknown; NAFB: no acid-fast bacilli.

Table 5 Chi-square analysis comparing pulmonary and extrapulmonary tuberculosis (TB) patient groups by sex, age, incoming patient status, local level government (LLG) area and treatment outcome

		TB type	
Variable	Frequency	Pulmonary	Extrapulmonary
Sex	*n*	Freq (%)	Freq (%)
Female	706	141 (20.0)	565 (80.0)
Male	783	180 (23.0)	603 (77.0)
Total	1489	321	1168
Pearson $X^2 = 2.00$, df = 1, $P = 0.16$			
Age category	*n*	Freq (%)	Freq (%)
0–14 years	376	11 (2.9)	365 (97.1)
15–54 years	905	278 (30.7)	627 (69.3)
Total	1281	289	992
Pearson $X^2 = 117.45$, df = 1, $P < 0.01$			
15–54 years	905	278 (30.7)	627 (69.3)
55+ years	168	30 (17.9)	138 (82.1)
Total	1073	308	765
Pearson $X^2 = 11.45$, df = 1, $P < 0.01$			
Patient status	*n*	Freq (%)	Freq (%)
New	1363	283 (20.8)	1080 (79.2)
Previously treated	114	35 (30.7)	79 (69.3)
Total	1477	318	1159
Pearson $X^2 = 6.15$, df = 1, $P = 0.01$			
Treatment outcome	*n*	Freq (%)	Freq (%)
Treatment success	605	169 (27.9)	436 (72.1)
Treatment failure	119	25 (21.0)	94 (79.0)
Total	724	194	530
Pearson $X^2 = 2.43$, df = 1, $P = 0.12$			
LLG area	*n*	Freq (%)	Freq (%)
Balimo Urban	249	43 (17.3)	206 (82.7)
Gogodala Rural	995	223 (22.4)	772 (77.6)
Bamu Rural	192	45 (23.4)	147 (76.6)
Total	1436	311	1125
Pearson $X^2 = 3.52$, df = 2, $P = 0.17$			

LLG, local level government; TB, tuberculosis.

Table 6 Tuberculosis (TB) treatment outcomes for all patients who had completed the 6-month treatment period, and where the treatment outcome was known

Treatment outcome	*n*	% (95% CI)
Cured	90	10.5 (8.6–12.7)
Treatment complete	521	60.8 (57.5–64.0)
Default	47	5.5 (4.1–7.2)
Treatment failure	5	0.6 (0.3–1.4)
Died	67	7.8 (6.2–9.8)
Transfer out	127	14.8 (12.6–17.4)
Total	857	100.0

while a study investigating presumptive TB in children hospitalised at Modilon in Madang Province found extrapulmonary TB to be the final diagnosis in 52.3% of paediatric TB cases [1, 23]. One study from PNG has commented on the prevalence of extrapulmonary TB in the country, noting that both under-diagnosis and over-diagnosis are possible outcomes in settings where diagnoses are predominantly symptom based [24]. The large proportion of extrapulmonary TB diagnoses in the Balimo region requires further investigation.

The 1:1.1 ratio of female to male TB cases identified in this study differs from the PNG national, South-East Asia and Western Pacific Region ratios, which in 2016 had estimated female:male incidence ratios of 1:1.7, 1:1.9 and 1:2.1, respectively [1]. Various factors could contribute to these differences, including TB contacts, health-seeking behaviour and smoking [25–28]. In PNG, the prevalence of tobacco smoking in males is 37.3% vs. only 14.5% in females [29]. As a result, it appears that smoking may not be a major risk factor affecting the female:male ratio in the Balimo setting. However, it should be noted that cooking fires are used extensively in this setting, which may affect more females and children.

The age distribution of TB cases in the Balimo region showed cases aged 0–14 years to be the largest group. In the analysis of pulmonary and extrapulmonary TB cases, the very low number of pulmonary cases in children aged up to 14 years is also of note. This corresponds with the general presentation of TB in children, which tends to be paucibacillary and have lymph node involvement [30]. However, from an epidemiological perspective high numbers of TB cases in children is concerning, as they tend to indicate recent *Mycobacterium tuberculosis* transmission, as well as ongoing transmission within the community [31]. The impact of TB in children in this region requires further research.

Given that many of the TB cases reported in this study are clinically diagnosed without bacteriological confirmation, there is the possibility of TB misdiagnosis among presenting patients. Furthermore, factors such as case detection bias should also be considered, particularly in relation to high case numbers in children, and of extrapulmonary TB. However, treatment outcomes suggest that over-diagnosis is not a major concern. More than 70% of TB patients with a known treatment outcome were classified as 'treatment success', meaning that they were either cured or successfully completed treatment, in the absence of classification as treatment failure. In a region where successful treatment is often the best supporting evidence for a TB diagnosis, this suggests that at

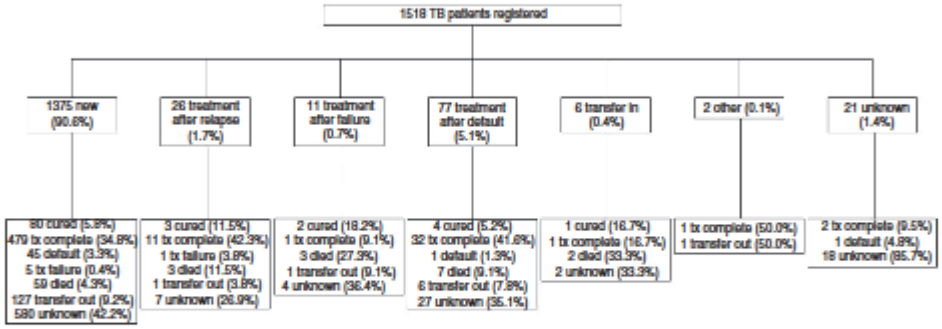

Figure 4 Incoming patient status and treatment outcomes for all tuberculosis (TB) patients registered during the study period. All unknown treatment outcomes are included, regardless of whether the patient had been under treatment long enough to have completed their course of medication.

Table 7 Univariate and multivariate logistic regression examining predictors of treatment failure in all tuberculosis (TB) patients. A total of 680 complete observations were included in the multivariate model

		Univariate			Multivariate	
Predictor variables		*n*	OR (95% CI)	*P*	OR (95% CI)	*P*
Sex	Female	343	1.0		1.0	
	Male	385	1.45 (0.97–2.16)	0.07	1.45 (0.94–2.22)	0.09
Age	0–14	188	1.0		1.0	
	15–24	140	0.62 (0.34–1.15)	0.13	0.81 (0.42–1.56)	0.53
	25–34	132	0.75 (0.41–1.37)	0.36	0.90 (0.46–1.73)	0.75
	35–44	102	0.62 (0.31–1.23)	0.17	0.67 (0.31–1.43)	0.30
	45–54	78	0.84 (0.42–1.70)	0.64	1.10 (0.52–2.37)	0.80
	55–64	64	1.18 (0.59–2.37)	0.64	1.63 (0.78–3.41)	0.20
	65+	12	1.41 (0.36–5.46)	0.62	1.65 (0.42–6.52)	0.48
Patient status	New	655	1.0		1.0	
	Prev. treated	67	1.43 (0.77–2.68)	0.26	1.47 (0.77–2.79)	0.24
TB type	Pulmonary	194	1.0		1.0	
	EP	530	1.46 (0.91–2.35)	0.12	1.47 (0.86–2.50)	0.16
LLG area	Balimo	110	1.0		1.0	
	Gogodala	492	1.97 (0.99–3.93)	0.06	2.49 (1.15–5.40)	0.02
	Bamu	104	3.00 (1.36–6.64)	0.01	4.11 (1.70–9.96)	<0.01

EP, extrapulmonary; LLG, local level government; OR, odds ratio; TB, tuberculosis.

BDH the recognition of non-laboratory confirmed cases of TB by clinicians is reasonable. Despite this, the use of non-TB treatments such as amoxicillin and aspirin has previously been described in the Balimo region [6], although whether these are used concomitantly with TB treatment is unknown. The possibility of symptom resolution even if extrapulmonary TB has been incorrectly diagnosed should therefore be considered. However, regardless of the accuracy of TB diagnoses in this setting, misdiagnoses of TB may in fact place greater burdens on the health system, particularly if symptoms are not resolved.

It is also of note that in this study, a relatively high proportion of pulmonary TB cases had a positive smear result, being 65.8% vs. 31% of pulmonary TB cases with bacteriological confirmation across PNG [1]. The low proportion of smear-negative pulmonary TB cases may reflect additional challenges in pulmonary TB diagnosis, particularly in this setting where chest X-ray was not available at the time of the study.

The increased likelihood of new patients being diagnosed with extrapulmonary TB, while previously treated patients were more likely to be diagnosed with pulmonary TB, may have several possible explanations. The higher proportion of pulmonary TB diagnoses in previously treated cases may be influenced by emerging drug resistance in the region. DR-TB is known to occur in Balimo and is likely to be more prevalent among pulmonary cases, particularly if these cases are more likely to truly be TB. An additional possibility for the increased likelihood of previous treatment among pulmonary cases is the status of the Balimo region as an area endemic for melioidosis [32–34], which frequently presents with pulmonary symptoms, and may initially be misdiagnosed as TB. Furthermore, limited diagnostic facilities in general result in challenges to infectious disease investigation and management in this region.

Treatment outcomes were unknown for just over 25% of patients who completed treatment at least 6 months before the end of the study period. This proportion may reflect the flexibility with which DOTS is often administered in the region, where patients may take their treatment packs back to their home village for the duration of treatment [6]. In this study, the only factor found to be significantly associated with poorer treatment outcomes was the LLG area in which a patient resided, with poorer outcomes more likely in the rural LLGs. This finding may be linked to the challenges associated with obtaining health care for those patients who have a rural residence. TB patients living in or around the Balimo Urban LLG will face substantially lower travel and economic burdens in obtaining care initially, and continuing treatment once diagnosed.

Conclusions

The extremely high reported incidence of TB, and particularly the high proportion of extrapulmonary TB, demonstrates a heavy burden of TB disease in the Balimo region. Increased understanding of the epidemiology of TB in this setting provides important information in the context of TB control and elimination in Western Province and PNG more broadly. Although improved resources and facilities are an urgent need at BDH, this study has also demonstrated the substantial success of healthcare workers (HEOs, clinical nurses and laboratory technicians) in diagnosing, treating and managing TB in this non-doctor-led model of care setting, and the dedication of these staff to this task. The burden of disease in this newly described TB-endemic region emphasises the need for the role of BDH to be considered in the broader Western Province TB control programme.

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References

1. World Health Organization. Global tuberculosis report 2017. Geneva, Switzerland: World Health Organization; 2017. (Available from: http://apps.who.int/iris/bitstream/10665/259366/1/9789241565516-eng.pdf?ua=1) [16 Jan 2018].
2. Gilpin CM, Simpson G, Vincent S *et al.* Evidence of primary transmission of multidrug-resistant tuberculosis in the Western Province of Papua New Guinea. *Med J Aust* 2008: 188: 148–152.
3. Simpson G, Coulter C, Weston J *et al.* Resistance patterns of multidrug-resistant tuberculosis in Western Province, Papua New Guinea. *Int J Tuberc Lung Dis* 2011: 15: 551–552.
4. McBryde E. Evaluation of risks of tuberculosis in Western Province Papua New Guinea. Barton, Australia: Department of Foreign Affairs and Trade; 2012. (Available from: https://www.burnet.edu.au/system/publication/file/3606/2012_Evaluation_of_Risks_of_Tuberculosis_in_Western_Province_PNG.pdf) [31 Oct 2017].
5. Aia P, Kal M, Lavu E *et al.* The burden of drug-resistant tuberculosis in Papua New Guinea: results of a large population-based survey. *PLoS ONE* 2016: 11: e0149806.
6. Diefenbach-Elstob T, Plummer D, Dowi R *et al.* The social determinants of tuberculosis treatment adherence in a remote region of Papua New Guinea. *BMC Public Health* 2017: 17: 70.
7. Horwood PF, Karl S, Mueller I *et al.* Spatio-temporal epidemiology of the cholera outbreak in Papua New Guinea, 2009-2011. *BMC Infect Dis* 2014: 14: 449.
8. The National. Authorities respond to cholera on Daru. Port Moresby, Papua New Guinea: The National; 2010. (Available from: http://www.thenational.com.pg/authorities-respond-to-cholera-on-daru/) [25 Oct 2017].
9. National Statistical Office. 2011 National population & housing census: ward population profile – southern region. Port Moresby, Papua New Guinea: National Statistical Office; 2014. (Available from: www.nso.gov.pg/index.php/document-library?view=download&fileId=64) [31 Oct 2017].
10. Government of Papua New Guinea. National Health Service standards for Papua New Guinea 2011–2020: volume 1: Government of Papua New Guinea; 2011. Available from: https://www.mindbank.info/item/1670 [5 Mar 2018].

11. Government of Papua New Guinea. National Health Service standards for Papua New Guinea 2011-2020: volume 2: Government of Papua New Guinea; 2011. (Available from: https://www.mindbank.info/item/1670) [8 Mar 2018].
12. Department of Health. Papua New Guinea: national tuberculosis management protocol. Port Moresby, Papua New Guinea: Department of Health; 2011. Available from: http://www.adi.org.au/wp-content/uploads/2016/11/National-Tuberculosis-Management-Protocol-PNG-2011.pdf [31 Oct 2017].
13. World Health Organization. Definitions and reporting framework for tuberculosis – 2013 revision (updated December 2014). Geneva, Switzerland: World Health Organization; 2013. (Available from: http://apps.who.int/iris/bitstream/10665/79199/1/9789241505345_eng.pdf?ua=1) [18 Jan 2018].
14. World Health Organization. *Treatment of Tuberculosis: Guidelines* (4th edn). Geneva, Switzerland: World Health Organization; 2010. (Available from: http://www.who.int/tb/publications/2010/9789241547833/en/) [5 Dec 2017].
15. National Statistical Office. Final figures – Papua New Guinea: national population & housing census 2011. Port Moresby, Papua New Guinea: National Statistical Office; 2013. (Available from: http://www.nso.gov.pg/index.php/document-library?view=download&fileId=65) [31 Oct 2017].
16. Sergeant E. *Epitools Epidemological Calculators*. Canberra, Australia: Ausvet Pty Ltd; 2017. (Available from: http://epitools.ausvet.com.au/).
17. LaMorte WW. *Epidemiology/Biostatistics Tools*. Boston, MA, USA: Boston University School of Public Health; 2006. (Available from: http://sphweb.bumc.bu.edu/otlt/mph-modules/ep/ep713_randomerror/Epi_Tools.xlsx).
18. National Statistical Office. Papua New Guinea: 2011 national report. Waigani, Papua New Guinea: National Statistical Office; 2015. (Available from: http://sdd.spc.int/en/resources/document-library?view=preview&format=raw&fileId=218) [31 Oct 2017].
19. World Health Organization. TB_notifications_2018-01-17.csv. Geneva, Switzerland: World Health Organization; 2018. (Available from: http://www.who.int/tb/country/data/download/en/).
20. Furin J, Cox H. Outbreak of multidrug-resistant tuberculosis on Daru Island. *Lancet Respir Med* 2016: 4: 347–349.
21. Kase P, Dakulala P, Bieb S. Outbreak of multidrug-resistant tuberculosis on Daru Island: an update. *Lancet Respir Med* 2016: 4: e40.
22. Chandler J. *'Ebola with Wings': Expert Raises Alarm over Deadly Tuberculosis Outbreak in PNG*. Sydney, Australia: ABC RN; 2016. (Available from: http://www.abc.net.au/radionational/programs/backgroundbriefing/experts-raise-alarm-over-deadly-tuberculosis-outbreak-in-png/7327018).
23. Watch V, Aipit J, Kote-Yarong T *et al.* The burden of presumed tuberculosis in hospitalized children in a resource-limited setting in Papua New Guinea: a prospective observational study. *Int Health* 2017: 9: 374–378.
24. Karki B, Kittel G, Bolokon I, Duke T. Active community-based case finding for tuberculosis with limited resources: estimating prevalence in a remote area of Papua New Guinea. *Asia Pacific J Public Health* 2017: 29: 17–27.
25. Mason PH, Roy A, Spillane J, Singh P. Social, historical and cultural dimensions of tuberculosis. *J Biosoc Sci* 2016: 48: 206–232.
26. Mason PH, Snow K, Asugeni R, Massey PD, Viney K. Tuberculosis and gender in the Asia-Pacific region. *Aust N Z J Public Health* 2017: 41: 227–229.
27. Watkins RE, Plant AJ. Does smoking explain sex differences in the global tuberculosis epidemic? *Epidemiol Infect* 2006: 134: 333–339.
28. Grandjean L, Crossa A, Gilman RH *et al.* Tuberculosis in household contacts of multidrug-resistant tuberculosis patients. *Int J Tuberc Lung Dis* 2011: 15: 1164–1169.
29. World Health Organization. WHO report on the global tobacco epidemic, 2017 – country profile Papua New Guinea. Geneva, Switzerland: World Health Organization; 2017. (Available from: http://www.who.int/tobacco/surveillance/policy/country_profile/png.pdf?ua=1).
30. Marais BJ. Tuberculosis in children. *J Paediatr Child Health* 2014: 50: 759–767.
31. Marais BJ, Obihara CC, Warren RM, Schaaf HS, Gie RP, Donald PR. The burden of childhood tuberculosis: a public health perspective. *Int J Tuberc Lung Dis* 2005: 9: 1305–1313.
32. Warner JM, Pelowa DB, Currie BJ, Hirst RG. Melioidosis in a rural community of Western Province, Papua New Guinea. *Trans R Soc Trop Med Hyg* 2007: 101: 809–813.
33. Warner JM, Pelowa DB, Gal D *et al.* The epidemiology of melioidosis in the Balimo region of Papua New Guinea. *Epidemiol Infect* 2008: 136: 965–971.
34. Diefenbach-Elstob TR, Graves PM, Burgess GW, Pelowa DB, Warner JM. Seroepidemiology of melioidosis in children from a remote region of Papua New Guinea. *Int Health* 2015: 7: 332–338.

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APPENDIX 7

STANDARD PERMISSIONS FOR USE OF ARCGIS ONLINE BASEMAPS AND GOOGLE MAPS

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APPENDIX 8

PUBLICATIONS, PRESENTATIONS, AND OTHER ACHIEVEMENTS THROUGHOUT DOCTORAL STUDIES

Publication:

Diefenbach-Elstob T, Graves P, Dowi R, Gula B, Plummer D, McBryde E, Pelowa D, Siba P, Pomat W and Warner J (2018) The epidemiology of tuberculosis in the rural Balimo region of Papua New Guinea. *Tropical Medicine & International Health.* In Press. https://doi.org/10.1111/tmi.13118

Conference presentations:

'Social and cultural determinants of TB treatment adherence in a remote region of Papua New Guinea' (June 2015) AIMS Tropical Division Scientific Meeting, Townsville Australia
– Oral presentation

'The sociocultural determinants of tuberculosis treatment adherence in the rural Balimo region of Papua New Guinea' (September 2015) Australasian Tropical Health Conference, Palm Cove, Australia
– Poster presentation

'Tuberculosis and drug resistance in the Balimo region of Papua New Guinea' (September 2015) Australasian Tropical Health Conference, Palm Cove, Australia
– Oral presentation

'Tuberculosis and spatial epidemiology in the Balimo region of Papua New Guinea' (December 2016) JCU CPHMVS 1st HDR Student Conference, Townsville, Australia
– Oral presentation

'Tuberculosis in rural Papua New Guinea: towards enhancing clinical diagnoses in a resource-limited setting' (March 2017) 6th Conference of International Union Against Tuberculosis and Lung Disease, Asia Pacific Region, Tokyo, Japan
– Poster presentation

'The epidemiology of presumptive tuberculosis cases in the rural Balimo district of Western Province, Papua New Guinea' (September 2017) CBMDT & CBTID 2017 Annual Scientific Retreat, Cairns, Australia
– Poster presentation

'The epidemiology of tuberculosis in the Balimo district of Western Province, Papua New Guinea' (September 2017) Australasian Tropical Health Conference, Cairns, Australia
– Oral presentation

'The epidemiology of tuberculosis in the Balimo district of Western Province, Papua New Guinea' (December 2017) PNG Impact, Port Moresby, Papua New Guinea
– Oral presentation

'The epidemiology of tuberculosis in the Balimo district of Western Province, Papua New Guinea' (December 2017) 2017 CPHMVS HDR Student Symposium, Townsville, Australia
– Oral presentation

'Tuberculosis in the Balimo region of Western Province, PNG' (May/June 2018) 7th Tuberculosis Control Symposium, TB-CRE, Sydney Australia
– Oral presentation

Other presentations and media:

2016 JCU 3-Minute Thesis (3MT) competition – Title: TB or not TB: that is the question

- College of Public Health, Medical and Veterinary Sciences – Finalist
- Division of Tropical Health and Medicine – Finalist

Brighter article: Battling a forgotten disease in remote Papua New Guinea – Available from: https://www.jcu.edu.au/brighter/articles/battling-a-forgotten-disease-in-remote-papua-new-guinea

Grants:

Graduate Research Scheme Grants-In-Aid 2015, Round 2 - $3240

Higher Degree by Research Enhancement Scheme – Grants-In-Aid 2016, Round 2 - $2500

Higher Degree by Research Enhancement Scheme – Grants-In-Aid 2017, Round 1 - $3000