Understanding the epidemiology and transmission of tuberculosis among adults in rural and urban Tanzania

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Prof. Dr. Martin Spiess
Dekan

Dedicated to my dearest family,

my mother Flora, my father Joseph and

my late grandmother Freda whose effort in academia inspired me to do this.

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2. Rutaihwa LK, Sasamalo M, Jaleco A, **Hella J,** et al., **Insights into the genetic diversity of *Mycobacterium tuberculosis* in Tanzania.** PLoS One. 2019 Apr 12;14(4):e0206334. doi: 10.1371/journal.pone.0206334
3. Mhalu G, Weiss MG, **Hella J**, Mhimbira F, et al., **Explaining patient delay in healthcare seeking and loss to diagnostic follow-up among patients with presumptive tuberculosis in Tanzania: a mixed-methods study**. BMC Health Serv Res. 2019 Apr 5;19(1):217. doi: 10.1186/s12913-019-4030-4.
4. Said K, **Hella J**, Ruzegea M, Solanki R, et al., **Immunologic-based diagnosis of latent tuberculosis among children less than 5 years of age exposed and unexposed to tuberculosis in Tanzania: Implications for tuberculosis infection screening.** Pediatr Infect Dis J. 2019 Apr;38(4):333-339. doi: 10.1097/INF.0000000000002131.
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7. Mhimbira F, Hiza H, Mbuba E, **Hella J**, et al., **Prevalence and clinical significance of respiratory viruses and bacteria detected in tuberculosis patients compared to household controls in Tanzania: A cohort study.** Clin Microbiol Infect. 2019 Jan; 25 (1): 107.e1-107.e7. doi: 10.1016/j.cmi.2018.03.019
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10. Mhimbira F, **Hella J**, Said K, Kamwela L, et al., **Prevalence and clinical relevance of helminth co-infections among tuberculosis patients in urban Tanzania**. PLoS Negl Trop Dis. 2017 Feb 8;11(2):e0005342. doi: 10.1371/journal.pntd.0005342.

Abbreviations

AFB	Acid fast bacilli
AIDS	Acquired immune-deficiency syndrome
aOR	Adjusted Odds Ratio
ART	Anti-retroviral therapy
BCG	Bacillus-Calmette-Guérin
BMI	Body mass index
CDC	Centre for Disease Control and Prevention
CI	Confidence interval
DOT	Directly observed therapy
EKNZ	Ethical Committee of North-western and Central Switzerland
ELISA	Enzyme-linked immunosorbent assay
EPTB	Extrapulmonary Tuberculosis
FBC	Full Blood Count
GIS	Geographical Information System
GPS	Geographical Positioning System
Hb	Haemoglobin
HIV	Human immunodeficiency virus
ICF	Informed Consent Form
IFN-γ	Interferon-gamma
IGRA	Interferon-gamma release assay
IHI	Ifakara Health Institute
INH	Isoniazid
IPT	Isoniazid preventive therapy
IQR	Interquartile range
IRB	Institutional Review Board
MDA	Mass Drug Administration
MDR-TB	Multi-drug resistant tuberculosis
MoHCDGEC	Ministry of Health, Community Development, Gender, Elderly and Children
MTBC	*Mycobacterium tuberculosis* complex
NBS	National Bureau of Statistics
NIMR	National Institute for Medical Research
NTD	Neglected Tropical Disease

NTLP	National Tuberculosis and Leprosy Program
NTP	National Tuberculosis Program
ODK	OpenDataKit
OR	Odds ratio
POC-CCA	Point-of-Care Circulating Cathodic Antigen
PTB	Pulmonary Tuberculosis
SD	Standard deviation
SES	Socio-economic status
sTfR	Soluble Transferrin Receptor
STH	Soil Transmitted Helminths
Swiss TPH	Swiss Tropical and Public Health Institute
TB	Tuberculosis
TST	Tuberculin skin test
WHO	World Health Organization

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Summary

Background: Tuberculosis (TB) is the leading cause of death in the world from a single infectious disease and resulted in about 1.5 million deaths in 2018. Globally, close to one third of the population is latently infected with *Mycobacterium tuberculosis* (Mtb), of which about 5-10% during their lifetime, will develop active TB depending on the age of infection. TB is an airborne disease transmitted by airways from one person to the next when an uninfected individual breathes in air containing Mtb expired from an infected person. One way to estimate the risk of TB transmission begins with measuring environmental levels of carbon dioxide (CO_2) levels exhaled by humans. CO_2 levels combined with social contact data allow calculation of the volumes of rebreathed air in order to estimate the potential for airborne disease transmission by considering time at risk, quanta of contagion and the number of people occupying the confined space etc.

Unfortunately, most TB patients are diagnosed at later stages of the disease, due to poor health-seeking behavior, inappropriate diagnostic investigations requested by health care providers, and limited access to better TB diagnostics which forces patients to seek relief by using self-prescribed medication. Delaying diagnosis and treatment of TB has important consequences for disease control at both the individual (poor treatment outcome) as well as the community level (continued transmission). Within the country, the prevalence of TB varies considerably across regions, and is higher among males, older persons, and those with lower socioeconomic status. Lastly, patients with TB often have additional comorbidities such as anemia, helminthiasis etc., which can result in poor treatment outcomes. Anemia of chronic disease is primarily found in patients with chronic disease status such as those with chronic immune activation such as TB and HIV-positive patients.

Objectives: The overall goal of this PhD project was to understand the epidemiology and transmission of tuberculosis in Tanzania by studying the infrastructure-related risk of TB transmission through measuring environmental CO_2 levels at locations of public importance; determining the TB diagnostic delay and its associated factors among TB patients; understanding differences in the epidemiology of TB and comorbidities among TB patients from rural and urban Tanzania; and investigating the association of hepcidin levels with coinfections, TB disease severity and progression from TB infection to disease among adults in Tanzania.

Methods: This PhD project was embedded in the ongoing hospital-based cohort of adult TB cases and controls in Temeke, Dar es Salaam and Ifakara, Morogoro, Tanzania. The field work for this PhD study was carried out between September 2016 and April 2018. The project had two designs; infrastructure-related and patient-related design. For objective 1, an observational study was carried out which

employed exposure assessment methods where we collected environmental data (CO2, geo-coordinates etc.,) from locations of public importance. We used the modified Wells-Riley equation to estimate the annual risk of TB transmission which was calculated as a function of time spent per year at a given location. For objectives 2-3, we analysed data from the ongoing cohort of adult TB patients and their household contacts (TB DAR). Briefly, recruitment of study participants began in 2013 at Temeke Regional Referral Hospital (Temeke district) and 2014 at the St. Francis Referral Hospital (Kilombero district). Participants collected baseline information and provided specimen for further analysis. Multivariate logistic regression models were used to find associations and presented as crude and adjusted odds ratio. Lastly, for objective 4, we designed a nested case-control study matched by age and sex. Descriptive statistics were used to summarize participants characteristics. We compared cases and controls using conditional logistic regression model to determine associations between outcomes of interest and various predictors.

Results: We found that the annual risks of TB transmission were highest among prison inmates (41.6%) and drivers (20.3%) in public transport. Lower transmission risks were found in central markets (4.8% for traders, but 0.5% for their customers), passengers on public transport (2.4%), public schools (4.0%), nightclubs (1.7%), religious (0.13%), and social halls (0.12%).

Diagnostic delay was positively associated with absence of chest pain (a*OR* = 7.97, 95% confidence intervals [*CI*] = 3.15 – 20.19), and presence of hemoptysis (a*OR* = 25.37, 95% *CI* = 11.15 – 57.74) and negatively associated with use of medication prior to TB diagnosis (a*OR* = 0.31, 95% *CI* = 0.14 – 0.71). Patients living far from pharmacies were less likely to visit a health care facility (incremental increase of distance versus visit to any facility: *OR* = 0.51, 95% *CI* = 0.28 – 0.96).

Patients from the rural setting were older (median age 37 years *vs.* 34 years, p=0.003), had a lower median body mass index (17.5 kg/m^2 *vs.* 18.5 kg/m^2, p<0.001), a higher proportion of recurrent TB cases (9% *vs.* 1%, p<0.001) compared to those from urban Tanzania. The overall prevalence of helminth co-infections was 22.9% with differences in the etiology of helminthiasis. The higher prevalence of helminthiasis in the urban setting (25.7% *vs.* 17.3%, p=0.018) was predominantly driven by *Strongyloides stercoralis* (17.0% *vs.* 4.8%, p<0.001) while *Schistosoma mansoni* infection (4.1% *vs.* 16.4%, p<0.001) dominated in the rural setting. Recurrent TB was associated with living in a rural setting (adjusted odds ratio [aOR] 3.97, 95% CI 1.16-13.67) and increasing age (aOR 1.06, 95% CI 1.02-1.10).

Anemia of chronic disease (ACD) was more frequent among cases than controls (59.8% vs. 26.1%), but iron-deficiency anemia more frequent in controls (10% vs. 1%). The median hepcidin level was higher in cases than controls (63.7 vs. 14.2 ng/mL), but coinfections with HIV, helminths, and respiratory pathogens did not show cumulative effects. Hepcidin was associated with more severe TB symptom scoring (coefficient 0.8, 95% confidence interval [CI] 0.5-1.2) and higher mycobacterial load

(0.7, 95% CI 0.4-1.0). Hepcidin was higher in TB cases and controls who developed TB compared to controls without TB ($p<0.001$), even when restricting to HIV-negative study participants.

Conclusions: From this PhD project, we have demonstrated that environmental CO_2 from locations of public importance, could identify locations with higher risk of TB transmission. This novel approach can be used by National TB Programs (NTP) to guide targeted infection control interventions for TB control in sub-Saharan Africa to reduce TB transmission. Adults from urban Tanzania, are still faced with significant TB diagnosis delay and that the use of medication prior to TB diagnosis was common. This provides a unique opportunity for inclusion of pharmacies in the formal referral systems from communities to TB diagnostic facilities. We found TB patients from rural Tanzania were likely to be older, with recurrent TB, with features of advanced TB disease due to a longer TB diagnosis delay and seek more frequently care from traditional healers. The overall prevalence of helminth co-infections among TB patients was higher in urban Tanzania, predominantly driven by *Strongyloides stercoralis* infection, but the prevalence of *Schistosoma mansoni* was higher in the rural Tanzania. NTPs should strive to understand fundamental differences in the TB epidemiology in different settings and translate that into specific interventions based on such differences. For example, the NTLP should build the capacity of traditional healers in rural Tanzania, for improving early detection of TB cases and referral for TB treatment. Lastly, we found hepcidin to be marginally upregulated by coinfections other than Mtb, and thus this could be used as a marker for identifying patient with severe TB disease and high-risk persons exposed to TB.

1. Introduction

It is estimated that close to one third of the world population is latently infected with *Mycobacterium tuberculosis* (Mtb) (Houben and Dodd, 2016), and about 1.5 million people died from tuberculosis (TB) in 2018 (World Health Organization, 2019). Globally, TB caused 10.0 million new cases in 2018 of which 8.6% were people living with Human immuno-deficient virus (HIV) infection. The disease remains among the 10 leading causes of death worldwide despite major steps taken in controlling the disease (World Health Organization, 2019). Among people who are latently infected with TB, 5-10% will develop active TB depending on the age of infection (Rieder, 1999; Vynnycky and Fine, 1997).

Progressing from latent TB infection (LTBI) to clinically active disease has been associated with many risk factors, including HIV co-infection, diabetes, silicosis, underweight, host-related genetic polymorphisms, and others (Rieder, 1999). Among these risk factors, HIV infection has been by far, the most important risk factor (Corbett et al., 2003; Selwyn et al., 1992). The HIV increases the lifetime risk of progression from Mtb infection to active TB by more than three times as compared to persons without HIV infection (Selwyn et al., 1992). HIV co-infection importantly contributes to the global increase of TB incidence: globally around 7.4% to 10% of TB cases are HIV-positive (Dye, 2006; World Health Organization, 2019).

1.1. Global burden of tuberculosis

Globally, TB is the leading cause of death from a single infectious disease and resulted in about 1.5 million deaths in 2018 (World Health Organization, 2019). The disease is considered as one of the oldest recorded human afflictions and has resulted in more deaths worldwide than any other causes combined in the last 200 years. The disease is a major public health problem in many developing countries despite presence of a vaccine (i.e., BCG) and antibiotics which could cure TB. In 2018, nearly 10 million people suffered from TB with this burden seen to be stabilizing over the last few years due to increased multilateral efforts put in TB control (World Health Organization, 2019).

There still large geographical differences in the burden of TB among the WHO regions, with the highest burden being in the South-East Asia (44%), followed by Africa (24%) and the Western

Pacific (18%). As per individual countries, there are still 30 countries which account for 87% of all cases notified in 2018 worldwide (World Health Organization, 2019) (Figure 1).

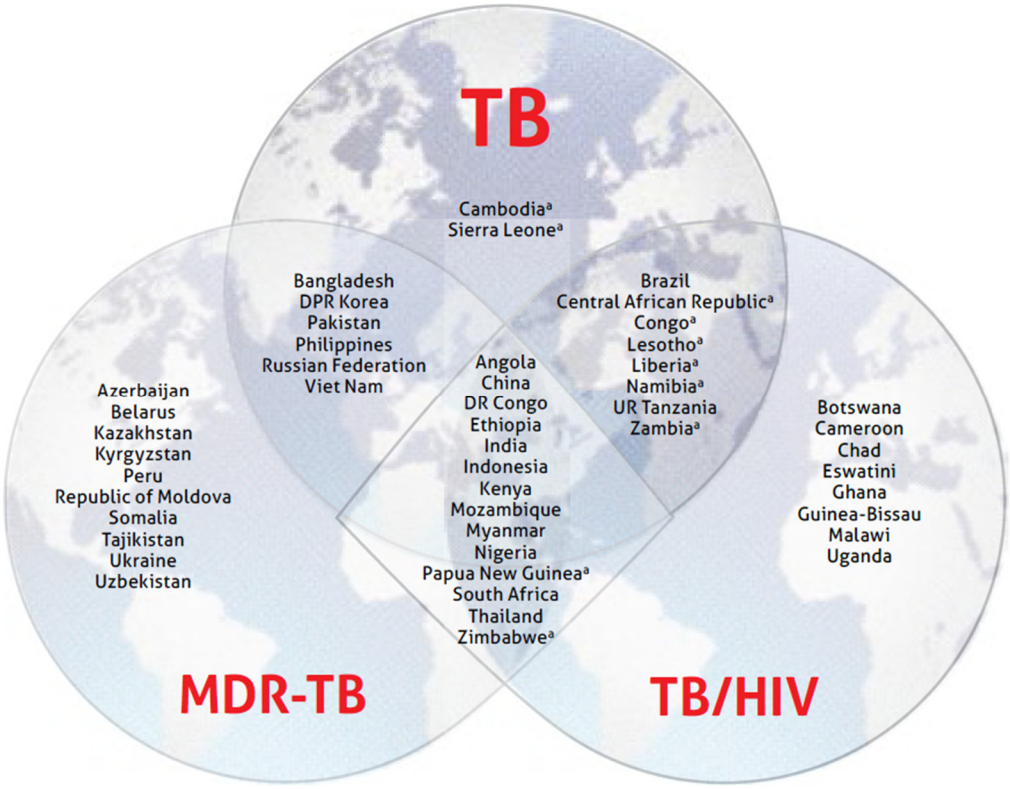

Figure 1. The three high-burden country lists for TB, TB/HIV and MDR-TB defined by WHO for the period 2016-2020 and their areas of overlap.

1.2. Burden of tuberculosis in Tanzania

Tanzania is ranked 14th among the 30 high TB burden countries according to WHO (World Health Organization, 2019). In 2018, the country had an estimated TB incidence of 253 cases per 100,000 population (Figure 2) (World Health Organization, 2019). In 2018, Tanzania had a total of 75,828 cases, among these over 96% of cases were newly diagnosed (World Health Organization, 2019). The disease is predominantly among people at the age group between 15-54 years and among men (59%) (MoHSW, 2014; World Health Organization, 2019).

Dar es Salaam has by far the highest number of notified TB cases in the country with 21% (about 14,620 cases) of all cases (69,623 cases) in 2017 (The National Tuberculosis and Leprosy Programme, 2017). The city is highly populated with approximately 4.3 million people (Statistics, 2014). The rapid and uncontrolled urbanisation has led to almost 70% of the population living in

informally developed settlements (UN-HABITAT, 2010), which poses a major challenge to the control of infectious diseases such as TB.

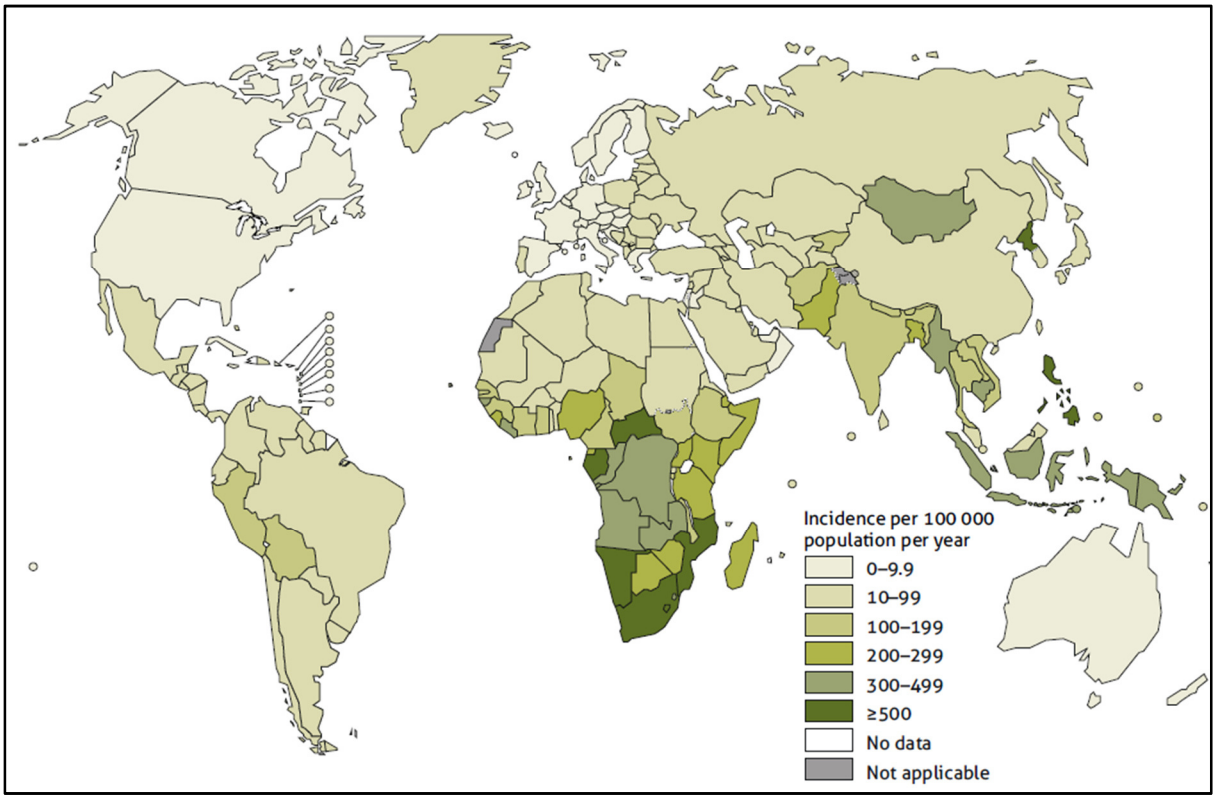

Figure 2. Estimated TB incidence rates in 2018 per 100,000 population.

1.3. Transmission of tuberculosis

TB is an airborne disease transmitted by airways from one person to the next when an uninfected individual breathes in air containing Mtb expired from an infected person (Rieder, 1999; Sultan et al., 1960; Wells, 1934). This is likely to occur when Mtb remain buoyant in air for a sufficient amount of time for the next person to breath in these infectious particles (Wells, 1934). Droplet nuclei with diameters ranging from 1µm to 5µm, have been identified to play a key role of suspension of Mtb in air until the particles are breathed in by the next person (Sultan et al., 1960; Wells, 1934). Such droplet nuclei have capabilities to remain suspended in air for several hours before dropping on the floor or evaporating (Fernstrom and Goldblatt, 2013).

It is known that transmission of airborne diseases essentially involves three stages; production, transportation and reception of aerosols. Coughing, talking and sneezing produce a large amounts of airborne aerosols (Loudon and Roberts, 1967; Wurie et al., 2016). Poor ventilation as that seen in

confined spaces facilitates suspension of infectious particles (droplet nuclei) and thus aiding in transportation from one person to the next (Beggs et al., 2003; Loudon and Roberts, 1967). Lastly, overcrowding conditions and other social mixing patterns among infectious cases and susceptible individuals facilitates that aerosols produced reach a susceptible individual (Rieder, 1999). Therefore, Mtb is highly transmissible in overcrowded locations where there is an increased probability of close contacts among humans and insufficient ventilation conditions which prolong the suspended droplet nuclei containing Mtb in circulating air.

1.4. Traditional and novel approaches to measure tuberculosis transmission

TB transmission lacks an appropriate *in vitro* test assay resulting in difficulties to measure transmission (Andersen et al., 2000). Historically, TB transmission has been investigated using contact tracing (Cook et al., 2012), molecular genotyping (Fenner et al., 2012; Guerra-Assunção et al., 2015; Stucki et al., 2012; Walker et al., 2013) and geo-temporal clustering analyses (Tiwari et al., 2006; Touray et al., 2010; Zelner et al., 2016). Molecular genotyping can only measure TB transmission resulting in secondary cases, where isolation of identical isolates does not automatically imply recent transmission (Nardell, 2004), and requires labour intensive culture facilities and high costs for the analyses. This leaves social contact tracing activities as the likely option, especially in a low income setting like Tanzania. Unfortunately, in such settings, social contact data are difficult to obtain (Verver et al., 2004; Wilkinson et al., 1997), particularly among high-risk groups such as chronic alcoholics, people with no permanent homes and illicit drug users (Asghar et al., 2009; Burki, 2010).

Carbon dioxide (CO_2) has recently been used as a proxy for measuring exhaled air to determine the potential risk for airborne transmission (Myatt et al., 2004; Rudnick and Milton, 2003) and specifically in TB transmission (Andrews et al., 2014, 2013; Rudnick and Milton, 2003; Wood et al., 2014). In this approach, environmental CO_2 levels combined with social contact data were used to estimate the proportion of shared re-breathed air that has been exhaled by someone else at indoor locations (Richardson et al., 2014; Rudnick and Milton, 2003). Until recent, only studies from Cape Town, South Africa have used this approach to estimate TB transmission. This group developed

portable CO_2 monitors which identified schools and public transportation as key TB hotspots (Andrews et al., 2014, 2013; Richardson et al., 2014; Wood et al., 2012). The annual risk of TB transmission in public transport was reported to range from 3.5% to 5%, being lowest in buses and highest in taxis (Andrews et al., 2013). In another study, the group estimated most TB infections to occur outside the households among the young adults aged 15 to 19 years (Andrews et al., 2014).

1.5. Mathematical models of tuberculosis transmission

Over the years, different mathematical models of TB transmission have been assessed in different settings. Early epidemiological models tried to estimate the effectiveness of TB control measures for interventions such as intensified case finding, effect of TB chemotherapy, effects of Bacillus Calmette–Guérin vaccine and Isoniazid prevention therapy (Waaler et al., 1962; Waaler and Piot, 1969). However, the epidemiology of TB continued to elude the vast TB community needing more insight into transmission dynamics especially in the high burden settings that exhibit the highest rates of transmission.

The intrinsic transmission dynamics driving the TB epidemics was well predicted by a research group from America, where it was seen that there exists three pools of sub-epidemics (fast progressors, slow progressors and re-activation) which explains why TB epidemics can take 100 years to stabilize (Blower et al., 1995). By far, the most effective mechanism to stop the epidemics in high-burden settings is by reducing transmission, as this reduces the three pools of the sub-epidemics (Blower et al., 1995). More emphasis has been placed on understanding models which will take aerobiology parameters to explain TB transmissions (Issarow et al., 2015; Rudnick and Milton, 2003; Wells, 1934; Wood et al., 2016). Three epidemiological models aimed to determine quanta of infection associated with TB transmissions. These models include the Mass Action model (Riley, 1974), the modified Wells-Riley model (Andrews et al., 2014; Beggs et al., 2003) and the Gammaitoni-Nucci Model (Beggs et al., 2003). However, all these models considered steady state conditions. Only recently, a group from Cape Town produced a flexible mathematical model (steady and non-steady state conditions) to predict TB transmission in South Africa (Issarow et al., 2015).

Introduction

1.6. Tuberculosis and helminth co-infection

Tanzania like most sub-Sahara countries, carries a large burden of the helminth infections, specifically soil-transmitted helminths. Being among the 30 high-burden TB countries worldwide, puts the country on the map with co-existence of the two diseases (i.e., TB and helminths infections). Largely, such diseases are poverty-related with a high-burden in poorly planned and developed neighborhood such as seen in the growing cities of sub-Sahara.

Tuberculosis and helminth infections are said to be diseases of the poor or those living in poverty, this is evidently clear by the consistently high incidence rates and prevalence of the diseases in regions like the sub Saharan African countries and those in the low and lower-income world bank categories (Figure 3). Because of this shared niche, the diseases and their co-occurrence warrant attention from the public health specialists at the population level to determine the influence of one disease on the other.

Immunologically, studies have shown that helminth-induced immune responses within human hosts results in suppressed immunological responses against M*tb* and makes it likely for TB disease progression (Figure 4). Thus latently infected individuals with helminth co-infections are at an increased risk for progressing to active TB disease (Babu and Nutman, 2016; Rafi et al., 2012). Helminth infections trigger a strong T-helper type 2 (Th2) immune response, which is a potent inhibitor for M*tb*-specific T helper type 1 (Th1) immune response (George et al., 2013; Rafi et al., 2012; Salgame et al., 2013). Interestingly, individuals with TB and helminths co-infection, usually presents with features of advanced TB disease at the time of TB diagnosis and are likely to remain with post TB treatment sequalae (Resende Co et al., 2007).

Introduction

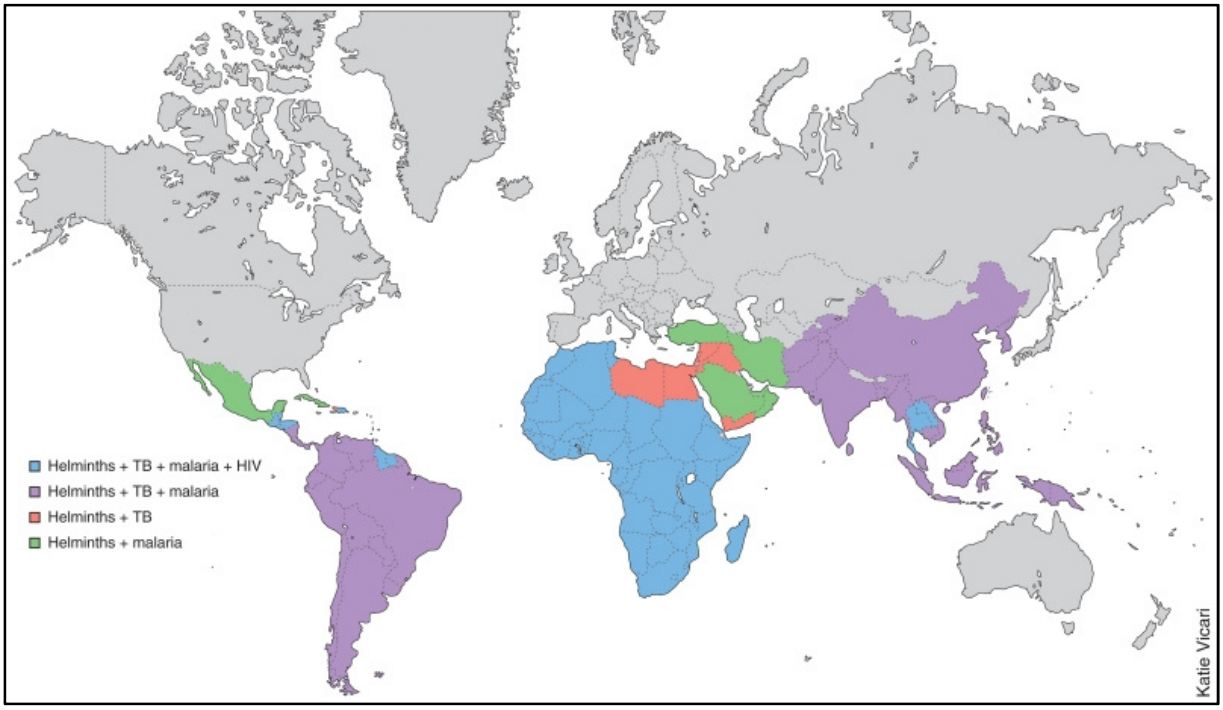

Figure 3. Geographic distribution of coinfection with helminths, tuberculosis, malaria and/or HIV infection among adults (Salgame et al., 2013).

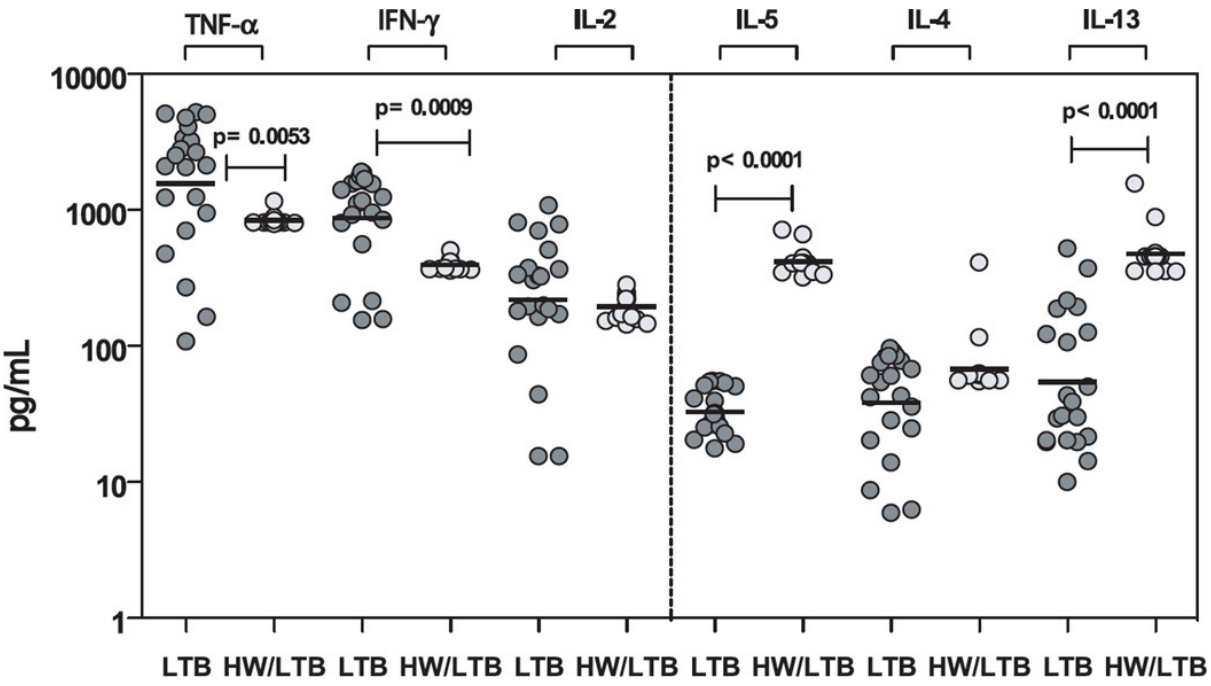

Figure 4. Alterations in plasma levels of Th1 and Th2 cytokines in latent TB patients with and without hookworm infection.

The plasma levels of Th1 - IFN-γ, IL-2, TNF-α and Th2 – IL-4, IL- 5, IL-13 cytokines were measured by ELISA in latent TB infected individuals with (HW/LTB; n=21) or without (LTB; n=21) concomitant hookworm infection. P values were calculated using the Mann-Whitney test (George et al., 2013).

Introduction
1.7. Anaemia and tuberculosis

Anaemia is highly prevalent in sub-Saharan Africa (World Health Organization, 2008). The aetiology of iron deficiency anaemia in sub-Saharan Africa is multifactorial; but largely it is thought to be low dietary iron intake and poor bioavailability induced by monotonous, cereal based diets (Zimmermann and Hurrell, 2007) exacerbated by chronic and sub-chronic infections, which may include TB in both its acute and latent form (Drakesmith and Prentice, 2012). Anemia is very common in individuals with TB, and was recently reported to be 64% in a cross sectional study in Dar es Salaam (Isanaka et al., 2012b; van Lettow et al., 2005), of which ≈50% was estimated to be due to iron deficiency (classified with a mean corpuscular volume MCV<80 fl). It appears both iron deficiency and iron sequestration play a role in the aetiology of TB.

TB in both latent and active stage is known to impact a systemic inflammatory state which can result into increased hepcidin concentration in the body. The high hepcidin concentration milieu of the body is associated with sequestration of iron into the reticulo-endothelial system causing a transient iron deficient state (Theurl et al., 2008). Increased risk of TB related deaths have been associated with iron deficiency and iron deficiency anaemia (Isanaka et al., 2012a). It has been shown in mouse models, that hepcidin possesses anti-mycobacterial activity (Drakesmith and Prentice, 2012; Sow et al., 2011). Proper understanding of hepcidin levels among TB patients with associated anemia will result into improved management of TB-related anemia states and ultimately improve TB treatment outcome.

Iron is a metabolite needed by intracellular organisms such as Mtb and thus propagating the infection. Iron supplementation during infection is not without risk and the safety of iron supplementation in areas of high infectious disease burden is uncertain. In areas with endemic malaria, WHO has recommended halting programs of universal iron supplementation (World Health Organization, 2007) due to an increased risk of serious adverse effects and hospitalizations in a trial of untargeted iron and folic acid supplementation in preschool children in Tanzania (Sazawal et al., 2006). In this trial, children receiving iron and folic acid, with or without zinc, were 12% more likely

to die or need treatment in hospital for an adverse event and 11% more likely to be admitted to hospital compared to placebo (Sazawal et al., 2006).

2. Rationale

As indicated by previous work, social contact patterns have been well studied in various settings to explain TB transmission (Horby et al., 2011; Johnstone-Robertson et al., 2011b; Mossong et al., 2008), and only one study integrated social and environmental data (environmental CO_2 as a proxy for shared re-breathed air) in a single setting in Cape Town, South Africa (Andrews et al., 2014). However, further work is needed to investigate transmission potential and social mixing patterns in different settings that are more representative for sub-Saharan Africa. A recent TB prevalence survey in Tanzania showed a higher prevalence of bacteriologically confirmed TB in rural than urban settings (316 vs. 276/100,000 populations) (Ministry of Health and Social Welfare, 2013).

As the WHO has set the ambitious goals of the End TB Strategy, novel approaches are needed, especially those that can be easily adapted in different (rural and urban) settings to implement TB controls measures that take into account different health seeking behaviors among patients, different rural-urban patient characteristics and other co-morbidities which may render TB treatment difficult. In 2013, we successfully initiated a cohort platform of adult TB patients and household contacts (controls) in the metropolitan setting of Dar es Salaam and rural setting of Ifakara, which offered an excellent opportunity for this PhD project.

This cohort (TB DAR) gave us the opportunity to implement a novel approach of studying TB transmission hotspots in the rapidly growing setting of urban Tanzania in Dar es Salaam by using environmental CO_2 data and other epidemiological data from Tanzania. Additionally, the cohort provided a platform to study TB diagnostic delay and associated factors among TB patients and further the understanding of existing fundamental differences of rural vs., urban TB patients in Tanzania. Lastly, we studied role of anaemia, co-infections and hepcidin among cases and controls in a matched design.

The novelty of this PhD project was its use of environmental data (CO_2 levels) to measure TB transmission with the inclusion of comparable epidemiological data collected in different settings of Tanzania which was the first in Tanzania. For the first time in this under-researched setting, we are able to explain how likely the TB transmission hotspots contribute in the burden of TB. Our findings

Rationale

explain the underlying epidemiology of the disease between rural and urban setting bearing in mind that the rural setting of Tanzania are less crowded but have more than 50% of the burden of disease according to the recent prevalence survey (Ministry of Health and Social Welfare, 2013).

3. Objectives

3.1 General objective

The overall objective of this PhD project was to understand the epidemiology and transmission of tuberculosis in Tanzania by studying the infrastructure-related risk of TB transmission through measuring environmental CO_2 levels at locations of public importance; determining the TB diagnostic delay and its associated factors among TB patients; understanding differences in the epidemiology of TB and comorbidities among TB patients from rural and urban Tanzania; and investigating the association of hepcidin levels with coinfections, TB disease severity and progression from TB infection to disease among adults in Tanzania.

3.2 Specific objectives

The specific objectives were to;

(i) study the infrastructure-related (locations of public importance) risk of TB transmission by integrating environmental CO_2 and social contact data;

(ii) determine the diagnostic delay of TB and its associated risk factors among newly diagnosed smear positive TB patients in Dar es Salaam;

(iii) understand differences in the epidemiology of TB and comorbidities among adult TB patients from rural and urban Tanzania; and to;

(iv) investigate the association of hepcidin levels with coinfections, TB disease severity and progression from TB infection to TB disease among adults in Tanzania.

4. Materials and methods

4.1 Study setting

Tanzania has population of more than 45 million people according to the latest census conducted in 2012 (NBS, 2013). The country had a total of 69,623 new TB cases notified to the NTLP in 2017, of these, nearly 21% cases originated from Dar es Salaam, making the city a national TB hotspot (The National Tuberculosis and Leprosy Programme, 2017).

Dar es Salaam is the commercial capital of Tanzania accounting for approximately 10% of the total population in the mainland Tanzania, with an estimated population size of 4.3 million people (NBS, 2013). The city consists of five administrative regions: Temeke, Kinondoni, Ilala, Ubungo and Kigamboni. The study area for the urban- setting was the Temeke district (Figure 5), with a land area of approximately 684 square kilometres and a population of 1,368,881 people (NBS, 2013).

Ifakara is the administrative capital of Kilombero district and the main trading centre for Kilombero and Ulanga districts within Morogoro region. The ward is located in the south central of Tanzania with a population of 55,956 people (NBS, 2013). Ifakara town was the study area for the rural setting.

4.2 Study design

4.2.1 Infrastructure-related design

Objective 1: This was an observational study in design employing exposure assessment methods. Briefly, we collected environmental data (CO_2 levels, geo-coordinates, time, etc.) from locations of public importance such as markets, prisons, night clubs, social halls and public transport using adult volunteers who carried CO_2 monitors at various parts of Dar es Salaam city (urban setting).

The microenvironments (location of public importance) were selected based on their use by the public and crowding conditions seen previously during our preliminary work in the region. To collect CO_2 and social contact data, we enrolled adult volunteers (age ≥18 years) who carried the CO_2 monitors to all locations of public importance in Dar es Salaam. At these locations, volunteers spent

time at different hours and days of the week and recorded the number of people present during each time point. They also recorded the social contact data in paper diaries.

Environmental data such as CO_2 levels was measured by CO_2/GPS monitors (Supplementary Figure 1). The monitors have been developed by the engineering department of the University of Cape Town (UCT), South Africa, and are designed for research purposes only. The monitors have been extensively tested in several studies in South Africa (Andrews et al., 2014, 2013; Wood et al., 2014), and have been calibrated and pilot tested in Tanzania proving to be safe and convenient for use by study participants. Briefly, each monitor has a built-in COZIR ambient transducer that measures CO_2 concentration, temperature and relative humidity (Andrews et al., 2014, 2013; Burki, 2010). Stored data can be exported in serial digital format, which is read in by a microcontroller device. In addition, each monitor has also a built-in global positioning system (GPS) receiver, which record geo-coordinates of locations where environmental data (CO_2 levels) have been recorded.

4.2.2 Patient-related design

Objective 2-3: Here we analysed data from the ongoing cohort of adult TB patients and their household contacts (TB DAR). Briefly, recruitment of study participants began in 2013 at Temeke Regional Referral Hospital (Temeke district) and 2014 at the St. Francis Referral Hospital (Kilombero district).

Eligibility criteria for TB-DAR study participants were as follows: 1) ≥18 years of age at recruitment, 2) sputum microscopy smear-positive TB (quantification grading at least scanty), 3) residency in Wailes I and II sub-districts of Temeke or residents of Kilombero or Ulanga districts and 4) attending the TB clinic at the Temeke District Hospital or St. Francis Referral Hospital. Patients with confirmed TB were started on standard TB treatment regimen within one day of diagnosis, as per the national guidelines (National Tuberculosis and Leprosy Program, 2013). Diagnostic and treatment services for TB were provided free of charge by the NTLP. Briefly, data were captured using the OpenDataKit application (www.opendatakit.org) on Android tablets, and data quality was monitored in real-time using the *odk_planner* tool (Steiner et al., 2016).

Materials and Methods

Objective 4: Here we created a nested case-control study within the TB DAR cohort where we selected 1:1 by matching age and sex of cases and controls. TB patients and controls were evaluated at the time of TB diagnosis (cases) or recruitment (controls), at six months (after completion of treatment for TB patients), and at 12 months. Clinical data were collected at each visit, and biological samples at the time of recruitment, as previously described (Hiza et al., 2017; Knopp et al., 2014; Mhimbira et al., 2017; Said et al., 2017).

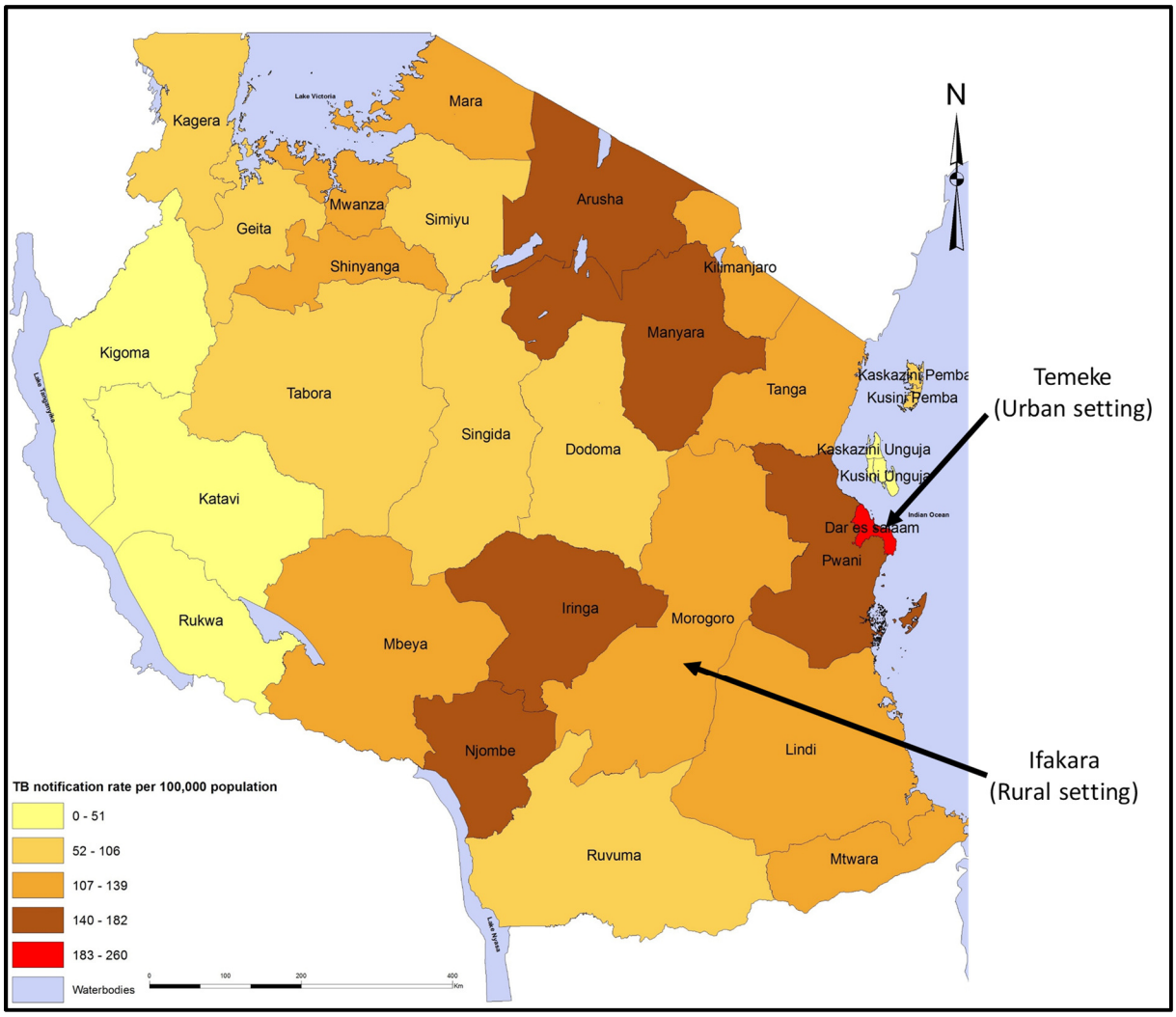

Figure 5. Map of Tanzania showing TB notification rates per 100,000 populations in 2015. Arrows show study sites

4.3 Laboratory procedures

Collected samples were transported daily from Temeke Regional Referral Hospital to the Bagamoyo Research and Training Center which is the laboratory of Ifakara Health Institute (IHI). Samples which were collected at the St. Francis Referral Hospital were immediately analysed by the IHI laboratory at Ifakara.

Materials and Methods

Bacteriological confirmation of TB was done by fluorescence LED microscope or by using Xpert MTB/RIF assay (Cepheid; Sunnyvale, USA) in all sputum samples. All sputum samples were sent to a biosafety level 2+ TB laboratory for solid culture on Lowenstein-Jensen medium at the BRTC. Sputum samples from Ifakara were preserved in cetylpyridinium chloride (CPC) and sent by post to BRTC in Bagamoyo and processed as previously described (Hiza et al., 2017).

Helminths confirmation were done from stool and urine samples collected at each site. From the rural site, the samples were sent to the IHI Helminth Laboratory in Ifakara. From the urban site, the samples were transferred to the Helminth Unit at BRTC. At each laboratory, samples were examined for helminth infection using standardized, quality-controlled procedures as previously described (Leuenberger et al., 2016; Mhimbira et al., 2017). The Kato-Katz method was performed in triplicate with thick stool smears from each sample. *Strongyloides stercoralis* infection was diagnosed by the Baermann method (Leuenberger et al., 2016). Microhaematuria was examined by reagent strips (Hemastix; Siemens Healthcare Diagnostics Inc; Tarrytown, USA). A point-of-care circulating cathodic antigen (POC-CCA) urine cassette test (ICT Diagnostics, Noordhoek, South Africa) was employed for rapid diagnosis of *S. mansoni* (WHO, 2013). *S. haematobium* eggs were detected using urine filtration (Lamberton et al., 2014; Mhimbira et al., 2017).

4.4 Statistical analysis

Objective 1: Descriptive statistics were used to describe distribution of shared re-breathed air based on environmental CO_2 and the number of people present at a given location (Richardson et al., 2014; Wood et al., 2014). We studied the time spent at different locations with poor ventilation (CO2 level ≥1,000 parts per million). For continuous variables, we used the unpaired Student's t-test (two sided). Lastly for objective 1, we used modified Wells-Riley equation to estimate the annual risk of TB transmission using environmental data we collected in Tanzania and previous published assumptions (Richardson et al., 2014; Wood et al., 2014).

Objective 2-3: Descriptive analyses were performed to summarize the data. For continuous variables, the Wilcoxon rank-sum or Student's *t*-tests were used, depending on the distribution from the two sites, and χ^2 or Fisher's exact tests for comparison of categorical variables, as appropriate.

Materials and Methods

Associations between dependent variables and independent variables were analysed using logistic regression models. Variables were included in the multivariate model if they were clinically and socially relevant and had a P-value of < 0.05 following univariate analysis. Results are presented as crude odds ratios (OR) and adjusted odds ratios (aOR).

Objective 4: Descriptive statistics were used to describe participant characteristics where groups were compared using χ^2 or Fisher's exact (in case of binary variables) and Kruskal-Wallis test (in case of continuous variables). Due to the matched design, we compared cases and controls using conditional regression model to determine associations between outcomes of interest and various predictors. All analyses were performed in Stata version 14 (Stata corporation, Texas, USA).

4.5 Ethics approval

The study (TB DAR) was approved by the institutional review board of the Ifakara Health Institute (IHI, reference no. IHI/IRB/04-2015), the Medical Research Coordinating Committee of the National Institute for Medical Research in Tanzania (NIMR, reference no. NIMR/HQ/R.8c/Vol. I/357), and the Ethics Committee of the Canton of Basel (EKNZ, reference no. UBE-15/42). All participants gave written informed consent before enrolment.

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5. Tuberculosis transmission in public locations in Tanzania: A novel approach to studying airborne disease transmission

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Short title: Tuberculosis transmission hotspots in Tanzania

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5.1 Abstract

Objectives – For tuberculosis (TB) transmission to occur, an uninfected individual must inhale the previously infected breath. Our objective was to identify potential TB transmission hotspots in metropolitan city of Dar es Salaam, Tanzania and to model the annual risk of TB transmission in different locations of public importance.

Methods – We collected indoor carbon dioxide (CO_2) data from markets, prisons, night clubs, public transportation, religious and social halls, and from schools. Study volunteers recorded social contacts at each of the locations. We then estimated the annual risks of TB transmission using a modified Wells-Riley equation for different locations.

Results – The annual risks of TB transmission were highest among prison inmates (41.6%) and drivers (20.3%) in public transport. Lower transmission risks were found in central markets (4.8% for traders, but 0.5% for their customers), passengers on public transport (2.4%), public schools (4.0%), nightclubs (1.7%), religious (0.13%), and social halls (0.12%).

Conclusion – For the first time in a country representative of sub-Saharan Africa, we modelled the risk of TB transmission in important public locations by using a novel approach of studying airborne transmission. This approach can guide identification of TB transmission hotspots and targeted interventions to reach WHO's ambitious End TB targets.

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5.2 Background

One quarter of the world's population is estimated to be infected with *Mycobacterium tuberculosis* (Mtb) (Houben and Dodd, 2016). Transmission of Mtb occurs when uninfected persons inhale infectious droplet nuclei from the infected (Rieder, 1999). Droplet nuclei with diameters of 1 to 5 µm can remain suspended in air for many minutes to hours (Wells, 1934), thus making Mtb highly transmissible in overcrowded locations with poor ventilation. For this and other reasons, tuberculosis (TB) remains a major public health problem worldwide.

One way to estimate the risk of TB transmission begins with measuring environmental levels of carbon dioxide (CO_2) levels (Andrews et al., 2014; Wood et al., 2014). CO_2 levels combined with social contact data allow calculation of the volumes of rebreathed air in order to estimate the potential for airborne disease transmission (Rudnick and Milton, 2003). A modified Wells and Riley equation can be used to estimate TB transmission by taking into account the re-breathed air fraction (estimated from indoor and outdoor CO_2 levels), time at risk, quanta of contagion and the number of people occupying the confined space (Andrews et al., 2014, 2013; Rudnick and Milton, 2003).

Until recently, only studies from Cape Town, South Africa have used this approach. Additional studies in settings more representative of sub-Saharan African countries, which have the highest burden of TB, are needed. We therefore studied potential TB transmission hotspots in metropolitan Dar es Salaam, Tanzania using this novel, CO_2-based approach to model the risk of TB transmission as the basis for planning intervention studies.

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5.3 Methods

5.3.1 Study locations and design

We used exposure assessment methods in Dar es Salaam where 22% of 62,952 new TB cases in Tanzania were notified in 2013, making the city a TB hotspot (MoHSW, 2014). Adult volunteers (age ≥18 years) carried CO_2 monitors to markets, nightclubs, social halls, religious venues, and on public transport. Volunteers collected data between November 2014 and August 2015, and they recorded the number of people at each location. We did not include health care facilities (HCF), as waiting rooms are usually open-air in Tanzania and as a study from South Africa indicated that the contribution of HCFs to the annual risk of TB transmission was small (Wood et al., 2012). We obtained written permission from the Ministry of Health and Social Welfare through the National Tuberculosis and Leprosy Program to collect the infrastructure-related CO_2 data at public locations. In addition, the Ministry of Home Affairs (Tanzania Prisons) issued permission to enter prisons for data collection.

Market - The largest market in Dar es Salaam, known as Kariakoo, has an underground floor, which is populated daily by 100-180 people during each hour of the day. Most retail traders elsewhere in Dar es Salaam make wholesale purchases of agricultural products from this market.

Prisons - The two largest facilities in the Dar es Salaam region with over 1,000 inmates were included. One prison is a short-term facility for the temporarily remanded who awaits court rulings, while the other is a long-term correctional facility. While inmates are outdoors during the day, at night they share cells that are occupied by approximately 40 inmates. Prison guards placed the monitors in different cells.

Night clubs - We collected data from eight of the largest popular nightclubs in the city that prohibit indoor cigarette smoking. The night clubs were soundproof and relied upon alternative ventilation mechanisms such as closed air conditioning systems.

Public transportation - Public transportation in Dar es Salaam largely relies upon two commuter bus designs with carrying capacities of 15 to 22 and 32 to 40 passengers.

Religious and social halls - Religious halls consist of mosques and churches with open ventilation complemented by fans for cooling. Social halls, in contrast, have closed ventilation systems that rely on air conditioning, and they are used for family events such as wedding receptions.

Schools - We collected data from both day and boarding schools in Dar es Salaam. The schools typically have open windows and no other type of ventilation. The classrooms hold 40 to 50 students in each room.

5.3.2 Data collection, definitions and statistical analysis

We collected social contact data using paper diaries and environmental CO_2 data from the monitors (Supplementary Figure 1) used in previous work elsewhere (Andrews et al., 2014, 2013; Wood et al., 2014). In cases of missing social contact data, trained field workers clarified with study participants and re-captured as much as participants could recall about their social contacts that were missing in the paper diaries e.g., times of sleep, waking up etc. Volumes of rebreathed air were calculated based on indoor and outdoor CO_2 levels at specific times, and accounted for the number of people present and assumed a respiration rate of eight breaths per minute (Andrews et al., 2014; Richardson et al., 2014; Wood et al., 2014). All analyses were performed in Stata software version 13.1 (Stata Corp; Texas, USA).

TB transmission risk was defined as the probability of an individual acquiring a primary infection that would result in formation of a Ghon complex in the lungs. Annual risk of transmission was defined as the probability of an individual acquiring a primary tuberculous infection in one year in a given location. We defined quantum as the number of infectious droplet required to infect 63.2% (i.e., $1- e^{-1}$) of susceptible individuals in an indoor environment (Beggs et al., 2010).

The calculation of shared rebreathed air (either as liter/minute or liter/hour) was based on environmental CO_2 data and the number of people present at a given location (Richardson et al., 2014; Wood et al., 2014). We studied the time spent at different locations with poor ventilation (CO_2 level ≥1,000 parts per million) (Andrews et al., 2013; Richardson et al., 2014). For continuous variables, we used the unpaired Student's *t*-test (two sided).

5.3.3 Modelling the annual risk of TB transmission

We used the modified Wells-Riley considering the work of Rudnick Milton on non-steady state situations to estimate the annual risk of TB transmission (Andrews et al., 2014, 2013; Rudnick and Milton, 2003). We parameterized the Equations 1 (a-c) based on previously published assumptions (Andrews et al., 2013; Wood et al., 2014) and based on data collected in this study (Supplementary Table 1). The annual risk (*AR*) of transmission was calculated as function of the time spent per year at a given location. We used 38,000 parts per million as the CO_2 levels in exhaled breaths (CO_2 breathed out) (Andrews et al., 2013; Wood et al., 2014). Briefly, we calculated the risk of TB transmission at each time (*P*) by using equation 1a:

$$P = 1 - e^{\left(\frac{-flqt}{n}\right)} \qquad \text{Equation: 1a}$$

Where rebreathed fraction, $f = \frac{CO2\ indoor - CO2\ outdoor}{CO2\ breathed\ out}$

P, probability of TB transmission; *f*, rebreathed fraction; *l,* number of infectious individuals in space; *q,* rate of generation of infectious quanta; *t,* duration of exposure; *n,* total number of contacts met by one infectious person (Andrews et al., 2014, 2013; Rudnick and Milton, 2003).

Then we calculated the annual exposure time (*T*) at each location using equation 1b:

$$T = \sum_a C_a\, t_a \qquad \text{Equation: 1b}$$

Where $\boldsymbol{C_a}$, is the annual number of visits at a given location, and $\boldsymbol{t_a}$, is the time spent during each visit at a given location (Andrews et al., 2013).

Finally, the annual risk of TB transmission was determined by including the annual time of exposure (1a) in the instantaneous risk of transmission (1b), and is summarised in the final equation:

$$AR = 1 - e^{\left(\frac{-flqT}{n}\right)} \qquad \text{Equation: 1c}$$

In summary, the calculation of the risk of transmission risk took into account (i) the time of exposure, (ii) the number of people at risk, (iii) the magnitude of infectious droplet generated, (iv) and the underlying prevalence of TB among people at risk.

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5.4 Results

Prisons had the highest mean CO2 level Summary statistics for CO_2 levels 1892 ppm, followed by night clubs (1488 ppm) and social halls (1262.9 ppm). The lowest mean CO2 levels were recorded in schools and religious halls (655 ppm and 629 ppm, respectively). Summary statistics for CO2 levels in Dar es Salaam's largest market, prisons, nightclubs, public transportation, religious settings and social halls, and schools are further detailed in Table 1.

5.4.1 Risk of TB transmission in the largest market

The mean value (standard deviation) of rebreathed air inside the market was 0.05 (0.02) L/min. At mean CO_2 levels of 730 ppm, the annual risk of TB transmission for a trader and customer were 4.8% and 0.5%, respectively (Figure 6). The mean annual risk of TB transmission was significantly higher for traders than for customers (4.9%, 95% confidence interval [CI]: 4.77, 5.05%, vs. 0.49%, CI: 0.48, 0.51%; P<0.001; Figure 7.

5.4.2 Risk of TB transmission in prisons

On average a prisoner rebreathed 0.14 (0.1) l/min of air from fellow prison inmates at night. A prison inmate rebreathed an average of 65 L (95% CI: 63.8, 67.8) of air per night from another inmate. At mean CO_2 level, the annual risk of TB transmission for a prisoner spending nights in a cell occupied by 40 prison inmates was 41.6% (Figure 6).

5.4.3 Risk of TB transmission in nightclubs

Poor ventilation conditions were observed in 15.8 (64.3%) hours out of 2.5 hours of observation, with a mean rebreathed air value of 0.2 L/min (Supplementary Figure 2). The mean rebreathed air was significantly higher during Friday night than Saturday night (0.5 vs 0.3 L/min, P<0.001). Assuming one infectious case per 100 people, the risk of TB transmission ranged from 0.02% for 2 hours to 0.03% for four hours. Assuming one spends four hours per weekend in a nightclub and quanta of contagion rate of 1.25/hour, we estimated the annual risk of TB transmission to be 1.7% inside a nightclub at a mean CO_2 level of 1,488 ppm (Figure 6).

Table 1. Carbon dioxide gas levels and annual risks of TB transmission at different locations of public importance in Dar es Salaam.
Carbon dioxide gas (CO_2) levels recorded at locations of public importance in Dar es Salaam, Tanzania and the annual tuberculosis (TB) transmission risk estimates based on varying assumptions of type and number of individuals, quanta generation rate, time of exposure and TB prevalence

Infrastructure	Type of individuals at risk	Mean CO_2 (SD), ppm	Number of people	Quanta, q/hour	Time of exposure in hours	TB prevalence per 100,000 population	Annual transmission risk (%)	
							Mean	95% CI
Market	Customers	730 (109)	100	1.25	208	295	0.49	0.48, 0.51
				1.25	208	528	0.89	0.86, 0.91
				12.70	208	295	4.90	4.80, 5.10
				12.70	208	528	8.60	8.40, 8.80
	Traders			1.25	1,112	295	4.91	4.77, 5.05
				1.25	1,112	528	8.59	8.35, 8.83
				12.70	1,112	295	38.61	37.73, 39.50
				12.70	1,112	528	56.80	55.70, 57.85
Prisons	Prisoners	1,892 (469)	40	1.25	3,200	295	38.40	37.60, 39.20
				1.25	3,200	528	55.50	54.60, 56.40
				12.70	3,200	295	95.20	94.80, 95.60
				12.70	3.200	528	98.60	98.40, 98.90
Night clubs	Club attendees	1,488 (787)	100	1.25	208	295	1.95	1.87, 2.03
				1.25	208	528	3.45	3.31, 3.60
				12.70	208	295	17.20	16.50, 17.90
				12.70	208	528	27.43	26.40, 28.40
Public transportation	Passengers	941 (246)	30	1.25	264	295	2.36	2.33, 2.39
				1.25	264	528	4.16	4.11, 4.22
				12.70	264	295	20.56	20.30, 20.8
				12.70	264	528	32.65	32.31, 32.99
	Drivers		30	1.25	2,640	295	20.29	20.04, 20.53
				1.25	2,640	528	32.27	31.93, 32.61
				12.70	2,640	295	80.63	80.25, 81.01
				12.70	2,640	528	91.24	90.96, 91.51

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Infrastructure	Type of individuals at risk	Mean CO_2 (SD), ppm	Number of people	Quanta, q/hour	Time of exposure in hours	TB prevalence per 100,000 population	Annual transmission risk (%)	
							Mean	95% CI
Religious halls	Attendees	629.31 (105)	100	1.25	104	295	0.13	0.12, 0.14
				1.25	104	528	0.23	0.21, 0.25
				12.70	104	295	1.30	1.20, 1.40
				12.70	104	528	2.30	2.12, 2.48
Social halls	Attendees	1,262.9 (149)	200	1.25	32	295	0.12	0.118, 0.125
				1.25	32	528	0.22	0.21, 0.223
				12.70	32	295	1.23	1.20, 1.26
				12.70	32	528	2.19	2.13, 2.24
Schools	Students	655 (230)	50	1.25	1,280	295	4.02	3.95, 4.09
				1.25	1,280	528	7.03	6.91, 7.14
				12.70	1,280	295	32.30	31.95, 32.66
				12.70	1,280	528	48.78	48.34, 49.23

Abbreviations: CO_2, carbon dioxide gas; 95% CI, 95% confidence interval; SD, standard deviation; ppm, parts per million; TB, tuberculosis

5.4.4 Risk of TB transmission on public transportation

Overall, commuters spent 62.5 (39.5%) hours of the total time under observation (158 hours) on buses in poorly ventilated conditions. The annual risk of TB transmission for a passenger making 264 bus trips could be up to 2.4% per year (Figure 6). The annual risk for a bus driver or conductor on a bus for 10 hours each day for 264 days was 20.3% (Figure 8). Overall, the mean annual TB transmission risk for a driver was significantly higher than for a passenger: 20.3 (95% CI: 20.0, 20.5%) vs. 2.36 (95% CI: 2.3, 2.4%; P<0.001, Table 1, Figure 8).

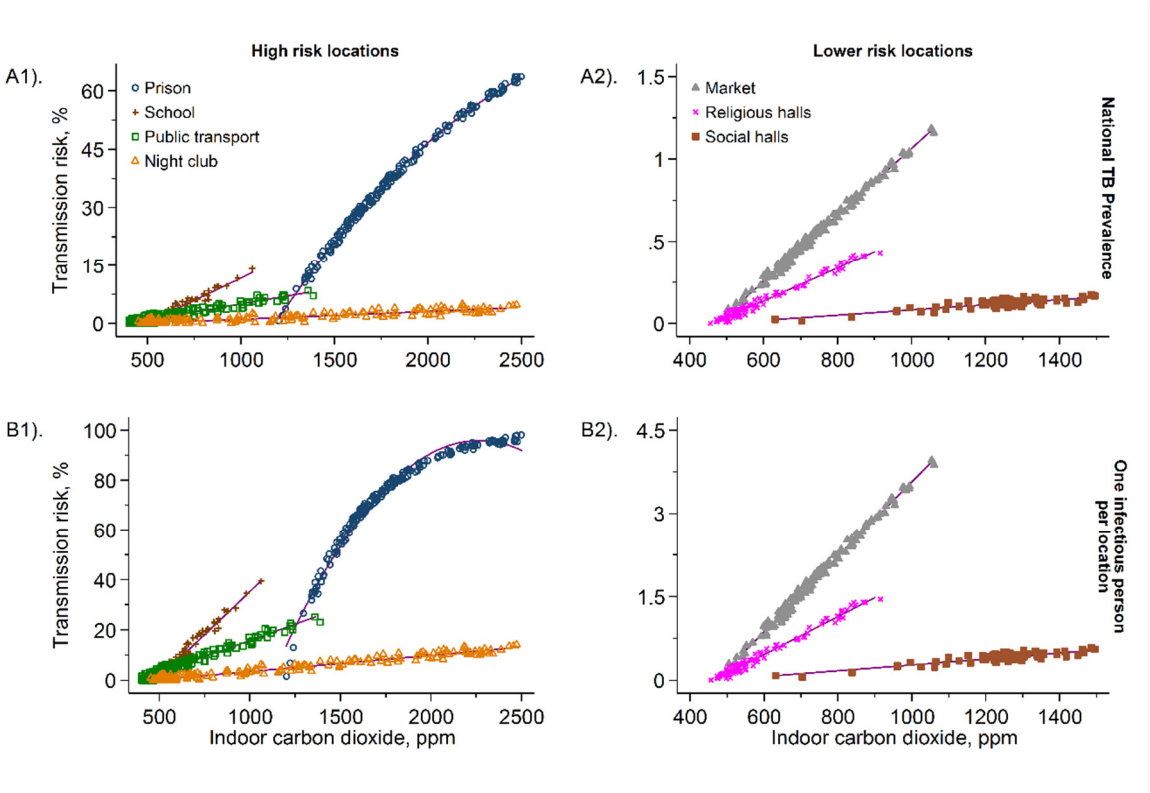

Figure 6. Annual tuberculosis transmission risk at locations of public importance in Dar es Salaam, Tanzania.
Annual tuberculosis transmission risk estimates based on varying environmental CO_2 levels (in parts per million, ppm) at higher risk (**Panels A1 and B1**) and lower risk locations (**Panels A2 and B2**, different scales), and under the assumption of the national TB prevalence (295 infectious cases/100,000 population, **Panels A**) and one infectious person at the location (**Panels B**). Lines indicate fitted risk of transmission. The model is based on quanta generation rate of 1.25/hour from infectious TB patient (Andrews et al., 2013). The mean outdoor CO_2 level at the locations was 431.1 ppm.

5.4.5 Risk of TB transmission at religious and social halls

Overall, mosques and churches were well ventilated, with CO_2 levels not exceeding 1,000 ppm. Assuming two hours spent each week in these religious halls, the mean annual risk of TB transmission was only 0.13%. Social halls had a mean CO_2 level of 1,262.9 (149) ppm (Table 1). However, the mean annual risk of TB transmission was only 0.12% with visits to social halls totaling 32 hours per year (Figure 6).

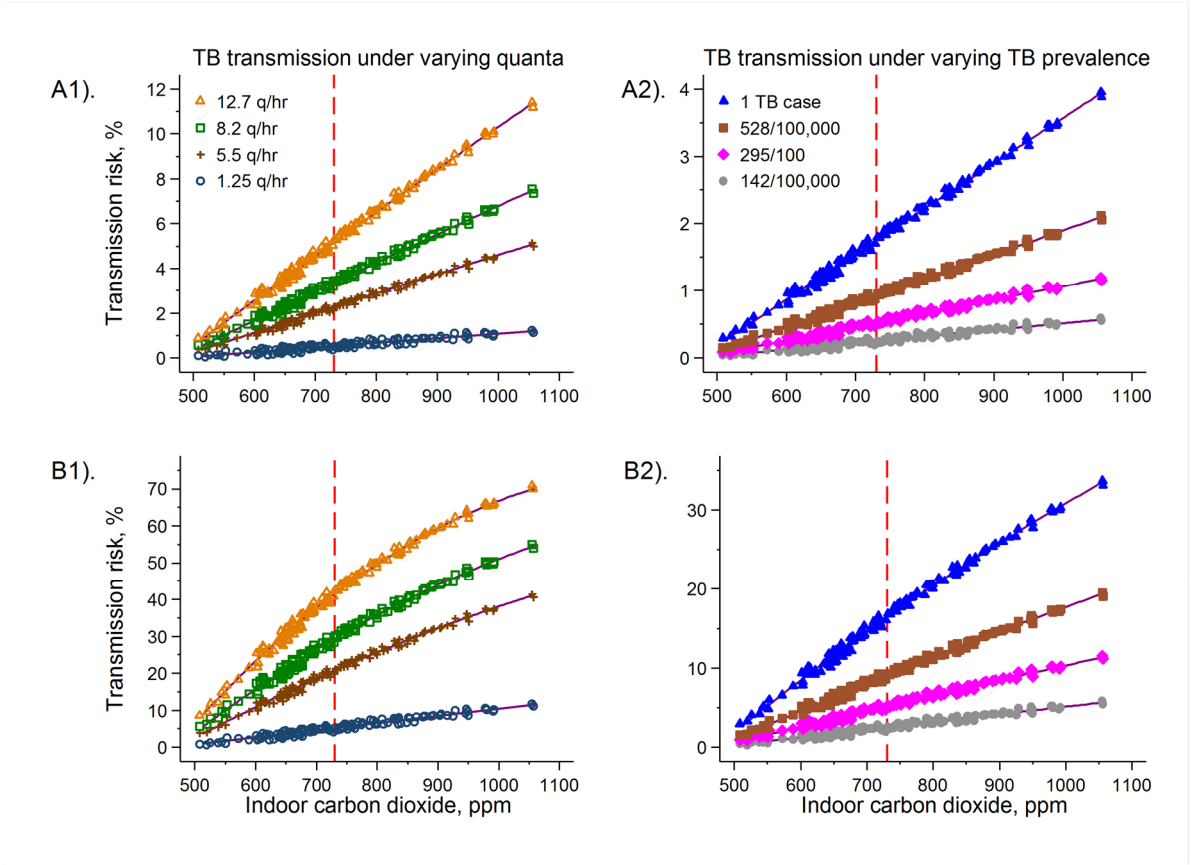

Figure 7. Comparison of TB transmission risks at the largest market in Dar es Salaam. Comparisons of TB transmission risks at a market in Dar es Salaam. Briefly, **Panels A1** and **A2** show estimates of annual risks of TB transmission at different quanta generation rates and TB prevalence for a customer who visits the market for one hour per week for one year. **Panel B1** and **B2** show the annual risks of TB transmission for a trader under different quanta generation rates and TB prevalence assuming a trader works 48 hours during each week over a period of 11 months in a year. The red dashed line show the mean CO_2 level in the market.

5.4.6 Risk of TB transmission in schools

We observed ventilation conditions at both day schools and boarding schools (Supplementary Figure 3). The annual risk of TB transmission associated with 1,280 hours spent in a class of 50 students at a mean CO_2 level of 655 ppm was estimated to be 4.02% (Figure 6).

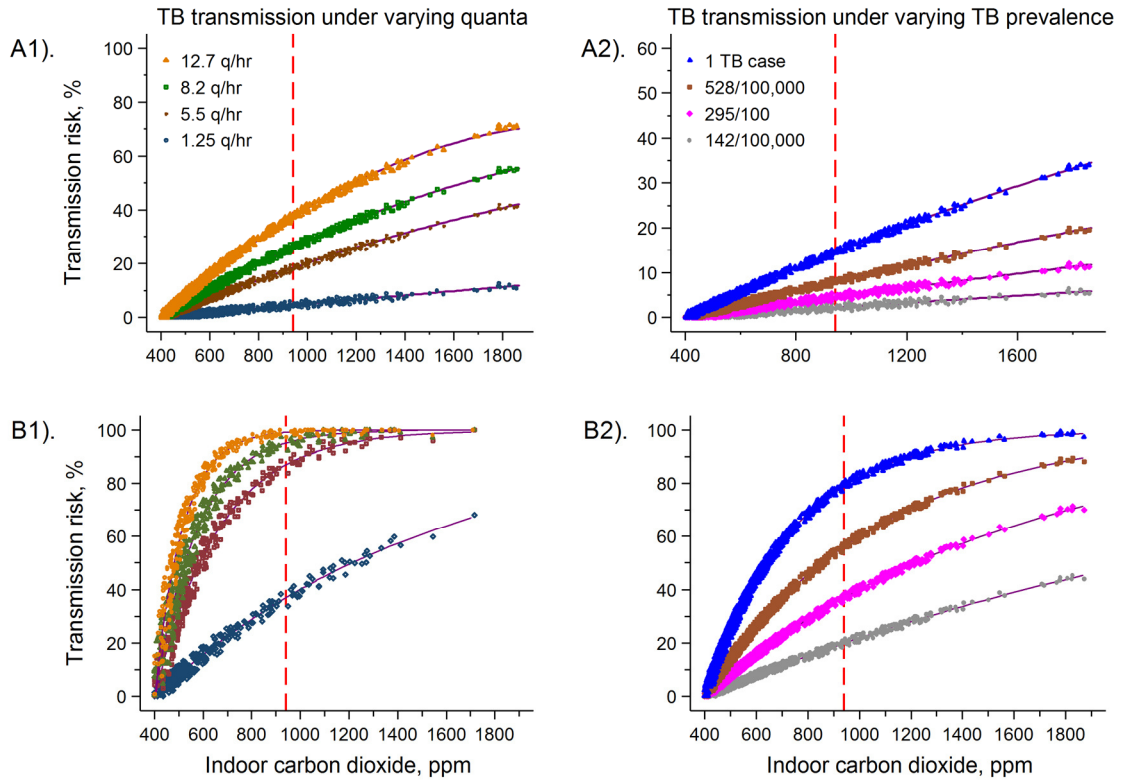

Figure 8. Comparison of TB transmission risks on public transportation in Dar es Salaam. A plot of annual TB transmission risks estimated from the public transport (commuter buses) in Dar es Salaam. Briefly, **Panels A1** and **A2** show estimates of annual risks of TB transmission under different quanta generation rates and TB prevalence for a passenger commuting on public transport for one year. **Panels B1** and **B2** show the annual risks of TB transmission for bus drivers and conductors working under different quanta generation rates and TB prevalence. Red dashed line show the mean CO_2 levels in the commuter buses.

5.5 Discussion

We found the highest annual risks of TB transmission from an urban setting of Dar es Salaam in prisons (41.6%), public transportation (20.3%), schools (4.02%), and nightclubs (1.7%), while risks were lowest in markets (0.5%), religious halls (0.13%) and social halls (0.12%). Generally, the annual transmission risk varies by location due to differing times spent in these venues and their (quite varied) ventilation situations. Our quantitative comparison of transmission risks has the potential to reveal why Dar es Salaam is a TB transmission hotspot in Tanzania.

Prisons unquestionably are sites with high risk of TB transmission. We saw TB transmission risks (up to 90%) that are similar to those seen in prisons in South Africa and Brazil (Johnstone-Robertson et al., 2011a; Urrego et al., 2015). These rates challenge the public health of surrounding communities. The risk of acquiring TB in the short-term correctional facility is approximately eight times higher than that of a trader at the Kariakoo market. Regular visits to nightclubs had TB transmission risks similar to those of a regular passenger in local public transportation which were comparable to those seen in South Africa (Andrews et al., 2013). The risks faced by personnel operating these commuter buses are approximately nine times higher.

At the largest market in Tanzania, we found that the annual risk among traders was ten times higher than that for customers. This market is a hotspot involving a lot of trading activities at the center of metropolitan Dar es Salaam. Due to this profile, we think this market can play a vital role in the control of TB in the country.

In the schools, we observed a lower risk of TB transmission than that seen at schools in Cape Town, South Africa, which has a different environmental profile compared to Dar es Salaam (Richardson et al., 2014) where, due to comparatively higher humidity and temperature, schools rely on open windows and are better ventilated.

This novel approach in the under researched area provides important findings for the control of TB epidemics (Andrews et al., 2013; Wood et al., 2014). This is the first study to compare TB transmission risk at important public locations using a non-traditional approach to study airborne

disease transmission. Perhaps the main limitation of this study lies in its collection of social contact data, which was difficult as seen in other settings with low socio-economic status. Furthermore, we did not take into account the role of immunology among susceptible persons at risk of infection and the virulence of Mtb bacilli. Finally, we also assumed that the air was uniformly distributed in a confined space.

Resources used in the campaign against TB are limited and need to be directed where there is maximum pay off (Rieder, 1999). Our novel approach can guide targeted infection control interventions for National TB Control Programs. This novel approach demonstrates a lower risk of TB transmission are associated with settings with adequate ventilation and a short total time of exposure such as religious and social halls, but a high risk in prisons, public transport and central markets. This study lays the foundation for the next TB transmission studies employing molecular epidemiology techniques or isolation of Mtb from bio-aerosols (Matuka et al., 2015; Wood et al., 2016) that will guide interventions to control TB and reach WHO's ambitious End TB targets by 2035.

5.6 Acknowledgements

Funding information: This work was supported by the Rudolf Geigy Foundation (Basel, Switzerland); the South African Medical Research Council (MRC) with funds from National Treasury under the Economic Competitiveness and Support Package [grant no. MRC-RFAUFSP-01-2013/CCAMP to CM and RW]; and the Bill & Melinda Gates Foundation [grant no. OPP1116641, CM and RW].

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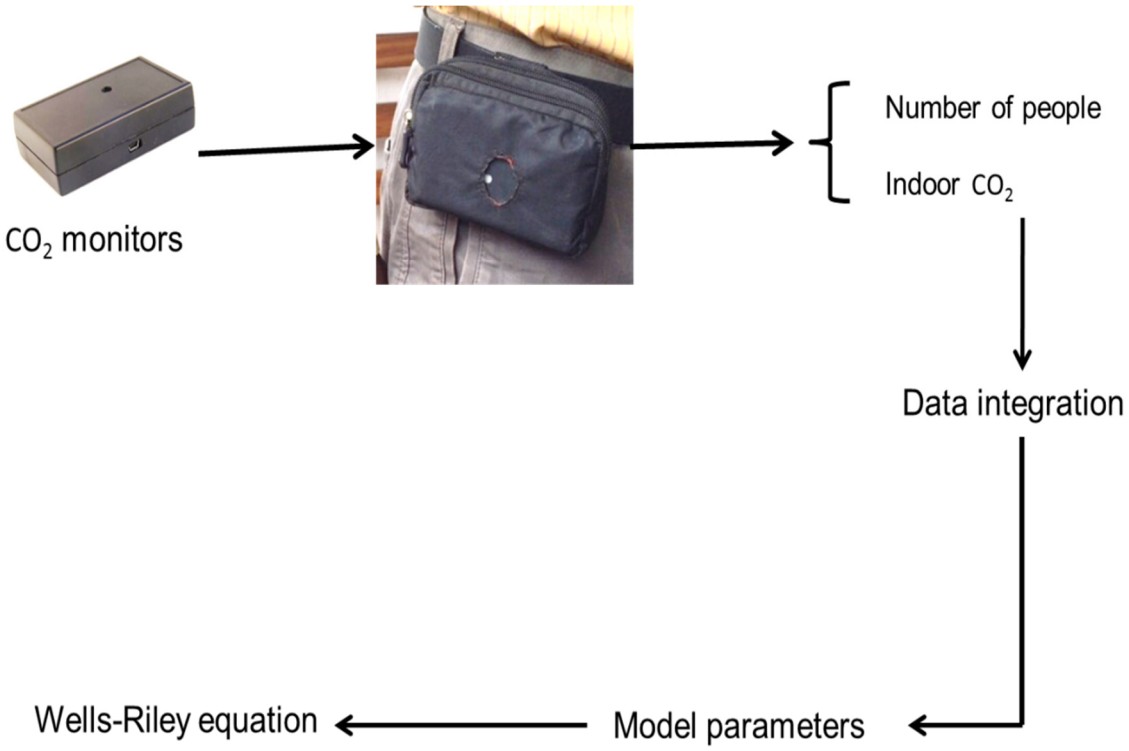
5.7 Supplementary information

Supplementary Figure 1. Work flow for data collection and parameterization.

Methods used for data collection and parameterization of the modified Wells-Riley equation. Briefly, participants carried the Carbon dioxide (CO_2) monitors and documented the number of people in paper diaries. CO_2 data and social contact data were then integrated in an Microsoft Access database. Parameterization was done using previously published results (Andrews et al., 2014; Escombe et al., 2008; Nardell et al., 1991), see Equations 1 (a-c) and Supplementary Table 1.



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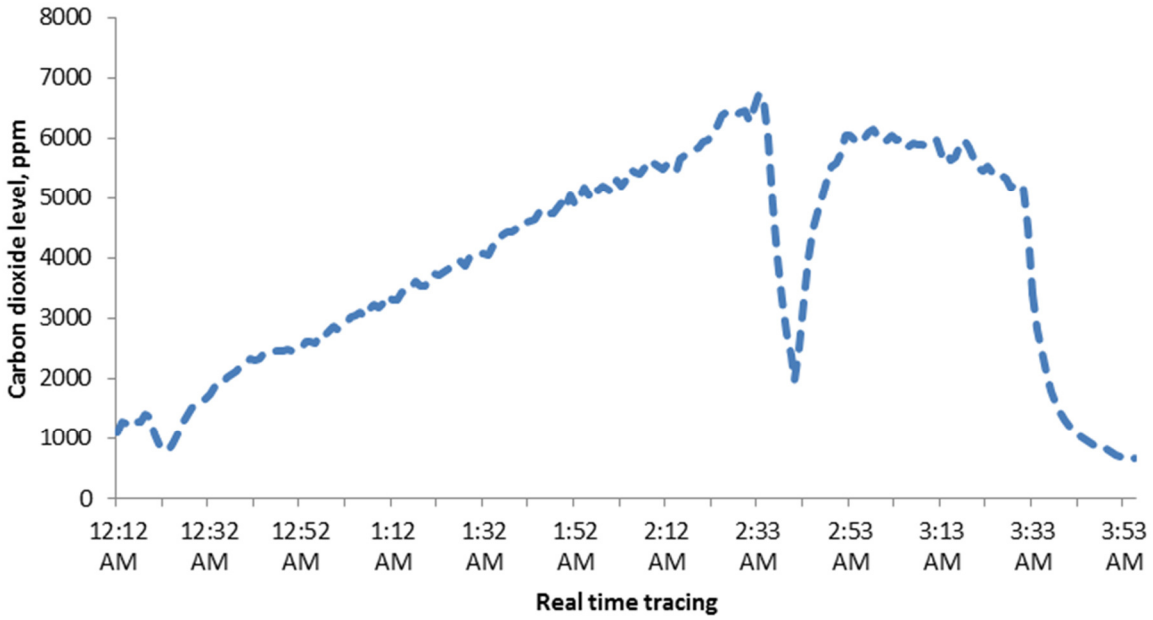
Supplementary Figure 2. Ventilation conditions from a nightclub in Dar es Salaam.

A real-time plot of carbon dioxide levels (y-axis) in parts per million (ppm) over time (x-axis) in 24 hours within a nightclub. The plot shows poor ventilation condition (>1,000 ppm) past midnight when the number of night club attendees increases. The sharp trough at 2.30 am to 2.50 am correspond to the time when the volunteer went outside before going back to the night club.



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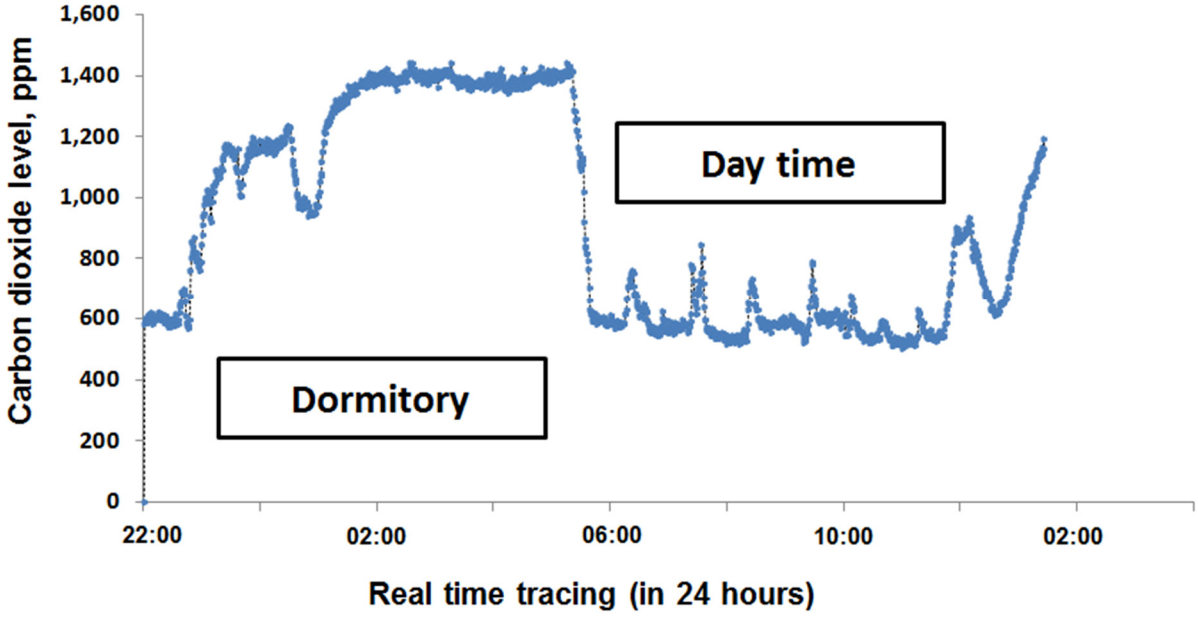
Supplementary Figure 3. Ventilation conditions at a college in Dar es Salaam.

A real-time plot of carbon dioxide levels in parts per million (ppm) over time from one college (students aged between 18 and 22 years) showing good ventilation conditions during day in the classrooms with open windows, but poor ventilation conditions in a dormitory during the night.



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Supplementary Table 1. Parameters used to estimate TB transmission in Dar es Salaam

Parameter description	Value	Reference(s)
Infectious quanta (*q*)		
Smear-positive TB patient (average)	1.25 q/hour	(Andrews et al., 2013)
Smear-positive TB patient co-infected with HIV	5.5 q/hour	(Escombe et al., 2008)
Smear-positive TB patient co-infected with HIV (average)	8.2 q/hour	(Andrews et al., 2013; Escombe et al., 2008)
Estimated from an office building	12.7 q/hour	(Nardell et al., 1991)
Infectious individuals in space (*I*)		
TB notification rate from NTLP	142/100,000 people	(MoHSW, 2014)
TB prevalence from NTLP	295/100,000 people	(Ministry of Health and Social Welfare, 2013)
TB prevalence estimates from WHO	528/100,000 people	(World Health Organization, 2015a)
One smear-positive patient	1 per location	-
Time spent in each location (*t*)	Varying by location	This study
Number of contacts at each time point (*n*)	Varying by location	This study
Rebreathed fraction (*f*)	Varying by location	This study

NTLP, National Tuberculosis and Leprosy Programme; TB, tuberculosis; WHO, World Health Organization

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6. Diagnostic delay and associated factors among patients with pulmonary tuberculosis in Dar es Salaam, Tanzania

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6.1 Abstract

Background: Tanzania is among the 30 countries with the highest tuberculosis (TB) burdens. Because TB has a long infectious period, early diagnosis is not only important for reducing transmission, but also for improving treatment outcomes. We assessed diagnostic delay and associated factors among infectious TB patients.

Methods: We interviewed new smear-positive adult pulmonary TB patients enrolled in an ongoing TB cohort study in Dar es Salaam, Tanzania, between November 2013 and June 2015. TB patients were interviewed to collect information on socio-demographics, socio-economic status, health-seeking behaviour, and residential geocodes. We categorized diagnostic delay into ≤ 3 or > 3 weeks. We used logistic regression models to identify risk factors for diagnostic delay, presented as crude (*OR*) and adjusted Odds Ratios (a*OR*). We also assessed association between geographical distance (incremental increase of 500 meters between household and the nearest pharmacy) with binary outcomes.

Results: We analysed 513 patients with a median age of 34 years (interquartile range 27 – 41); 353 (69%) were men. Overall, 444 (87%) reported seeking care from health care providers prior to TB diagnosis, of whom 211 (48%) sought care > 2 times. Only six (1%) visited traditional healers before TB diagnosis. Diagnostic delay was positively associated with absence of chest pain (a*OR* = 7.97, 95% confidence intervals [*CI*] = 3.15 – 20.19; *P* < 0.001), and presence of hemoptysis (a*OR* = 25.37, 95% *CI* = 11.15 – 57.74; *P* < 0.001) and negatively associated with use of medication prior to TB diagnosis (a*OR* = 0.31, 95% *CI* = 0.14 – 0.71; *P* = 0.01). Age, sex, HIV status, education level, household income, and visiting health care facilities (HCFs) were not associated with diagnostic delay. Patients living far from pharmacies were less likely to visit a HCF (incremental increase of distance versus visit to any facility: *OR* = 0.51, 95% *CI* = 0.28 – 0.96; *P* = 0.037).

Conclusions: TB diagnostic delay was common in Dar es Salaam, and was more likely among patients without prior use of medication and presenting with hemoptysis. Geographical distance to HCFs may have an impact on health-seeking behaviour. Increasing community awareness of TB signs and symptoms could further reduce diagnostic delays and interrupt TB transmission.

Key words: Tanzania, tuberculosis, diagnostic delay, health-seeking, geographic information system, pharmacy, transmission

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6.2 Background

Tuberculosis (TB) control remains a major public health challenge in low-income countries with a high incidence of TB, particularly where HIV prevalence is also high (Cummings, 2007). In these settings, TB accounts for approximately 40% of adult deaths. In almost half of these cases, the disease remains undiagnosed until death (Gupta et al., 2015). TB patients are often diagnosed at the later stages of the disease, due to health-seeking behaviour, inappropriate diagnostic investigations requested by health care providers, and limited diagnostic capacities at health care facilities (HCFs) (Senkoro et al., 2015; World Health Organization, 2006). Patients experiencing TB symptoms may initially seek relief by using self-prescribed medication or by consulting a health care provider who does not request TB investigations despite repeated visits (Rabin et al., 2013). The economic burden of seeking care remains a barrier for TB patients (Chen et al., 2015).

Delaying diagnosis and treatment of TB has important consequences for disease control at both the individual as well as the community level. At the individual level, a patient with a delayed diagnosis of TB risks advanced disease states and worse treatment outcomes. At community level, a patient with delayed diagnosis is infectious to close contacts; an untreated smear-positive TB case infects approximately 15 people annually (Storla et al., 2008; World Health Organization, 2015b). Delay in seeking care, therefore, promotes continued TB transmission (Creswell et al., 2015). The complex pathway to care, from the onset of symptoms to diagnosis and treatment, may result in delays in seeking care and contribute to patient morbidity and mortality (Kapoor et al., 2012; Senkoro et al., 2015).

Tanzania is among the 30 countries with the highest TB burdens (World Health Organization, 2015c). TB case detection in Tanzania largely relies on passive case detection when patients present themselves to HCFs, which likely leads to longer diagnostic delays (Ngadaya et al., 2009). The first national TB prevalence survey in Tanzania in 2012 showed that only 30% of people with TB symptoms sought health care and almost half of these did not undergo diagnostic procedures for TB (Senkoro et al., 2015). To better understand this situation, we studied the extent of diagnostic delay of TB, its associated risk factors and factors contributing to health-seeking behaviour among newly

diagnosed smear-positive adult pulmonary TB (PTB) patients in Dar es Salaam, an urban region in Tanzania with a high TB burden.

6.3 Methods

6.3.1 Study setting and study population

We collected data from an ongoing prospective cohort of smear-positive adult PTB patients (≥ 18 years) in Dar es Salaam, Tanzania (TB-DAR). TB-DAR was initiated in November 2013 to study the epidemiology and molecular epidemiology of TB in the densely populated Temeke district in Tanzania's commercial capital, Dar es Salaam. Dar es Salaam, with a population of about 4.4 million and rapid urbanization (The United Republic of Tanzania, 2012), accounted for nearly 22% of the 65 732 TB cases notified in 2013 to the National Tuberculosis and Leprosy Programme (NTLP) in Tanzania (Ministry of Health and Social Welfare, 2014; World Health Organization, 2014). The Temeke district has approximately 1.4 million people and reported 4,373 TB cases in 2014 (Ministry of Health and Social Welfare, 2014). The main sources of income in the district are food crop sales and small businesses (Prime Minister's Office, Regional Administration and Local Government, 2014).

Eligibility criteria for TB-DAR study participants were as follows: 1) ≥18 years of age at recruitment, 2) sputum microscopy smear-positive TB (quantification grading at least scanty), 3) residency in Wailes I and II sub-districts of Temeke and 4) attends the TB clinic at the Temeke District Hospital or one of its associated TB satellite treatment centers, Tambukareli and Pasada. Patients with confirmed TB were started on standard TB treatment regimen within one day of diagnosis, as per the national guidelines (National Tuberculosis and Leprosy Program, 2013). Diagnostic and treatment services for TB are provided free of charge by the NTLP.

For this analysis, we included newly diagnosed smear-positive adult PTB cases who were enrolled between November 2013 and June 2015. The selection of eligible patients is shown in Figure 1. Of the 525 smear-positive PTB patients enrolled in the TB-DAR Study during this time period, we excluded 12 (2.3%) patients for the following reasons: 10 (2%) relapse patients, 1 (0.2%) loss to follow-up and 1 (0.2%) treatment failure (Figure 9).

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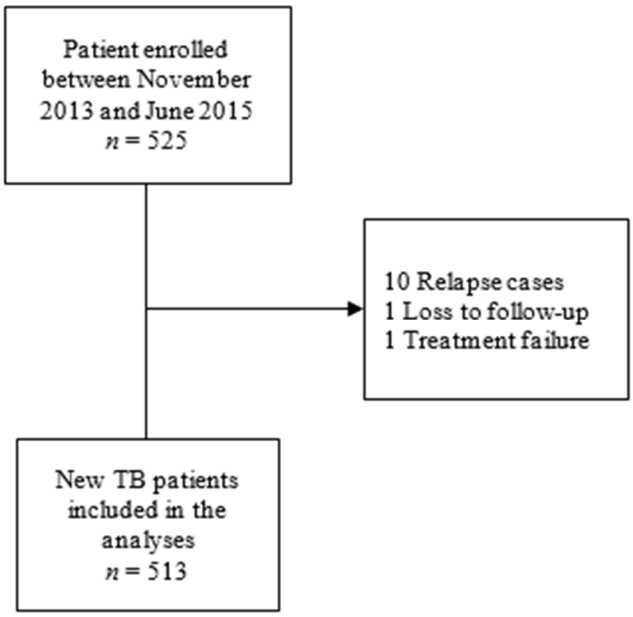

Figure 9. Flow chart of patient selection

According to the national guidelines (National Tuberculosis and Leprosy Program, 2013), presumptive PTB cases (outpatient or inpatient) are counseled to bring sputum samples to the laboratory; patients with a smear-positive result or smear-negative TB cases with clinical features suggestive of TB (and/or based on additional tests such as chest X-ray) are immediately started on TB treatment.

6.3.2 Data collection and definitions

Clinical officers with extensive experience in clinical research interviewed patients using standardized questionnaires (Steiner et al., 2016). Officers collected data on socio-demographic and socio-economic characteristics and on health-seeking behaviour at the time of enrolment. In ascertaining signs and symptoms suggestive of TB, we posed specific questions (patient ever having felt sick of any symptom such as coughing, chest pain, fever, weight loss, hemoptysis, night sweats) and patients were asked to recall the duration of symptoms (in weeks) (Aoki et al., 1985). Duration of diagnostic delay was calculated based on the longest reported TB-related symptom.

A new adult TB case was defined as a patient aged ≥ 18 years with newly diagnosed smear-positive (at least scanty) pulmonary TB. Sputum microscopy was performed using fluorescent light-

emitting diode (LED) microscopy and the quantitative scoring system was based on the number of acid-fast bacilli (AFB) according to the national guidelines (Lumb et al., 2013; National Tuberculosis and Leprosy Program, 2013): scanty, 1+, 2+, and 3+. Diagnostic delay was defined as the time from onset of any TB-related symptom (patient ever having felt sick of any symptom such as coughing, chest pain, fever, weight loss, hemoptysis and night sweat) until the time of TB diagnosis (Aoki et al., 1985; Chen et al., 2014; Lawn et al., 1998; World Health Organization, 2006). Health Care Facilities (HCFs) were defined as places that provide health care, including hospitals, health centers and dispensaries. Prior medication was defined as the use of any self-prescribed or prescribed medication prior to TB diagnosis and any medication other than anti-TB, antiretroviral therapy (ART) or co-trimoxazole (for patients co-infected with HIV). Multiple HCF visits were defined as seeking health care from formal health providers more than twice.

6.3.4 Statistical analysis

Descriptive analyses were performed to summarize the data. For the purpose of this study, we categorized diagnostic delay into two categories: three weeks or less and more than 3 weeks. We considered three weeks as a cutoff for diagnostic delay based on the distribution of the delay in our study population (Supplementary Figure 4) and the range of diagnostic delay reported in a systematic review (Sreeramareddy et al., 2009). Associations between dependent variables (delay of ≤ 3 and > 3 weeks) and independent variables (such as age, sex, occupation, household income, household size, TB symptoms, prior use of medication, and number of HCF visits) were analyzed using logistic regression models. Variables were included in the multivariate model if thought to be clinically or socially relevant or had a *P*-value of < 0.05 following univariate analysis. Result are presented as crude (*OR*) and adjusted odds ratios (a*OR*). All analyses were performed in Stata software version 13.1 (Stata Corporation, Collage Station, Texas, USA).

6.3.5 Geographical analysis

We collected geocodes using Android tablets (Samsung) for all known pharmacies, dispensaries (public/private), and hospitals, identified during extensive fieldwork in the study area (Figure 10). In addition, all study participants' households were geocoded. We generated maps using the open source

software QGIS version 2.10.1 (QGIS Development Team, 2015). We then calculated the Euclidean distances in meters as a linear distance matrix between participants' households and the nearest pharmacy. The resulting Euclidean distances were then imported into STATA software for further analyses. To overcome separation in conventional logistic models, we created penalized likelihood models using the firth logit command in STATA and assessed the association between an incremental increase of 500 meters (distance between household and the nearest pharmacy) with binary outcomes (prior use of medication, diagnostic delay and any visit to a HCF) (Coveney, 2015). Results were presented as unadjusted *OR*s (Figure 11).

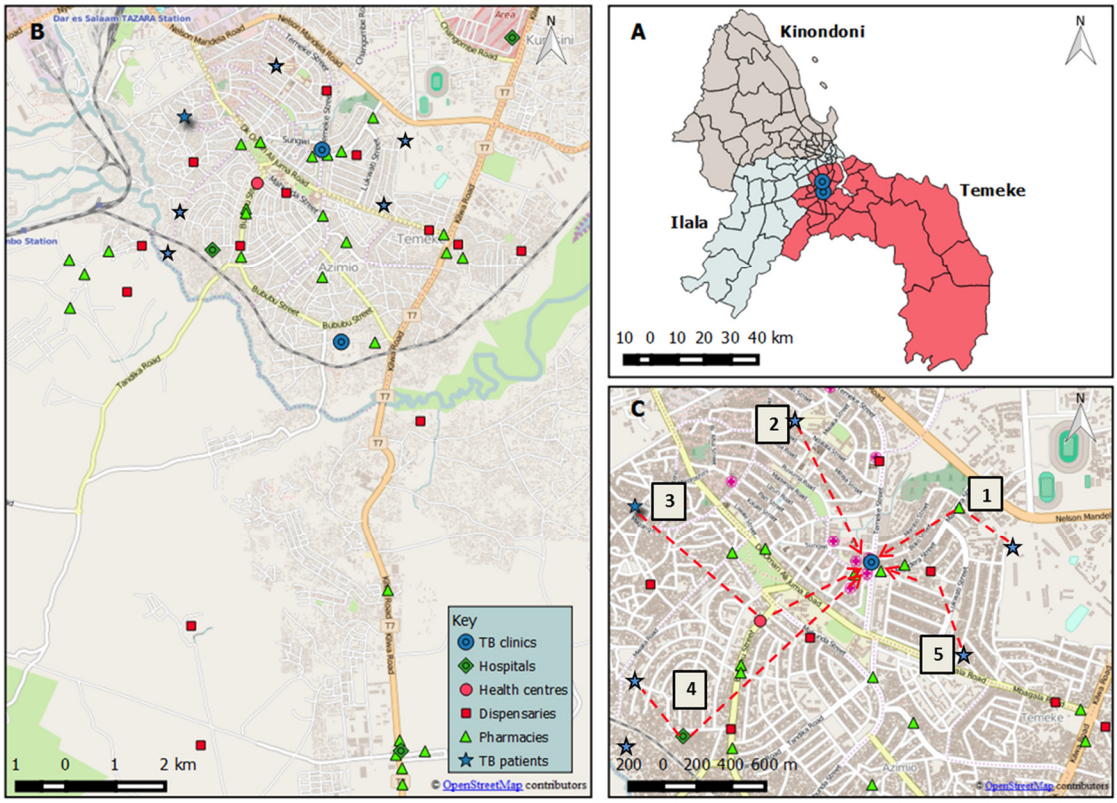

Figure 10. Geographical analyses of health care facilities (HCFs) and pathways to care of patients with tuberculosis (TB) symptoms in the study area, Temeke District, Dar es Salaam, Tanzania.
Panel A: Localization of the two governmental TB clinics which serve as recruitment sites in the study area (in red). **Panel B**: Spatial distribution of pharmacies and HCFs in the study area**. Panel C**: Five examples of possible pathways to care of patients with TB symptoms seeking care. Various types of HCFs as the entry point into the health care system (single or multiple visits) until final diagnosis at the TB clinic are presented.

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6.4 Results

6.4.1 Patient characteristics

We analysed 513 newly diagnosed smear-positive adult PTB patients. The median age was 34 years (interquartile range [IQR]: 27 – 41 years, range 18 – 79); 353 (69%) were men and 147 (29%) were HIV-positive (Table 1). The proportion of HIV was higher among women compared to men (43%, versus 22%; $P < 0.001$). Of the 147 HIV-positive patients, 37 (25%) were on ART prior to TB diagnosis and 86 (59%) started ART within seven weeks of TB diagnosis; ART information was unknown for 24 (16%) patients. Overall, 92 patients (18%) had no formal education. More than half of the patients (341; 66%) were semi-skilled workers. Most patients lived in rented houses (288; 56%). The median household size was three persons (IQR: 2 – 4 persons) and 408 (80%) patients earned < 200 USD per month.

The most commonly reported symptom was coughing (511 patients; 99.6%), followed by weight loss (493; 96%), night sweats (487; 94%), fever (475; 93%), chest pain (411; 80%), and hemoptysis (147; 29%) (Table 2). The median duration of cough was three weeks (IQR: 3.0 – 3.0). All other symptoms had a median duration of two weeks (IQR: 1.0 – 3.0).

6.4.2 Health-seeking behaviour and diagnostic delay

About 444 (87%) patients reported seeking care from at least one of the formal health care providers; of these, 211 (48%) sought care at a HCF three times or more in relation to TB symptoms prior to TB diagnosis. Only six (1%) reported to have sought care from traditional healers and 63 (12%) did not seek care until they presented to the HCFs of the study recruitment centers, where they were diagnosed with TB. The median age of those who sought care three times or more was 35 years (IQR: 29 – 44). More men than women sought formal care three times or more (67% versus 33%). Almost all patients reported cough (Table 2). The majority of patients reported having taken medication prior to TB diagnosis (461 patients; 90%); amoxicillin was the most common drug of choice (432; 94%), followed by ciprofloxacin (5; 1%).

The median diagnostic delay time was three weeks (IQR: 3.0 – 3.0, range 1 – 45 weeks); 28 (5%) patients had a diagnostic delay of more than four weeks. By the third week of presenting with

TB symptoms, 442 (86%) patients had been diagnosed with the disease. Figure S1 shows the distribution of the diagnostic delay in the study population.

6.4.3 Patient factors associated with diagnostic delay

In the multivariate analysis, diagnostic delay of > 3 weeks was more likely among patients who did not have chest pain (a*OR* = 7.97, 95% *CI* = 3.15 – 20.19; *P* < 0.001), and who presented with hemoptysis (a*OR* = 25.37, 95% *CI* = 11.15 – 57.74; *P* < 0.001), and less likely among patients who used medication prior to TB diagnosis (a*OR* = 0.31, 95% *CI* = 0.14 – 0.71; *P* = 0.03) as shown in Table 2. Age, sex, HIV status, occupation, level of income, household size, and visits to HCF were not associated with diagnostic delay (Table 3).

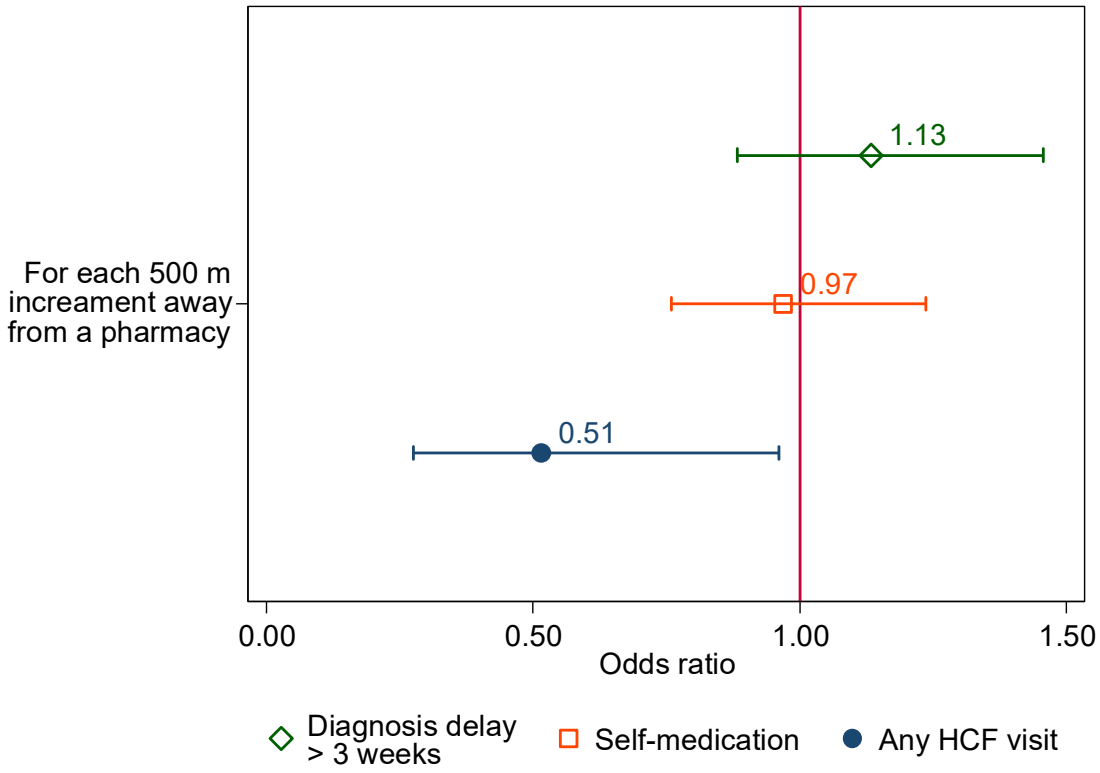

Figure 11. Association between health-seeking behaviour and geographical distances to pharmacies. The regression coefficient plot (with corresponding 95% confidence intervals) shows the association of the Euclidean distance between participants' households to the nearest pharmacy with purchase of medication prior to TB diagnosis, diagnostic delay (> 3 weeks), and visit of any HCF before TB diagnosis. Odds Ratios (*OR*) above 1 indicate that the factor is more likely with increasing distance away from the nearest pharmacy, and an *OR* below 1 that the factor is more likely the closer the household is to a pharmacy.
HCF, health care facility

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Table 2. Patient characteristics of new smear-positive adult pulmonary tuberculosis (TB) cases in the Temeke District, Dar es Salaam, Tanzania.

Variable	All *n* = 513	Delay duration (weeks)		Prior medication		HC facility visits	
n (%)		≤ 3	> 3	Not used	Used	≤ 2 visits	> 2 visits
Total	513 (100)	406 (79)	107 (21)	52 (10)	461 (90)	297 (58)	216 (42)
Age, years							
18 – 24	91 (18)	73 (18)	18 (17)	6 (12)	85 (18)	58 (20)	33 (15)
25 – 45	333 (65)	264 (65)	69(64)	37 (71)	296 (64)	199 (67)	134 (59)
>45	89 (17)	69(17)	20 (19)	9 (17)	80 (17)	40 (13)	49 (23)
Sex							
Male	353 (69)	280 (69)	73 (68)	38 (73)	315 (68)	209 (70)	144 (67)
Female	160 (31)	126 (31)	34 (32)	14 (27)	146 (32)	88 (30)	72 (33)
HIV status							
Positive	146 (28)	119 (29)	27(25)	16 (31)	130 (28)	73 (25)	73 (34)
Negative	367 (72)	287 (71)	80 (75)	36 (69)	331 (72)	224 (75)	143 (66)
Education							
No education	92 (18)	73 (18)	19 (18)	8 (15)	84 (18)	46 (15)	46 (21)
Primary/Secondary	404 (79)	316 (78)	88 (82)	44 (85)	360 (78)	243 (82)	161 (75)
University	17 (3)	17(4)	0 (0)	0 (0)	17 (4)	8 (3)	9 (4)
Occupation							
Unemployed or h/wife	103 (20)	86 (21)	17 (16)	4 (8)	99 (21)	46 (15)	57 (26)
Unskilled labour	69 (13)	52 (13)	17 (16)	11 (21)	58 (13)	38 (13)	31 (14)
Semiskilled labour	341 (67)	268 (66)	73 (68)	37 (71)	304 (66)	213 (72)	128 (60)
Household income							
<$200 per month	408 (80)	329 (81)	79 (74)	37 (71)	371 (80)	225 (76)	183 (85)
≥$200 per month	105 (20)	77 (19)	28 (26)	15 (29)	90 (20)	72 (24)	33 (15)
BMI category, kg/m^2							
<18.5	36 (7)	29 (7)	7 (7)	3 (6)	33 (7)	25 (8)	11 (5)
18 – 24.9	273 (53)	204 (50)	69 (64)	20 (38)	253 (55)	163 (55)	110 (51)
25 – 29.9	154 (30)	129 (32)	25 (23)	19 (37)	135 (29)	78 (26)	76 (35)
>30	50 (10)	44 (11)	6 (6)	10 (19)	40 (9)	31 (11)	19 (9)
Household size, persons							
≥4	137 (27)	123 (30)	14 (13)	3 (6)	134 (29)	56 (19)	81 (37)
2 – 3	331 (64)	256 (63)	75 (70)	43 (83)	288 (63)	208 (70)	123 (57)
Single	45 (9)	27 (7)	18 (17)	6 (12)	39 (8)	33 (11)	12 (6)
House ownership							
Own	225 (44)	178 (79)	47 (21)	17 (33)	208 (45)	127 (43)	98 (45)
Rented	288 (56)	228 (79)	60 (21)	35 (67)	253 (55)	170 (57)	118 (55)
Coughing							
No	2 (0.5)	2 (0.5)	0	0 (0)	2 (0.4)	1 (0.3)	1(0.5)
Yes	511 (99.5)	404 (99.5)	107(100)	52 (100)	459 (99.6)	296(99.7)	215 (99.5)
Fever							
No	38 (7)	37 (9)	1 (1)	3 (6)	35 (8)	33 (11)	5 (2)
Yes	475 (93)	369 (91)	106 (99)	49 (94)	426 (92)	264 (89)	211 (98)
Chest pain							
No	102 (20)	69 (17)	33 (31)	40 (77)	62 (14)	94 (32)	8 (4)
Yes	411 (80)	337 (83)	74 (69)	12 (23)	399 (87)	203 (68)	208 (96)
Haemoptysis							
No	336 (71)	324 (80)	42 (39)	52 (100)	314 (68)	185 (62)	181 (84)
Yes	147 (29)	82 (20)	65 (61)	0	147 (32)	112 (38)	35 (16)
Night sweat							
No	26 (5)	25 (6)	1 (1)	1 (2)	25 (5)	24 (8)	2 (1)
Yes	487 (95)	381 (94)	106 (99)	51 (98)	436 (95)	273 (92)	214 (99)
Unexplained weight loss							
No	20 (4)	18 (4)	2 (2)	1 (2)	19 (4)	15 (5)	5 (2)
Yes	493 (96)	388 (96)	105 (98)	51 (98)	442 (96)	282 (95)	211 (98)

BMI, Body Mass Index; h/wife, housewife; TB, tuberculosis; HC facility, health care facility

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Table 3. Associations of diagnosis delay (defined as >3 weeks) with socio-demographic and clinical characteristics among new pulmonary TB patients.

Characteristic	Cases *n* (%)	Unadjusted		Adjusted	
		***OR* (95% *CI*)**	***P*-value**	**a*OR* (95% *CI*)**	***P*-value**
Age, years			0.7		0.7
18 – 24	91 (18)	1		1	
25 – 45	333 (65)	1.06 (0.59 – 1.89)		0.81 (0.39 – 1.67)	
>45	189 (17)	1.18 (0.57 – 2.41)		1.08 (0.44 – 2.66)	
Sex			0.9		0.6
Female	160 (31)	1		1	
Male	353 (69)	0.97 (0.61 – 1.53)		0.84 (0.46 – 1.51)	
HIV status			0.4		0.5
Negative	366 (71)	1		1	
Positive	147 (29)	0.81 (0.50 – 1.30)		0.83 (0.46 – 1.52)	
Occupation			0.4		0.1
Unemployed, or h/wife	103 (20)	1		1	
Unskilled labor	69 (13)	1.65 (0.78 – 3.52)		1.24 (0.48 – 3.26)	
Semiskilled labor	341 (67)	1.38 (0.77 – 2.46)		0.69 (0.32 – 1.51)	
Household income			0.1		0.3
<$200 per month	408 (80)	1		1	
≥$200 per month	105 (20)	1.51 (0.92 – 2.49)		1.34 (0.73 – 2.47)	
Household size, persons			<0.001		0.2
≥4	137 (27)	1		1	
2 – 3	331 (64)	2.57 (1.40 – 4.74)		1.38 (0.67 – 2.83)	
Single	45 (9)	5.86 (2.60 – 13.21)		1.81 (0.71 – 4.59)	
Visits to a HCF			<0.001		0.9
>2	216 (42)	1		1	
≤2	297 (58)	3.31 (2.01 – 5.46)		0.99 (0.52 – 1.90)	
Prior purchase of medication			0.001		0.01
No	52 (10)	1		1	
Yes	461 (90)	0.37 (0.20 – 0.68)		0.31 (0.14 – 0.71)	
Chest pain			0.002		<0.001
Yes	411 (80)	1		1	
No	102 (20)	2.18 (1.34 – 3.54)		7.97 (3.15 – 20.19)	
Hemoptysis			<0.001		<0.001
No	366 (71)	1		1	
Yes	147 (29)	6.11 (3.87 – 9.66)		25.37 (11.15 – 57.74)	

OR, Odds Ratio; aOR adjusted Odds Ratio; 95% CI, 95% Confidence Interval; h/wife, housewife; HCF, health care facility

Model was adjusted for age, sex, HIV status, occupation, household income, household size, visit to HCF, prior use of medication, chest pain and hemoptysis

6.4.4 Health-seeking behaviour and geographical distance to pharmacies

The map in Figure 10 shows the spatial distribution of pharmacies and HCFs in the study area, and offers examples of the complex pathways to care until final TB diagnosis. We counted a total of 35 pharmacies within the catchment area of the study. The median Euclidean distance from a participant's household to the nearest pharmacy was 412.3 meters (IQR: 254 – 781, range 6 – 4 820).

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More than half of participants' households (303; 59%) were found within 500 meters of their nearest pharmacy; 137 (26.7%) were between 500 and 1 000 meters to the nearest pharmacy and only 73 (14.2%) participants' households were more than 1 000 meters away from a pharmacy. For each incremental increase of 500 meters away from a pharmacy, the odds of visiting a HCF decreased (*OR* = 0.51, 95% *CI* = 0.28–0.96; *P* = 0.037, comparing patients with any visit to a HCF versus no visit prior to TB diagnosis, Figure 11). Geographical distance did not seem to have an effect on the use of medication prior to TB diagnosis or on the degree of diagnostic delay.

6.5 Discussion

We studied diagnostic delay among adult infectious TB patients in an urban district of Dar es Salaam, Tanzania, with a high TB notification rate. We found that multiple visits to HCFs and prior use of prescribed or self-prescribed medication was frequent (41% and 90%) and that multiple visits were more likely among patients living closer to HCFs.

We showed that 21% of patients delayed seeking care for TB symptoms. The median diagnostic delay of three weeks in our study was shorter compared to other delay studies conducted in sub-Saharan Africa, Asia as well as Eastern Europe (Irani et al., 2007; Mfinanga et al., 2008; Rabin et al., 2013; Saifodine et al., 2013; Thakur and Murhekar, 2013; Yimer et al., 2014). A systematic review from low- and high-income settings reported a median total delay of 3 – 26 weeks (Sreeramareddy et al., 2009). Our diagnostic delay (time from onset of symptoms until diagnosis) was also shorter than the treatment delay (time from onset of symptoms until treatment) reported in a study conducted in six rural and urban districts in Tanzania (median treatment delay time was 10 – 14.5 weeks) (Hinderaker et al., 2011). In our study, we disregarded the treatment delay because all patients were strictly put on TB treatment within one day of confirming TB diagnosis, as per the guidelines of the NTLP.

We found that more than three quarters of patients sought formal care prior to being diagnosed with TB, with nearly half of them seeking care more than twice. This indicates that a high proportion of TB patients is seen by several different health care providers before receiving a TB diagnosis,

suggesting a low suspicion index among health care providers. The first national TB prevalence survey in Tanzania reported that one third of patients suspected of having TB sought care before the survey, which confirms that many infectious TB cases are missed by the health care system in Tanzania (Senkoro et al., 2015). Furthermore, a study from Uganda showed that the proportion of patients who sought care before being diagnosed with TB can be as high as 80% (Lambert and Van der Stuyft, 2005).

We also found that use of medication prior to TB diagnosis was associated with shorter diagnostic delays. This could be explained by the fact that patients who did not use medication prior to TB diagnosis could not afford any health care service, feared the stigma of being known to be sick, or did not have adequate knowledge about signs and symptoms of TB. Indeed, inadequate knowledge about TB and stigma has previously been associated with longer diagnostic delays (Biya et al., 2014; Odusanya and Babafemi, 2004; Shete et al., 2015; Sreeramareddy et al., 2009; World Health Organization, 2006). In contrast, studies from Georgia, Ethiopia and Angola reported that the use of antibiotics prior to TB diagnosis was associated with a prolonged delay (Belay et al., 2012; Paz-Soldan et al., 2014; Rabin et al., 2013). This difference can be explained by the fact that we defined use of medication as any medication prior to TB diagnosis, rather than antibiotics alone, as patients may not accurately remember the type of medication used in the past. Furthermore, patients may tend to wait for improvement of symptoms before seeking health care in these specific study settings and countries, but not in our setting of Dar es Salaam.

Patient factors associated with diagnostic delay may vary considerably across settings and countries (Makwakwa et al., 2014; Mfinanga et al., 2008; Saifodine et al., 2013; Segagni Lusignani et al., 2013; Shete et al., 2015; Storla et al., 2008; Ukwaja et al., 2013). In our study, diagnostic delay was not reduced among HIV-positive patients despite the widely implemented TB/HIV collaborative activities by the National AIDS Control Programme and the NTLP in Tanzania (National Tuberculosis and Leprosy Program, 2013). We also found that chest pain was associated with a shorter diagnostic delay, in contrast to studies from Afghanistan and Brazil (Maciel et al., 2010; Sabawoon et al., 2012). Hemoptysis, which usually presents at the later stage of disease (Kardjito and

Grange, 1980), was associated with a prolonged delay in our study. Patients presenting with hemoptysis may tend to ignore other TB symptoms until the disease is advanced and hemoptysis has developed. Systematic reviews reported the opposite trend, possibly because hemoptysis may necessitate health care providers to investigate for TB early on (Cai et al., 2015; Storla et al., 2008). Consistent with our results, previous studies found no association of diagnostic delay with patients' sex (Shete et al., 2015) or low-income class (Storla et al., 2008).

We also investigated the impact of the geographical distance on the health-seeking behaviour of patients with TB symptoms using geographic information system (GIS) tools. GIS is increasingly being used to describe the epidemiology of diseases and to map HCFs for strategic planning of public health measures (MacPherson et al., 2013; Sabde et al., 2011; Theron et al., 2015). We found that patients living closer to a pharmacy tended to visit facilities more frequently. This suggests that the availability of health care services (public/private) affects use by the resident population. In line with our results, a study from Tanzania showed that residing at a distance of more than 5 km from a TB diagnostic center was associated with diagnostic delay (Mfinanga et al., 2008). In addition, a systematic review found that a longer walking distance to a HCF was correlated with patient delay, defined as the time from onset of the first TB symptoms (usually described as the onset of persistent coughing) until the date of the first HCF consultation (Cai et al., 2015).

Our study has several limitations. First, we had to rely on patients' recall of previous interactions with the health care system. This could have introduced a recall bias influencing the accuracy of the data. However, our interviewers were well trained in patient interviews and experienced in conducting scientific studies. Therefore, the collected information was likely to be accurate. Second, our findings may be specific to urban settings in Tanzania such as Dar es Salaam as well as to the study period and, therefore, may not be generalizable. Finally, we did not collect detailed information on the pathway to care at the individual level, such as dates and types of HCF visited, patients' reasons for delay or socio-cultural factors underlying the delay in seeking care.

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In conclusion, we found that TB diagnostic delay was common in urban Tanzania, possibly due to passive case detection practice. We also found that the use of medication prior to TB diagnosis was common, which potentially poses challenges in the rational use of medication. Since many patients repeatedly accessed health care services before being diagnosed with TB, our study re-emphasizes the need for improved diagnostic capacities, as well as training and re-training of health care workers to increase awareness and timely diagnosis of TB. Consequent systematic screening for TB at HCFs at any care level could further reduce the diagnostic delay to interrupt TB transmission in the community. To support the implementation of active case detection interventions, future research should focus on health-seeking behaviour and how it is affected by cost factors and by the role of socio-cultural and other community determinants (Hargreaves et al., 2011; Lönnroth et al., 2009; Maske et al., 2015; Ngadaya et al., 2009).

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6.6 Declarations

Ethics approval and consent to participate

The study was approved by the institutional review board of the Ifakara Health Institute (IHI, reference no. IHI/IRB/04-2015), the Medical Research Coordinating Committee of the National Institute of Medical Research in Tanzania (NIMR, reference no. NIMR/HQ/R.8c/Vol. I/357), and the Ethics Committee of the Canton of Basel (EKNZ, reference no. UBE-15/42). All participants gave written informed consent before enrolment.

Consent for Publication

All participants gave written informed consent before enrolment for their data to be published.

Availability of data and material

The datasets analysed and material used during this study are available from the corresponding author on reasonable request.

Competing interest

None of the authors have any competing interests to declare.

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Authors' Contributions

KS, FM, JH, LF designed the study. KS, FM, TM, JH, NK, MC, EM, GM collected the data. KS, FM, JH did the analysis. KS, FM, JH, TM, NK, MC, EM GM and LF wrote and reviewed the manuscript. All the authors authorized the submission and publication of the manuscript.

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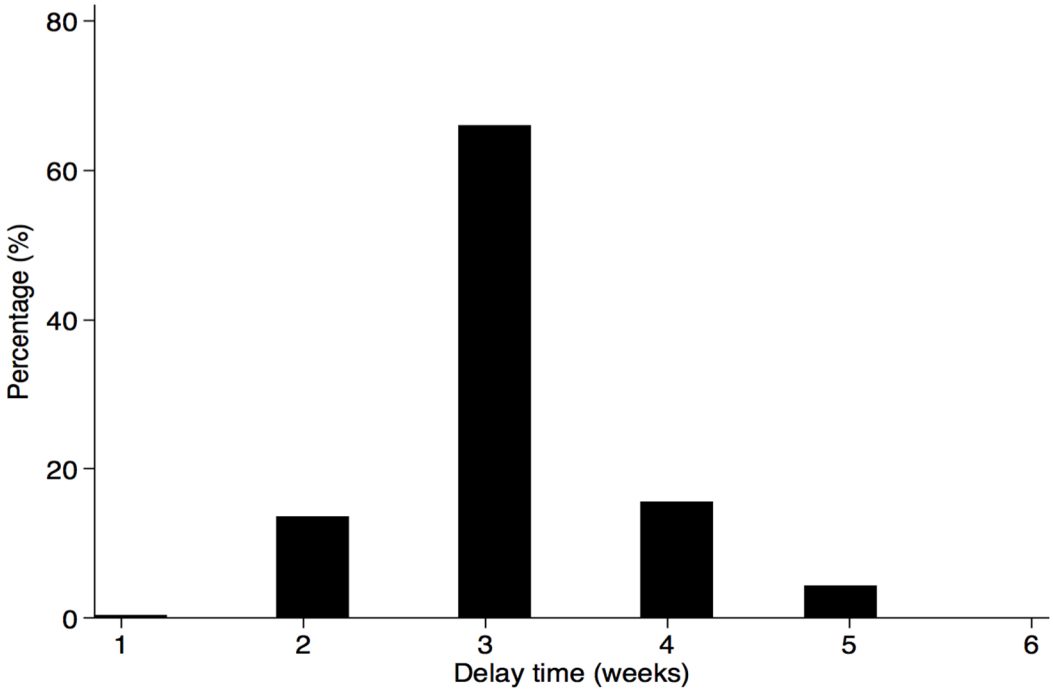
6.7 Supplementary materials

Supplementary Figure 4. Histogram of delay time reported among 507 TB patients



Six patients with delays of >5 weeks were excluded in this figure (one patient with a delay of 6 weeks, one patient with eight weeks, and three patients with 12 weeks, and one patient with a delay of 45 weeks)

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7. Distinct clinical characteristics and helminth co-infections in adult tuberculosis patients from urban compared to rural Tanzania

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7.1 Abstract

Background: Differences in rural and urban settings could account for distinct characteristics in the epidemiology of tuberculosis (TB). We comparatively studied epidemiological features of TB and helminth co-infections in adult patients from rural and urban settings of Tanzania.

Methods: Adult patients (≥18 years) with microbiologically confirmed pulmonary TB were consecutively enrolled into two cohorts in Dar es Salaam, with ~4.4 million inhabitants (urban), and Ifakara in the sparsely populated Kilombero district with ~400,000 inhabitants (rural). Clinical data were obtained at recruitment. Stool and urine samples were subjected to diagnose helminthiases using Kato-Katz, Baermann, urine filtration, and circulating cathodic antigen tests. Differences between groups were assessed by χ^2, Fisher's exact, and Wilcoxon rank sum tests. Logistic regression models were used to determine associations.

Results: Between August 2015 and February 2017, 668 patients were enrolled, 460 (68.9%) at the urban and 208 (31.1%) at the rural site. Median patient age was 35 years (interquartile range [IQR] 27-41.5 years), and 454 (68%) were males. Patients from the rural setting were older (median age 37 years *vs.* 34 years, p=0.003), had a lower median body mass index (17.5 kg/m^2 *vs.* 18.5 kg/m^2, p<0.001), a higher proportion of recurrent TB cases (9% *vs.* 1%, p<0.001), and in HIV/TB co-infected patients a lower median CD4 cell counts (147 cells/µl *vs.* 249 cells/µl, p=0.02) compared to those from urban Tanzania. There was no significant difference in frequencies of HIV infection, diabetes mellitus, and haemoglobin concentration levels between the two settings. The overall prevalence of helminth co-infections was 22.9% (95% confidence interval [CI] 20.4-27.0%). The significantly higher prevalence of helminth infections at the urban site (25.7% *vs.* 17.3%, p=0.018) was predominantly driven by *Strongyloides stercoralis* (17.0% *vs.* 4.8%, p<0.001) and *Schistosoma mansoni* infection (4.1% *vs.* 16.4%, p<0.001). Recurrent TB was associated with living in a rural setting (adjusted odds ratio [aOR] 3.97, 95% CI 1.16-13.67) and increasing age (aOR 1.06, 95% CI 1.02-1.10).

Conclusions: Clinical characteristics and helminth co-infections pattern differ in TB patients in urban and rural Tanzania. The differences underline the need for setting-specific, tailored public health interventions to improve clinical management of TB and comorbidities.

Keywords: Co-infection, Helminth infection, Recurrent tuberculosis, Schistosomiasis, Tanzania, Tuberculosis

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7.2 Background

Worldwide, tuberculosis (TB) is the leading cause of mortality from an infectious disease surpassing human immunodeficiency virus (HIV) infection (World Health Organization, 2016). Globally, the burden of TB is decreasing, but mortality due to TB remains high with 1.4 million TB deaths and an estimated 10.4 million new cases in 2015 (World Health Organization, 2016). The global TB case detection rate is below 63% and even lower in Tanzania with a detection rate ranging from 42% to 54% (Ministry of Health and Social Welfare, 2013; WHO, 2015a). This is partly due to frequent delays in TB diagnosis in low-income settings (Ngadaya et al., 2009; Senkoro et al., 2015; Sreeramareddy et al., 2009; Storla et al., 2008; WHO, 2006) ranging from 25 to 185 days (Ngadaya et al., 2009; Sreeramareddy et al., 2009; Storla et al., 2008). Delay in TB diagnosis is associated with increased transmission in the community (Golub et al., 2006). A deeper understanding of the epidemiology of TB is needed in order to reach the ambitious vision of the End TB strategy of zero TB discrimination, disease suffering, and deaths by 2035 (Uplekar et al., 2015; WHO, 2015b).

In Tanzania the prevalence of TB varies considerably across regions, and is higher among males, older persons, and those with lower socioeconomic status (Ministry of Health and Social Welfare, 2013). Studies have shown different epidemiological features of TB in urban and rural settings due to differences in health-seeking behaviour, knowledge of TB transmission, gender roles, socioeconomic status, and disease burden (Deribew et al., 2010; Shen et al., 2012; van der Hoeven et al., 2012). Rural-urban characteristics and living conditions could account for differences in the epidemiology of TB in Tanzania and elsewhere. Comorbidities such as HIV and helminth co-infections contribute to different treatment outcomes among TB patients (Faurholt-Jepsen et al., 2013; Isanaka et al., 2012b, 2012a). We studied differences in the epidemiology of TB and comorbidities such as helminth co-infections and severe anaemia from two population-based TB cohort platforms established since 2013 in Tanzania (Mhimbira et al., 2017).

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7.3 Methods

7.3.1 Study settings

We included adult patients (≥18 years) from an ongoing prospective cohort of bacteriologically confirmed pulmonary TB patients in Tanzania (TB-DAR). TB-DAR was initiated in 2013 as a platform to study clinical and molecular epidemiology of TB in Tanzania. The study has two recruitment sites (Figure 12): one urban site in the densely populated Temeke district in Tanzania's economic capital, Dar es Salaam, with ~4.4 million inhabitants, and one in the rural site found within the Ifakara ward in the sparsely populated Kilombero district with ~400,000 inhabitants (NBS, 2013).

7.3.2 Study sites

Urban site (Dar es Salaam)

The urban site is located in the Temeke district in Dar es Salaam inhabited by about 1.4 million inhabitants (Figure 12). Patient recruitment was done at the TB clinic of Temeke District Hospital, which is one of three regional referral hospitals in the city. The hospital is the largest healthcare facility in the district that provides specialized care and treatment for the Temeke population. Recruitment of patients started in November 2013 and is ongoing in the second part of 2017 (Mhalu et al., 2015; Mhimbira et al., 2017; Said et al., 2017; Steiner et al., 2016).

Rural site (Ifakara)

The rural site is located in the Kilombero district with a population of about 407,000 people (NBS, 2013). Recruitment was done at the Chronic Disease Clinic of Ifakara (CDCI), a clinic for patients infected with HIV and/or TB at the Saint Francis Referral Hospital (Figure 12). The hospital is the largest healthcare facility of the Kilombero district in the Morogoro region, located in southern Tanzania, and provides care for residents of Kilombero and the nearby Ulanga district (Letang et al., 2017). Patient recruitment started in August 2015 and is ongoing at the time of manuscript writing.

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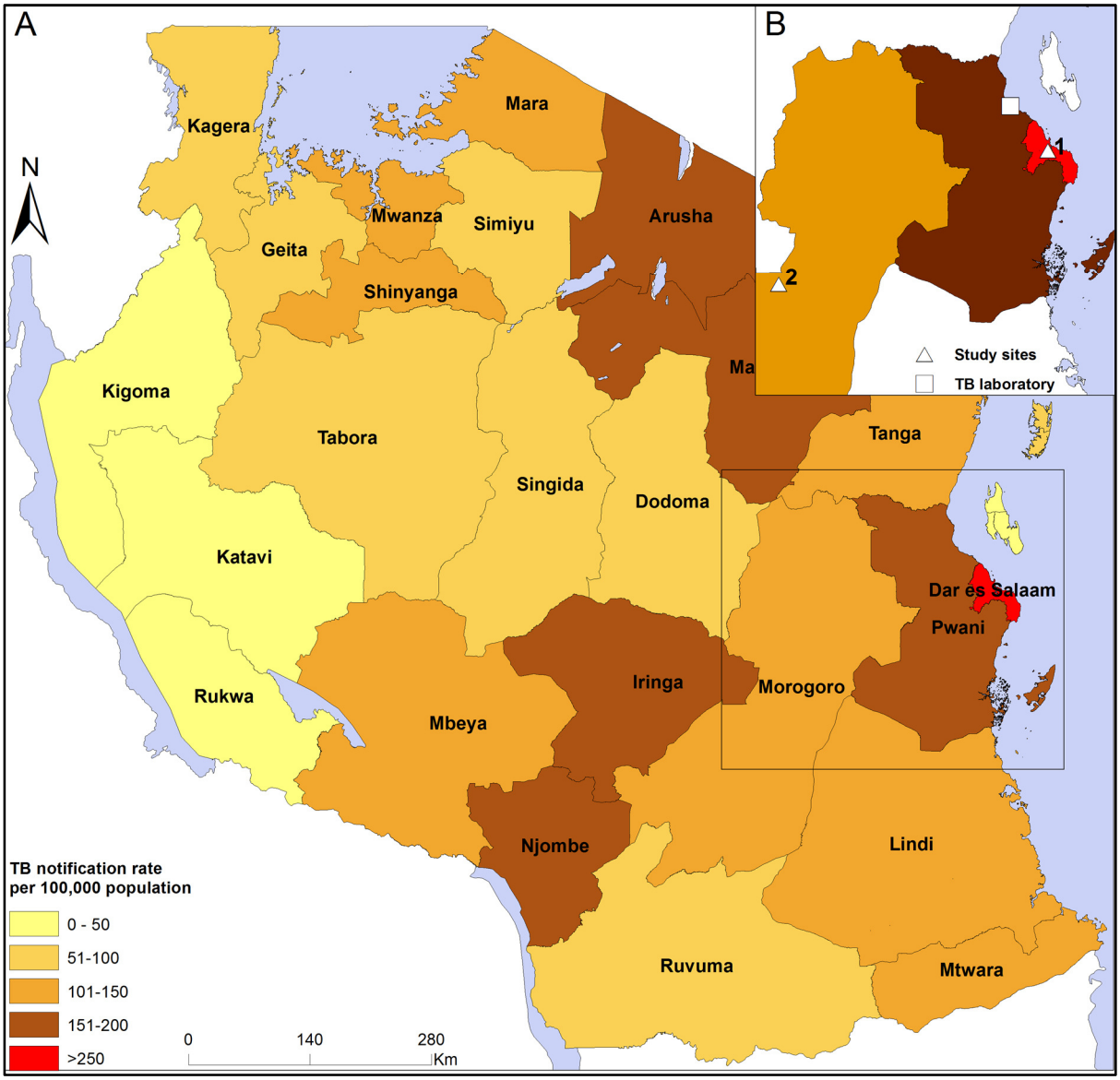

Figure 12. Map of Tanzania showing the regional tuberculosis (TB) notification rates, the locations of the study sites and the TB laboratory.
A: Overview. **B:** Study site 1 (urban), Temeke district, Dar es Salaam region (triangle); study site 2 (rural), Ifakara, Kilombero district (triangle); and the tuberculosis laboratory in Bagamoyo, Pwani region.

7.3.3 Study population

We analysed data collected from patients who were consecutively enrolled between August 1, 2015 and February 28, 2017. Patients were eligible for enrollment if they had an age of 18 years or above, lived within the study area, and gave written informed consent. Patients who were severely ill were excluded. Of the 672 bacteriologically confirmed TB patients enrolled in the TB-DAR study during this time period, we excluded four patients due to missing complete clinical information (Figure 13).

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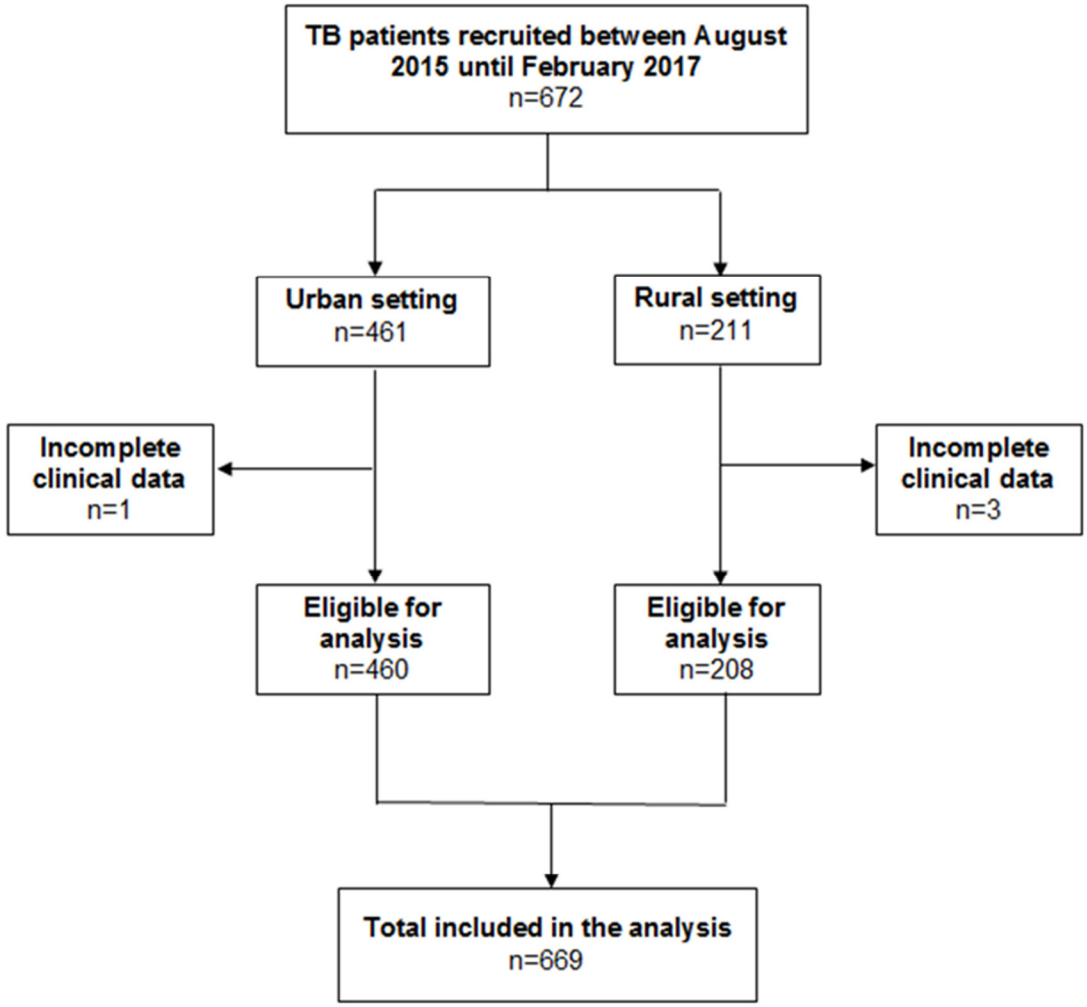

Figure 13. Selection of study patients.

7.3.4 Study procedures

At recruitment, patients with confirmed pulmonary TB were interviewed and underwent physical examination as previously described (Mhimbira et al., 2017; Said et al., 2017). After recruitment, patients were seen by the study doctor at 6 and 12 months after initiating TB treatment. Of note, TB treatment was supervised by home-based or facility-based direct observation (patient-centered approach) according to the National guideline (National Tuberculosis and Leprosy Program, 2013). Clinical data and biological specimens (sputum, serum, plasma, stool, and urine samples) were collected from all TB patients for laboratory analysis.

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7.3.5 Laboratory procedures

Microbiological investigations

Bacteriological confirmation of TB was done by examining the presence of acid fast bacilli (AFB) under fluorescence LED microscope or by using Xpert MTB/RIF assay (Cepheid; Sunnyvale, USA) in all sputum samples. AFB smear-positive results were graded according to published guidelines (Lumb et al., 2013; National Tuberculosis and Leprosy Program, 2013). All sputum samples were sent to a biosafety level 2+ TB laboratory for solid culture on Lowenstein-Jensen medium at the Bagamoyo Research and Training Center (BRTC), Bagamoyo, Tanzania (Figure 12). Sputum samples from Ifakara were preserved in cetylpyridinium chloride (CPC) and sent by post to BRTC in Bagamoyo and processed as previously described (Hiza et al., 2017).

Helminth investigations

For the diagnosis of helminth infections, stool and urine samples were collected once from each patient before the start of TB treatment. From the rural site, the samples were sent to the Ifakara Health Institute Helminth Laboratory in Ifakara, Tanzania. From the urban site, the samples were transferred to the Helminth Unit at BRTC in Bagamoyo. At each laboratory, samples were examined for helminth infection using standardized, quality-controlled procedures as previously described (Leuenberger et al., 2016; Mhimbira et al., 2017). The Kato-Katz method was performed in triplicate with thick stool smears from each sample to diagnose *Ascaris lumbricoides,* hookworm, *Schistosoma mansoni*, and *Trichuris trichiura* infection. *Strongyloides stercoralis* infection was diagnosed by the Baermann method (Leuenberger et al., 2016). Microhaematuria was examined by reagent strips (Hemastix; Siemens Healthcare Diagnostics Inc; Tarrytown, USA). Additionally, a point-of-care circulating cathodic antigen (POC-CCA) urine cassette test (ICT Diagnostics, Noordhoek, South Africa) was employed for rapid diagnosis of *S. mansoni* (WHO, 2013). *S. haematobium* eggs were detected using urine filtration (Lamberton et al., 2014; Mhimbira et al., 2017). For quality control, 10% of the Kato-Katz slides were randomly selected and re-examined by a senior laboratory technician at each site (Leuenberger et al., 2016) (Leuenberger et al., 2016).

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Other laboratory investigations

HIV screening was done using the Alere Determine HIV rapid test (Alere; San Diego, USA) following national HIV testing algorithms; the Uni-gold HIV rapid test (Trinity Biotech; Wicklow, Ireland) served as a confirmatory test in the event of a positive screening test. In HIV-positive patients, CD4 cell counts were determined by flow cytometry (FACS Calibur, Becton Dickinson Biosciences; San Jose, USA) within 3 hours after blood was drawn. A full blood cell count was done with a Sysmex XP-300 (Sysmex Corporation; Kobe, Japan). Blood tests were performed at the Ifakara Health Institute (IHI) laboratories under regular supervision by the quality assurance team.

7.3.6 Data collection and definitions

At the time of recruitment, clinical officers from the Ifakara Health Institute with extensive experience in clinical research performed physical examinations and interviewed patients using standardized questionnaires (Mhimbira et al., 2017; Said et al., 2017). We collected sociodemographic, clinical, and socioeconomic data from all patients. Data were entered using tablets via the OpenDataKit application (www.opendatakit.org). Data quality was monitored in real-time using the "*odk_planner*" tool (Steiner et al., 2016).

A TB patient was defined as new detection of *Mycobacterium tuberculosis* in the sputum by smear microscopy or Xpert MTB/RIF assay (National Tuberculosis and Leprosy Program, 2013). A new TB patient was defined as a person, who had never been treated or whose prior treatment for TB had lasted less than 1 month (WHO, 2013). Recurrent TB patients (relapse patients) were persons who had been treated previously for TB and had been declared cured or had completed their most recent course of treatment, who then presented with a recurrent episode of TB (either a true relapse or a new episode of TB caused by reinfection) (WHO, 2013).

Severe anaemia was defined as haemoglobin (Hb) <8.5 g/dl. Diabetes mellitus was defined as a random or fasting blood glucose level of ≥11.1 mmol/l or 7 mmol/l (American Diabetes Association, 2004). Patients were considered co-infected with helminths if eggs or larvae of the following species in stool or urine microscopy were present: *A. lumbricoides*, *Enterobius vermicularis*, *Hymenolepis diminuta*, hookworm, *S. haematobium*, *S. mansoni*, *S. stercoralis*, and *T. trichiura*. Additionally, *S.*

mansion infection was defined as a positive POC-CCA urine cassette test. Two forms of helminth infection were distinguished: schistosomiasis, due to infection with either *S. mansoni* or *S. haematobium*; and other helminthiases, which included infection with *A. lumbricoides, E. vermicularis, H. diminuta*, hookworm, *S. stercoralis,* or *T. trichiura*. Occupational risk for acquiring helminth infection was defined as working in rice fields, car washing, or sand harvesting pits, and fishing in rivers or still bodies of natural freshwater. TB diagnosis delay was recorded whenever more than 3 weeks elapsed between occurrence of patient's first TB symptom(s) and TB diagnosis was made (Said et al., 2017).

7.3.7 Statistical and geographical analysis

Descriptive statistics were employed for characterizing patients. For continuous variables, the Wilcoxon rank-sum or Student's *t*-tests were used, depending on the distribution from the two sites, and χ^2 or Fisher's exact tests for comparison of categorical variables, as appropriate. We set the threshold of a statistically significant difference at an alpha level of 0.05. Univariate and multivariate logistic regression models were fitted to assess the association between recurrent TB with different epidemiological characteristics among TB patients. Additionally, we analysed the association of helminth co-infections and different predictors in a logistic regression model adjusted for age, sex, HIV infection, individual deworming history, occupational risk, and site (urban/rural). All analyses were performed in Stata version 13.1 (Stata Corporation; College Station, USA).

We collected geo-coordinates using Android tablets from the two study sites and the TB laboratory. The Tanzania regions shapefiles were obtained from the Tanzanian National Bureau of Statistics, and merged with the corresponding annual TB notification rates in 2015 obtained from the National Tuberculosis and Leprosy Programme. We used ArcGIS version 10.4 (Esri; Redlands, USA) to produce the map.

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7.4 Results

7.4.1 Comparison of patient characteristics in the urban and rural setting

We studied 668 patients enrolled between August 2015 and February 2017, 460 (68.9%) at the urban and the remaining 208 (31.1%) at the rural site (Table 4). Their median age was 35 years (interquartile range [IQR] 27-41.5 years), and 454 (68.0%) were males. Rural patients were older than those from the urban setting (median age 37 years, IQR 27-46 years, *vs.* 34 years, IQR 27-40 years; p=0.003). Prevalence of HIV infection was similar in the two settings (59/208 [28.4%] in the rural *vs.* 108/460 [23.5%] in the urban setting, p=0.18). Among HIV-positive patients from the rural setting, start of anti-retroviral treatment (ART) was delayed after HIV diagnosis compared to those from the urban setting (median time to start of ART 24 days [IQR 11-64 days] *vs.* 14 days [IQR 11.5-20.5 days], p=0.001). The proportion of a contact history with a TB case was higher in rural compared to urban patients (20.2%, 95% CI 14.7-25.6% *vs.* 12.8%, 95% CI 9.8-15.9%, p=0.014). Body mass index (BMI) at the time of TB diagnosis was significantly lower among rural patients than their urban counterparts (median 17.5 kg/m^2 [IQR 16.2-19.6 kg/m^2] *vs.* 18.5 kg/m^2 [IQR 17-20.3 kg/m^2], p<0.001).

At the time of TB diagnosis, mean Hb levels were comparable in both groups. Among HIV-positive TB patients, the median CD4 cell count was lower in the rural compared to the urban setting (147 cells/µl [IQR 84-246 cells/µl] *vs.* 249 CD4 cells/µl [IQR 131-450 cells/µl], p=0.02). Among 630 patients whose blood glucose levels were tested, 17/630 (2.5%) were diagnosed as diabetic. Prevalence of diabetes in the TB patients did not differ between the two settings.

TB patients in the rural setting were less likely to self-access anthelminthic medications than urban patients (73/208 [35.1%] *vs.* 304/460 [66.1%], p<0.001) and had less access to mass deworming campaigns in the last 12 months at the time of recruitment (10/208 [4.8%] *vs.* 73/460 [15.9%], p<0.001).

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Table 4. Sociodemographic and clinical characteristics of adult tuberculosis (TB) patients.
Sociodemographic and clinical characteristics of adult tuberculosis (TB) patients at the time of diagnosis, enrolled between August 2015 and February 2017 in Dar es Salaam (urban) and Ifakara (rural), Tanzania.

Characteristics	All	Urban	Rural	P-value
Total	**668**	**460**	**208**	
Age groups in years, n (%)				0.001
18-24	112 (16.8)	78 (17.0)	34 (16.4)	
25-33	199 (29.8)	151 (32.8)	48 (23.1)	
34-43	220 (32.9)	155 (33.7)	65 (31.3)	
≥44	137 (20.5)	76 (16.5)	61 (29.3)	
Sex, n (%)				0.81
Female	214 (32.0)	146 (31.7)	68 (32.7)	
Male	454 (68.0)	314 (68.3)	140 (67.3)	
HIV status, n (%)				
Negative	497 (74.4)	352 (76.5)	145 (69.7)	
Positive	167 (25.0)	108 (23.5)	59 (28.4)	0.18
Unknown	4 (0.6)	0	4 (1.9)	
Time to ART initiation in days, median (IQR)	15 (11-35)	14 (11.5-20.5)	24 (11-64)	0.001
Education level, n (%)				0.85
No/primary	552 (82.6)	381 (82.8)	171 (82.2)	
Secondary/university	116 (17.4)	79 (17.2)	37 (17.8)	
Occupation, n (%)				<0.001
Unemployed	257 (38.5)	100 (21.7)	157 (75.5)	
Employed	411 (61.5)	360 (78.3)	51 (24.5)	
Smoking status [1], n (%)	166 (24.9)	122 (26.5)	44 (21.2)	0.18
Alcohol abuse [2], n (%)	158 (23.7)	105 (22.8)	53 (25.5)	0.46
People in the household, n (%)				<0.001
≤3	428 (64.1)	315 (68.5)	113 (54.3)	
>3	240 (35.9)	145 (31.5)	95 (45.7)	
History of TB contact, n (%)	101 (15.1)	59 (12.8)	42 (20.2)	0.014
Household monthly income, n (%)				0.032
<100 USD	459 (68.7)	328 (71.3)	131 (63.0)	
≥100 USD	209 (31.3)	132 (28.7)	77 (37.0)	
Patient category				<0.001
New case	643 (96.3)	454 (98.7)	189 (90.9)	
Recurrent case	24 (3.5)	6 (1.3)	18 (8.7)	
Return after lost to follow-up case	1 (0.2)	0	1 (0.5)	
BMI (kg/m^2), median (IQR)	18.3 (16.7-20.3)	18.5 (17-20.4)	17.5 (16.2-19.6)	<0.001
BMI categories in kg/m^2, n (%)				0.001
Underweight, <18.5	362 (54.2)	229 (49.8)	133 (63.9)	
Normal, 18.5-24.9	278 (41.6)	205 (44.6)	73 (35.1)	
Overweight, 25.0-29.9	21 (3.1)	20 (4.4)	1 (0.5)	
Obese, ≥30	7 (1.1)	6 (1.3)	1 (0.5)	
Hb level in g/dl, median (IQR)	11.0 (9.7-12.5)	11.1 (9.9-12.6)	11.0 (9.3-12.1)	0.27
Body fat in %, median (IQR)	10.8 (8.0-14.8)	10.6 (7.8-14.8)	11.5 (8.3-14.8)	0.087
Diagnosis delay in weeks, median (IQR)	4 (3-8)	4 (3-6)	8 (4-12)	<0.001
Diagnosis delay in weeks, n (%)				<0.001
≤3 weeks	174 (26.1)	153 (33.4)	21 (10.1)	
>3 weeks	492 (73.9)	305 (66.6)	187 (89.9)	
Visiting traditional healers, n (%)	86 (12.9)	26 (5.7)	60 (28.9)	<0.001
Helminth factors, n (%)				

Characteristics	All	Urban	Rural	P-value
Occupational risk [3]	274 (41.0)	232 (50.4)	42 (20.2)	<0.001
Individual deworming [4]	377 (56.4)	304 (66.1)	73 (35.1)	<0.001
Part of mass drug campaign [4]	83 (12.4)	73 (15.9)	10 (4.8)	<0.001

ART, antiretroviral therapy; BMI, body mass index; HIV, human immunodeficiency virus; Hb, haemoglobin level; IQR, inter-quartile range

USD, United States Dollars (1 USD=2,171 Tanzanian Shillings, June 2016)

[1] Defined as current smoking

[2] Alcohol use defined as drinking alcohol regularly—at least three standard bottles of beer (or equivalent) per day

[3] Occupational risk for helminth infection defined as working in rice fields, car washing, harvesting sand, or fishing

[4] Deworming practice in past 12 months

7.4.2 Comparison of recurrent TB cases in the urban and rural setting

In the rural setting, 18/208 (10.0%) were recurrent (relapse) TB cases compared to 6/460 (1.3%) in the urban setting. Patients with recurrent TB were older than patients with a first TB episode (47 years *vs.* 35 years), 22 of the 24 patients with recurrent TB were males, and 14 were not employed. Rural recurrent TB patients had a higher median body fat percentage than those from the urban setting (11.9% *vs.* 7.1%, p=0.005). Characteristics of recurrent TB patients are described in Table 5.

In a multivariate logistic regression model, we found that rural patients were more likely to have recurrent TB than urban patients (adjusted odds ratio [aOR] 3.97, 95% CI 1.16-13.67, p=0.029). Furthermore, for each 1-year increase in age, the adult TB patient risk of developing recurrent TB increased by 6% (aOR 1.06, 95% CI 1.02-1.10, p=0.001). We found that patients who were underweight (BMI <18.5 kg/m^2) had a higher risk of recurrent TB (aOR 2.97, 95% CI 0.85-10.30, p=0.087; Table 6).

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Table 5. Characteristics of recurrent TB cases in urban and rural Tanzania.
Patients enrolled between August 2015 and February 2017 in Dar es Salaam (urban) and Ifakara (rural)

Characteristics	All	Urban	Rural	P-value
Total, n (%)	24 (100)	6 (25.0)	18 (75.0)	<0.001
Age in years, median (IQR)	47 (33.5-53)	39 (29-47)	47.5 (38-55)	0.20
Male sex, n (%)	22 (91.7)	6 (100)	16 (88.9)	0.55
HIV infection, n (%)	5 (20.8)	1 (16.7)	4 (22.2)	1.0
Education level, n (%)	5 (0.9)			0.55
No/primary school	21 (87.5)	15 (83.3)	6 (100)	
Secondary/university	3 (12.5)	3 (16.7)	-	
Occupation, n (%)				0.002
Unemployed	14 (58.3)	-	14 (77.8)	
Employed	10 (41.7)	6 (100)	4 (22.2)	
Smoking [1], n (%)	3 (12.5)	2 (33.3)	1 (5.6)	0.25
Alcohol use [2], n (%)	4 (16.7)	-	4 (22.2)	0.54
Diabetes mellitus	1 (5.3)	-	1 (7.7)	-
People in the household, n (%)				0.99
≤3	17 (70.8)	4 (66.7)	13 (72.2)	
>3	7 (29.2)	2 (33.3)	5 (27.8)	
History of TB contact, n (%)	7 (29.2)	1 (16.7)	6 (33.3)	0.63
Household monthly income, n (%)				0.99
<100 USD	18 (75.0)	5 (83.3)	13 (72.2)	
≥100 USD	6 (25.0)	1 (16.7)	5 (27.8)	
BMI in kg/m^2, median (IQR)	16.9 (16.3-18.4)	16.5 (15.7-17.0)	17.1 (16.5-	0.14
Body fat in %, median (IQR)	10.9 (7.4-14.9)	7.1 (5.9-8.5)	11.9 (10.2-	0.005
Hb level in g/dl, median (IQR)	10.8 (10-12)	11.2 (7.2-12.9)	10.5 (10.1-12)	0.79
Visiting traditional healers, n (%)	5 (20.8)	1 (16.7)	4 (22.2)	0.99
Previous use of antibiotics, n (%)	18 (75.0)	3 (50.0)	15 (83.3)	0.14
Any helminth infection, n (%)	5 (22.7)	2 (50.0)	3 (16.7)	0.21

n, number; IQR, interquartile range; HIV, human immunodeficiency virus; USD, United States Dollars (1 USD=2,171 Tanzanian Shillings, June 2016)

[1] Defined as current smoking

[2] Alcohol use defined as drinking alcohol regularly—at least three standard bottles of beer (or equivalent) per day

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Table 6. Factors associated with recurrent tuberculosis (TB) among TB cases in urban and rural Tanzania.

Characteristics	Crude			Adjusted		
n (%)	OR	(95% CI)	P-value	OR	(95% CI)	P-value
Demographics						
Age (years)	1.06	(1.04-1.09)	<0.001	1.06	(1.02-1.10)	0.001
Male sex	4.42	(1.18-16.5)	0.027	3.04	(0.73-12.6)	0.13
BMI <18.5kg/m^2	2.47	(0.99-6.11)	0.051	3.0	(0.85-10.3))	0.087
Body fat (%)	0.98	(0.91-1.04)	0.50	-	-	-
***Social* characteristics**						
Higher education level [1]	0.67	(0.2-2.29)	0.52	-	-	-
Employed	0.44	(0.2-0.99)	0.046	1.52	(0.47-4.87)	0.49
Monthly income >200	0.76	(0.31-1.89)	0.56	-	-	-
Living in the rural	6.79	(2.73-16.86)	<0.001	3.97	(1.16-13.67)	0.029
Household members >3	0.76	(0.32-1.8)	0.53	-	-	-
History of TB contact	2.5	(1.03-6.04)	0.04	1.66	0.55-5.03	0.37
Individual deworming [2]	0.38	(0.17-0.89)	0.026	0.59	(0.19-1.76)	0.34
Occupational risk [3]	1.04	(0.46-2.34)	0.92	-	-	-
Smoking	0.48	(0.15-1.50)	0.21	-	-	-
Alcohol abuse	0.70	(0.25-1.96)	0.50	-	-	-
Comorbidities						
HIV infection	1.38	(0.66-2.88)	0.39	0.51	(0.19-1.38)	0.13
Diabetes mellitus	2.93	(0.52-16.60)	0.23	3.25	(0.43-4.65)	0.25
Severe anaemia [4], g/dl	1.07	(0.34-3.37)	0.91	-	-	-
Haematuria	0.3	(0.02-5.10)	0.41	-	-	-
Any helminth infection	0.99	(0.38-2.65)	1.0	-	-	-

OR, odds ratio; CI, confidence interval; HIV, human immunodeficiency virus; BMI, body mass index

[1] Higher education level consists of TB patients who completed their secondary of university education
[2] Individual deworming habit in the last 12 months prior to TB diagnosis
[3] Occupational risk for helminth infection defined as working in rice fields, car wash, harvesting sand, or fishing
[4] Severe anaemia defined as blood haemoglobin (Hb) level <8.5 g/dl

Logistic regression model adjusted for age, sex, BMI, employment status, setting (urban/rural), history of TB contact in the household, individual deworming, HIV infection and diabetes

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Table 7. Frequency distribution of helminth infection among adult TB patients in Dar es Salaam (urban) and Ifakara (rural), Tanzania

Helminth infection	All, n (%)	Urban		Rural		P-value
		n (%)	95% CI	n (%)	95% CI	
Total	668 (100)	460 (68.9)		208 (31.1)		
Any helminth infection [1]	154 (23.1)	118 (25.7)	21.7-29.6	36 (17.3)	12.2-22.5	0.018
Soil-transmitted helminths						
Strongyloides stercoralis	89 (13.3)	79 (17.2)	14.4-21.5	10 (4.8)	1.9-7.8	<0.001
Hookworm	45 (6.7)	26 (5.7)	3.7-8.1	19 (9.1)	5.3-13.2	0.12
Ascaris lumbricoides	2 (0.3)	-	-	2 (1.0)	-	-
Trichuris trichiura	5 (0.8)	2 (0.4)	-	3 (1.4)	-	-
Schistosomiasis						
Schistosoma mansoni [2]	15 (2.3)	8 (1.7)	0.57-3.1	7 (3.4)	0.9 to 5.9	0.21
Schistosoma mansoni [3]	53 (7.9)	19 (4.13)	2.4-6.2	34 (16.4)	11.4-21.6	<0.001
1+	19 (43.2)	0	-	19 (63.3)	-	-
2+	6 (13.6)	0	-	6 (20.0)	-	-
3+	19 (43.2)	14 (100)		5 (16.7)	-	-
Schistosoma haematobium [4]	19 (2.8)	16 (3.5)	1.9-5.4	3 (1.4)	-	-
Multiple helminth infection						0.63
None	471 (70.5)	322 (70.0)	65.8-74.2	149 (71.6)	65.5-77.7	
Mono-infection	159 (23.8)	108 (23.5)	19.6-27.4	51 (24.5)	18.7-30.3	
Infection with ≥2 species	16 (2.4)	10 (2.2)	0.86-3.5	6 (2.9)	0.62-5.2	

TB, tuberculosis; POC-CCA, point-of-care circulating cathodic antigen

[1] Including POC-CCA positive tests (*Schistosoma mansoni*)

[2] Based on stool microscopy

[3] Based on POC-CCA test only

[4] Based on urine filtration

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7.4.3 Comparison of helminth co-infections patterns and associated risk factors in the urban and rural settings

The overall prevalence of helminth co-infections in TB patients was 23.1%. As As shown in Table 7, the prevalence of helminth co-infections was significantly higher in the urban compared to the rural setting (25.7%, 95% CI 21.7-29.6% vs. 17.3%, 95% CI 12.2-22.5%; p=0.02). The rural setting had a higher prevalence of *S. mansoni* than the urban setting and a lower prevalence of *S. stercoralis*. Figure 14 shows the distinctive pattern of helminth co-infections.

In a multivariate analysis, TB patients from the urban setting had significantly higher odds of having any helminth infection at the time of TB diagnosis compared to those from the rural setting (aOR 2.44, 95% CI 1.44-4.14, p=0.001). TB patients who had taken anthelminthic medication within 12 months prior to TB diagnosis had lower odds of having acquired a helminth infection (aOR 0.59, 95% CI 0.39-0.88, p=0.009). Neither age, sex, HIV infection, occupational risk, nor any other cofactor was associated with helminth infection.

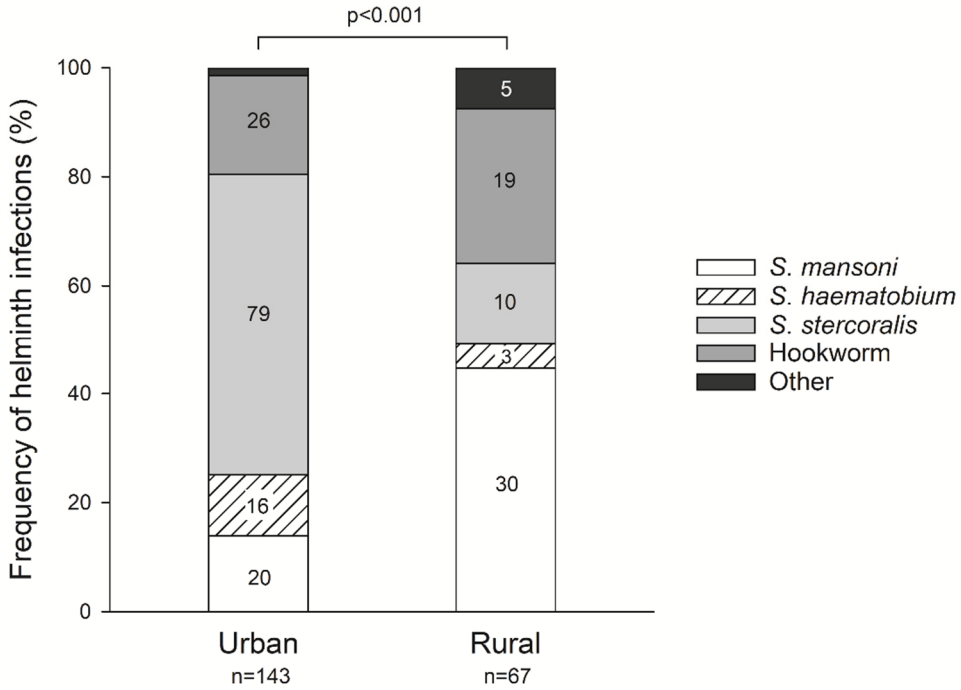

Figure 14. Frequency distribution of helminth species among adult TB patients co-infected with any helminth in an urban and rural setting in Tanzania (in percentage).
Numbers on bars represent absolute numbers, the category "Other" includes *Ascaris lumbricoides* and *Trichuris trichiura*. All positive helminth results were analysed (including results from patients with multiple helminth infection).

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7.5 Discussion

Among the 668 TB patients from the urban and rural settings in Tanzania we studied, patients from the rural setting were older, had a lower average BMI, and lower median CD4 cell counts in case of HIV co-infection. Moreover, patients from the rural setting had more frequently recurrent TB. Whereas schistosomiasis was higher in the rural setting, the overall prevalence of helminth infections was higher in the urban setting, especially due to *S. stercoralis*.

We found differences in patient characteristics between urban and rural settings, such as age, BMI, occupation, as well as health seeking behavior (e.g., use of antibiotics prior to TB diagnosis and consultation of traditional healers) as reported previously from rural Tanzania (Verhagen et al., 2010). The difference in time between HIV diagnosis and ART initiation in the two sites is likely explained by greater distances of rural patients to the treatment center, a factor that has been shown to contribute in treatment delay (Mfinanga et al., 2008; Verhagen et al., 2010). Rural patients comparatively had increased likelihood of visiting traditional healers due to poorer access to health facilities (Sandler, 1990). The resulting delay in seeking medical care has been shown to contribute to ongoing TB transmission (Ngadaya et al., 2009; Sreeramareddy et al., 2009; Verhagen et al., 2010; Wandwalo and Mørkve, 2000).

Helminth co-infections have been associated with rural residence (Alemu and Mama, 2017; Walson et al., 2010). In our recent work in Tanzania, we also found a positive association between TB and helminth co-infections in an urban setting (Mhimbira et al., 2017). The observed higher prevalence of helminths in the urban setting could be due to the rapid growth of the city with poor urban planning and hygiene control. This results in slum-like dwellings in large parts of the city with higher risk of infection with helminths (UN-HABITAT, 2010). The overall prevalence of TB and helminth co-infections we observed was comparable to previous studies elsewhere in sub-Saharan Africa (Alemu and Mama, 2017; Babu and Nutman, 2016; Dinardo et al., 2016; Mhimbira et al., 2017; Rafi et al., 2012; Salgame et al., 2013). The prevalence of *S. stercoralis*, the principal driver of helminthiasis in the urban setting in the current study, was comparable to that seen in previous investigations from urban Tanzania and rural Ghana (Mhimbira et al., 2017; Yelifari et al., 2005). The

high prevalence of schistosomiasis we observed in the rural setting is likely to be setting-specific, with Ifakara town being close to the Kilombero river. This was also shown in rural areas of the Democratic Republic of the Congo (Madinga et al., 2015), especially among those who came in regular contact with natural open freshwater bodies (e.g., fishermen and rice famers) (Mazigo et al., 2012; Tukahebwa et al., 2013). Environmental conditions and administration of anthelminthic medication initiated on an individual basis or during mass deworming campaigns differed across our two settings, and these two factors affect prevalence and patterns of helminth co-infections. When designing public health interventions, such differences must be taken into consideration for further improvement of clinical outcomes in TB patients, as helminth co-infections alter clinical presentation and immune response to infection (Dinardo et al., 2016; Mhimbira et al., 2017; Rafi et al., 2012; Salgame et al., 2013).

The association of recurrent TB with older age and living in certain areas has also been observed elsewhere (Gadoev et al., 2017; Hung et al., 2015), and particular comorbidities, such as HIV and diabetes mellitus, have been associated with recurrent TB (Gadoev et al., 2017; Sanghani and Udwadia, 2013; Wurie et al., 2016). Recurrent TB is more common in HIV-positive than HIV-negative patients (Gadoev et al., 2017; Jasmer et al., 2004; Sonnenberg et al., 2001), and is related to poor treatment outcomes (Gadoev et al., 2017). Smoking has been associated with a three-fold increased risk of developing recurrent TB in India (Thomas et al., 2005). However, this finding could not be confirmed in the present study. Because most recurrent cases of TB occur within 12 months of completion of treatment, follow-up after completion of treatment is important (Guerra-Assuncao et al., 2015; Thomas et al., 2005).

A limitation of our study was its inability to differentiate reinfection from relapse among recurrent TB cases because we did not have sputum samples from prior episodes that would have made possible differentiation by molecular genotyping of *M. tuberculosis* isolates. Drug resistance information was also lacking, except for rifampicin resistance tested by Xpert MTB/RIF assay. Although multiple techniques were used for identification of helminth co-infections, the investigation

of a single stool sample in our study could have resulted in underestimation of the true prevalence of infection, especially of hookworm, *T. trichiura* and *A. lumbricoides* (Knopp et al., 2008).

7.6 Conclusions

The differences in clinical and socio-demographic characteristics of TB patients in urban and rural Tanzania underline that public health interventions need to be tailored to a given setting to improve clinical outcomes of TB and mitigate the risk of co-infections. TB patients in the rural Tanzania are likely to be older with more recurrent TB cases, have more limited access to anthelminthic medication individually, have a longer TB diagnosis delay, and seek more frequently care from traditional healers. The overall prevalence of helminth co-infections in TB patients was higher in the urban setting, predominantly driven by *S. stercoralis* infection, but the prevalence of *S. mansoni* was higher in the rural setting. These observations may guide public health interventions that target, for example, traditional healers in the rural setting, aiming to improve early detection of TB cases and referral for anti-tuberculosis treatment. On the other hand, screening and treatment of helminths among TB patients should be improved, especially in the urban setting.

7.7 Declarations

Ethics approval and consent to participate

The study was approved by the institutional review board of the Ifakara Health Institute (IHI; reference no. IHI/IRB/04-2015), the Medical Research Coordinating Committee of the National Institute of Medical Research in Tanzania (NIMR; reference no. NIMR/HQ/R.8c/Vol. I/357), and the Ethics Committee of the Northwestern and Central Switzerland (EKNZ; reference no. UBE-15/42). All patients gave written informed consent before enrolment into the study.

Consent for publication

Not applicable.

Availability of data and material

The datasets that were used for analysis and preparation of this manuscript are not publicly available due the national policy on data sharing. The datasets will be available from the corresponding author

upon reasonable request where concerned parties will sign a data transfer agreement approved by the Medical Research Coordinating Committee.

Competing interest

The authors declare that they have no competing interests.

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Author's contribution

Conceived and designed the study: GS, JH, EL, FM, LR, MS, KS, SG, KR, MT, MW and LF. JH, GS, YM, GM, MW and LF analysed the data. GS, JH, FM, KS, FB and RN contributed clinical data. MS, LK, FB, SK and LR contributed laboratory data. JH and GS prepared the first draft of the manuscript. LR, GM, CH, JU, MT, MW and LF contributed in the first major revision of the manuscript. All authors contributed in final manuscript revisions and approved the final version.

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8. Anemia in tuberculosis cases and household controls from Tanzania: Contribution of disease, coinfections, and the role of hepcidin

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8.1 Abstract

Background: Tuberculosis (TB) induces a systemic inflammatory state affecting iron homeostasis. Patients with TB often have additional comorbidities such as anemia which can result in poorer treat outcomes. We studied the contribution of anemia and the role of the iron regulatory hormone hepcidin among TB patients and household contacts.

Methods: We analyzed serum samples from 102 TB cases and 98 controls without TB, matched by age/sex, for hepcidin, iron, and inflammation parameters. Five controls developed TB within 12 months. We used linear regression to assess associations.

Results: Anemia of chronic disease (ACD) was more frequent among cases than controls (59.8% vs. 26.1%), but iron-deficiency anemia more frequent in controls (10% vs. 1%). The median hepcidin level was higher in cases than controls (63.7 vs. 14.2 ng/mL), but coinfections with HIV, helminths, and respiratory pathogens did not show cumulative effects. Hepcidin was associated with more severe TB symptom scoring (coefficient 0.8, 95% confidence interval [CI] 0.5-1.2) and higher mycobacterial load (0.7, 95% CI 0.4-1.0). Hepcidin was higher in TB cases and controls who developed TB compared to controls without TB ($p<0.001$), even when restricting to HIV-negative study participants.

Conclusions: ACD was the predominate etiology in TB patients suggesting limited benefit from iron supplementation. Increased hepcidin levels long before active disease, indicating altered iron metabolism, may be a marker for developing disease among TB-exposed individuals. Clinical management of anemia and nutrition interventions in TB patients need to be considered to improve the clinical course and outcomes.

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8.2 Introduction

One-quarter of the world's population is estimated to be infected with *Mycobacterium tuberculosis* (Houben and Dodd, 2016). Patients with TB often have additional comorbidities such as anemia which can result in poor treatment outcomes (Isanaka et al., 2012a, 2012b). The global prevalence estimation of anemia was 33% in 2010, with higher estimates in sub-Saharan Africa (Kassebaum et al., 2014). Anemia is mainly caused by low dietary iron intake and low bioavailability, but chronic parasitic infection and inflammation also contribute to the burden of disease (Zimmermann et al., 2005). Anemia of chronic disease (ACD), is primarily found in patients with chronic immune activation such as TB and HIV-positive patients (Kerkhoff et al., 2016b).

TB induces a chronic systemic inflammatory state, which triggers hepcidin synthesis from hepatocytes and predominately macrophages, influencing iron homeostasis (Girelli et al., 2016; Nemeth et al., 2004; Sow et al., 2011). As the key regulator of iron homeostasis (Drakesmith and Prentice, 2012; Nemeth et al., 2004), hepcidin drives the process of ACD by restricting the availability of iron for erythropoiesis (Armitage et al., 2014; Minchella et al., 2014). The hormone does this by internalizing and degrading the iron exporter ferroportin in macrophages, hepatocytes and enterocytes, which results in sequestration of iron into the reticuloendothelial system and reduced dietary iron absorption (Girelli et al., 2016; Nemeth et al., 2004; Wang and Babitt, 2016). The acquisition of iron from the host is a crucial prerequisite for the survival and replication of many pathogens, including *M. tuberculosis* (Drakesmith and Prentice, 2012; Voss et al., 1999). Clinical studies showed that iron deficiency (ID) and anemia were associated with increased TB recurrence and mortality in HIV-positive TB patients (Isanaka et al., 2012a, 2012b). In addition, hepcidin possibly plays a role in the immune response by inhibiting invasion of pathogens, having shown direct antimicrobial activity (Michels et al., 2015; Shin and Jo, 2011).

We studied the contribution of ACD and iron deficiency anemia (IDA) in TB patients in a high TB incidence country, Tanzania, where we compared TB patients to persons in contact households who were free of active disease. We also investigated the association of hepcidin levels with coinfections, disease severity, and progression from TB infection to disease.

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8.3 Methods

8.3.1 Study setting

Study participants were part of an ongoing prospective cohort of bacteriologically confirmed pulmonary TB patients in Tanzania (TB-DAR) initiated in October 2013. The study site is located in the densely populated Temeke district of Dar es Salaam. With approximately 4.4 million people, Dar es Salaam is a TB hotspot region in Tanzania notifying 22% of all TB cases in Tanzania (MoHSW, 2014). The details of the study setting have been described previously (Hiza et al., 2017; Mhimbira et al., 2016, 2017; Said et al., 2017; Steiner et al., 2016). Participants included as TB cases were sputum smear-positive adult TB patients (>18 years), while those included as controls were exposed household contacts who did not have active TB.

8.3.2 Selection of study participants

Among 359 TB patients recruited between 2014 and 2015, we randomly selected 103 TB cases, and 103 household controls exposed to a TB case but without active disease, matched according to age and sex. The final analysis included 200 participants, 102 cases and 98 controls, for whom serum samples and hemoglobin results were available.

8.3.3 Study procedures and data collection

TB patients and controls were evaluated at the time of TB diagnosis (cases) or recruitment (controls), at six months (after completion of treatment for TB patients), and at 12 months. Clinical data were collected at each visit, and biological samples at the time of recruitment, as previously described (Hiza et al., 2017; Knopp et al., 2014; Mhimbira et al., 2017; Said et al., 2017). Briefly, data were captured using the OpenDataKit application (www.opendatakit.org) on Android tablets, and data quality was monitored in real-time using the *odk_planner* tool (Steiner et al., 2016). Serum samples were taken at the time of TB diagnosis before starting TB treatment (cases) or at the time of recruitment (controls), and stored at -80° C. Xpert MTB/RIF (Cepheid, USA) was used to rule out TB in controls. Stool and urine samples were collected for diagnosis of helminths at the time of recruitment (Knopp et al., 2014; Mhimbira et al., 2017), as were nasopharyngeal swabs (Copan, USA)

to detect respiratory pathogens. All study participants were screened for malaria using a rapid diagnostic test (Diagnostics Malaria P.f. MRDT, ICT Diagnostics, South Africa).

8.3.4 Laboratory investigations

Full blood counts were done with a MS4 Vet hematology analyzer (Diamond Diagnostics, Massachusetts, USA). These blood tests were performed at the Temeke Regional Referral Hospital laboratory and inflammation parameters were obtained at the Labor Risch, Bern (Switzerland) using the Siemens Nephelometer BN II (soluble transferrin receptor) and the Cobas 6000, Roche diagnostics, Switzerland (all other parameters). We used a commercial assay (DRG Hepcidin 25 bioactive, HS ELISA GmbH, Marburg, Germany) to determine hepcidin levels (Kroot et al., 2010). HIV screening was done using Alere Determine HIV rapid test, and the Uni-gold HIV (Trinity Biotech, USA) rapid test served as a confirmatory test in case of a positive screening test.

The Kato-Katz method (in triplicates), Baermann technique (in duplicates), urine filtration (in duplicates), and circulating cathodic rapid antigen test (POC-CCA; Rapid Medical Diagnostics, South Africa) were used to diagnose helminths (*Strongyloides stercoralis, Trichuris trichiura, Schistosoma mansoni, S. haematobium, Ascaris lumbricoides*, hookworm) (Knopp et al., 2014; Mhimbira et al., 2017). We analyzed the nasopharyngeal swabs to detect respiratory pathogens using a multiplex real-time PCR with a broad panel of 16 viral (Anyplex II RV16) and seven bacterial (Allplex panel 4) respiratory pathogens (Supplementary Table 2) according to the manufacturer's instructions (Seegene, Seoul, South Korea) (Kim et al., 2013).

8.3.5 Definitions

World Health Organization (WHO) criteria were used to classify the severity of anemia: no anemia (hemoglobin [Hb] 13.0 g/dL for men, 12.0 g/dL for women), mild anemia (11.0–12.9 g/dL for men, 11.0–11.9 g/dL for women), moderate anemia (8.0–10.9 g/dL for men and women) or severe anemia (8.0 g/dL for men and women). We used published definitions of ACD and IDA (Kerkhoff et al., 2016b), where, patients with anemia were then classified into one of three mutually exclusive groups (Kerkhoff et al., 2016b; Weiss and Goodnough, 2005): ACD, IDA, or combined ACD and IDA

(ACD+IDA) (Supplementary Figure 5). Briefly, ferritin levels were used to distinguish ACD from IDA: ferritin >336.2 ng/mL defined patients with anemia as ACD, and ferritin <30 ng/mL as IDA (Kerkhoff et al., 2016b). Hepcidin levels further distinguished between ACD and ACD+IDA. We also used IDA definitions based on single laboratory parameters (Koulaouzidis et al., 2009; Minchella et al., 2015a; World Health Organization, 2001).

The mycobacterial load in TB patients was defined based on sputum smear microscopy results (quantitative scoring) (Lumb et al., 2013). In order to grade the clinical severity of TB, we adopted a previously published clinical TB score (Mhimbira et al., 2017; Wejse et al., 2008), the score was then categorized into mild (score of 1-5) and severe (score of ≥6). Helminth infection was defined as infection with any helminth species, and respiratory infection as detection of any respiratory viral or bacterial pathogen.

8.3.6 Statistical analysis

Cases and controls were compared using conditional regression which accounts for the matched study design. Other groups were compared using chi-square or Fisher's exact (binary variables) and Kruskal-Wallis (continuous variables) tests. We also used nonparametric tests for trend analysis across ordered groups. In addition, we performed logistic regression models to determine associations between binary outcomes (anemia versus no anemia, ACD versus no anemia, anemia based on hepcidin levels versus no anemia) and patient characteristics (body mass index [BMI], viral respiratory and helminth infection), and conditional regression models for the outcome TB (case-control matching). Odds ratios (ORs) were presented as unadjusted ORs and adjusted for HIV and BMI (aOR). Finally, we performed linear regression to assess associations between log laboratory parameter (hepcidin, procalcitonin, and hemoglobin levels) and progression to disease (cases, controls with and without TB) and TB disease severity (symptom scoring, mycobacterial load), as well as to assess associations between log hepcidin levels and hematological parameters. All analyses were performed in Stata version 14.0 (Stata corporation, Texas, USA).

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8.3.7 Ethical approval

The study was approved by the institutional review board of the Ifakara Health Institute (IHI, reference no. IHI/IRB/04-2015), the Medical Research Coordinating Committee of the National Institute for Medical Research in Tanzania (NIMR, reference no. NIMR/HQ/R.8c/Vol. I/357), and the Ethics Committee of the Canton of Basel (EKNZ, reference no. UBE-15/42). All participants gave written informed consent before enrolment.

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8.4 Results

We analyzed data from 102 cases and 98 controls. The median age was 32.9 years (interquartile range [IQR] 26.2-40.1); 152 (76.0%) were male. Of the 24 HIV-positive participants (12.0%), two were on antiretroviral therapy at the time of recruitment. The median BMI was lower among cases than controls (17.4 vs. 25.2 kg/m2). Coinfections with HIV, helminths, and respiratory pathogens were equally distributed in cases and controls (Table 8). Malaria screening was negative in all study participants. Among the controls, 5 (5.1%) developed active TB during the follow-up time of 12 months, with a median time of seven months (range 5.5-8.0) between recruitment and TB diagnosis.

Table 8. Patient characteristics of tuberculosis (TB) patients (cases) and household contact controls without TB (controls) in Tanzania.

Characteristic	Cases	Controls	*P* value [1]
All	**102**	**98**	
Age, years, median (IQR)	33.0 (26.0-40.0)	32.7 (26.2-40.1)	-
Male sex, n (%)	78 (76.5)	74 (75.5)	-
BMI, kg/m [2], median (IQR)	17.4 (15.8-19.2)	25.2 (22.1-28.5)	0.002
Education, n (%)			0.30
No/primary	78 (76.5)	80 (81.6)	
Secondary/university	24 (23.5)	18 (18.4)	
Coinfections, n (%)			
HIV infection	13 (12.8)	11 (11.2)	0.64
Helminth infection [3]	32 (31.4)	21 (21.4)	0.14
Any viral respiratory pathogen [4]	25 (24.5)	19 (19.4)	0.24
Any bacterial respiratory pathogen [4]	41/98 (41.8)	18/29 (62.1)	0.59
Malaria	0	0	-

BMI, body mass index; IQR, interquartile range

[1] Accounts for case-control matching (values for the matching variables age and sex are not shown)

[2] At the time of tuberculosis diagnosis (cases) or enrolment (controls)

[3] Soil-transmitted and intestinal helminths, as determined by stool microscopy, Baermann test, urine filtration, and rapid urine antigen test

[4] As determined by a panel of 16 viral respiratory and seven bacterial species (molecular detection in nasopharyngeal specimens)

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8.4.1 Prevalence of anemia and hematological characteristics

The overall median Hb concentration was 12.5 g/dL (IQR 10.9-13.7), and significantly lower in cases compared to controls (12.05 vs. 13.0, p<0.001, Table 9). Prevalence of anemia was significantly higher in cases compared to controls (62.2% vs. 37.8%, p<0.001) (Supplementary Figure 6). Particularly moderate and severe anemia were more common in cases than controls.

Table 9. Hematological, iron, and inflammatory parameters among cases and controls

Parameter (unit)	**No. included** [1] Cases / controls	**Median (IQR)** Cases	Control	***P* value** [2]
Iron (µmol/L)	99 / 91	4.4 (3.3-7.1)	12.9 (8.9-17.3)	<0.001
Ferritin (ng/mL)	101 / 89	309.8 (162.2-601.2)	103.5 (59.5-159.5)	<0.001
sTfR (mg/L)	89 / 75	1.8 (1.4-2.2)	1.4 (1.2-1.8)	0.001
Transferrin (g/L)	99 / 94	1.6 (1.4-2.0)	2.5 (2.3-2.8)	<0.001
Hepcidin (ng/mL)	81 / 65	63.7 (22.0-121.9)	14.2 (4.5-27.4)	0.003
CRP (mg/L)	99 / 94	67.8 (36.5-116.9)	1.6 (0.6-6.0)	<0.001
Procalcitonin (µg/L)	90 / 68	0.07 (0.04-0.17)	0.019 (0.019-0.03)	0.009
Hemoglobin (g/dL)	102 / 98	12.05 (10.3-12.9)	13.0 (11.5-14.3)	<0.001
MCV (f/L)	102 / 98	75.5 (68.6-82.8)	81.2 (76.0-86.3)	0.001
MCH (pg/cell)	102 / 98	25.0 (22.4-27.5)	26.3 (23.8-29.1)	0.027
MCHC (g/dL)	102 / 98	33.1 (32.0-34.0)	32.7 (31.2-33.9)	0.014
Red blood cell distribution width (f/L)	63 / 82	14.9 (13.8-16.7)	14.7 (13.5-15.7)	0.064

CRP, C-reactive protein; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; sTfR, soluble transferrin receptor

[1] Patients with an available laboratory result
[2] Accounts for case-control matching

Hematological, iron, and inflammatory parameters are shown for TB cases and controls in Table 2 (stratified by sex in Supplementary Figure 7A and Supplementary Table 4 and by anemia severity in Supplementary Table 3 and Supplementary Figure 7B). The median soluble transferrin receptor (sTfR) concentrations were significantly higher among cases than controls (1.8 mg/L, IQR [1.4-2.2] vs. 1.4 mg/L, IQR [1.2-1.8], p=0.001). sTfR concentrations were also higher in cases and controls who had a more severe degree of anemia (mild to severe) as opposed to study participants without anemia (Supplementary Table 3). Similarly, the levels of the acute phase proteins procalcitonin and C-reactive protein (CRP) were higher in cases than in controls (Table 9). Median CRP concentration was significantly lower among controls without anemia than it was among controls with mild and

moderate/severe anemia (respectively 0.9 vs. 3.3 vs. 4.1 mg/L, p=0.024). Among TB cases, CRP levels were elevated across groups (varying with anemia status), thus reflecting the stronger inflammatory response in TB patients induced by *M. tuberculosis* infection (Supplementary Table 3).

8.4.2 Types of anemia and risk factors

ACD was the most common cause-specific type of anemia among study participants (81/185, 43.8%), and significantly more common among cases than controls (59.8% vs. 26.1%, overall p<0.001) as shown in Table 10. Multifactorial anemia (mixed ACD and IDA) was the second most common type of anemia where 13 (7.0%) participants had this type of anemia. IDA anemia was the third most common type of anemia (10/185, 5.4%), while controls had a significantly higher proportion of IDA anemia than cases (10.2% vs. 1%).

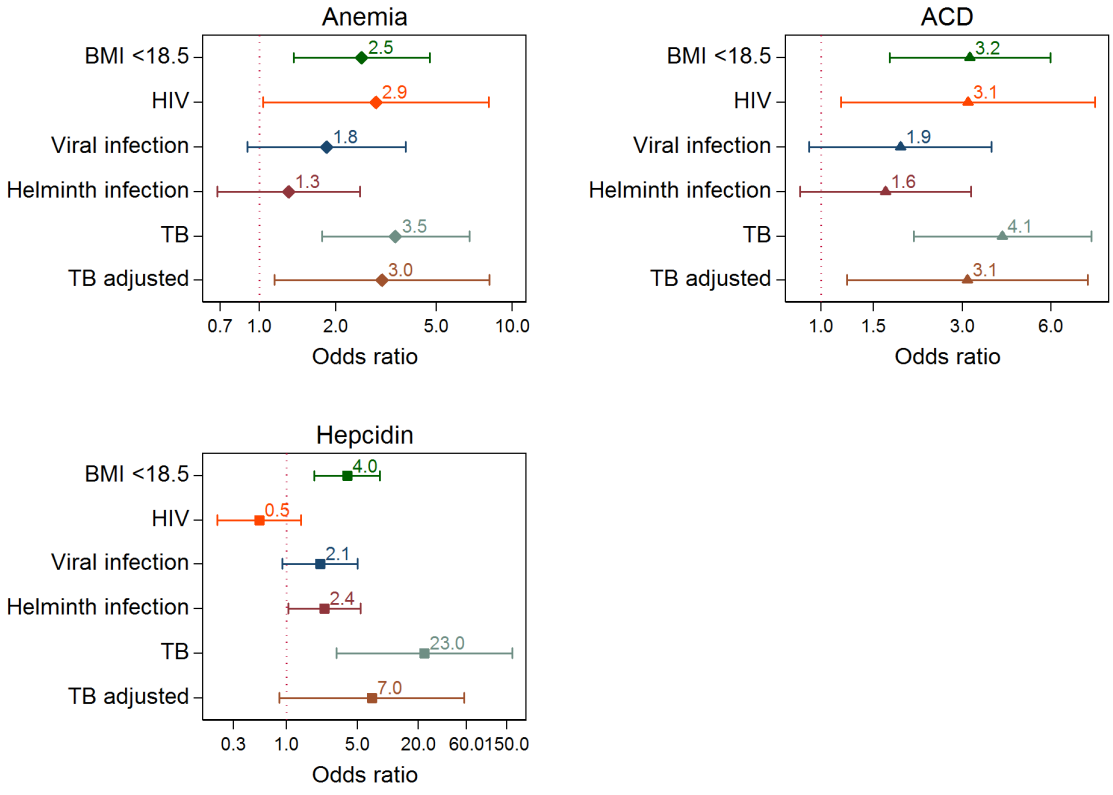

Figure 15. Associations of anemia with patient characteristics and coinfections (HIV, viral respiratory and helminth infection).
Anemia based on hemoglobin levels vs. no anemia ("Anemia"); anemia of chronic disease vs. all other forms ("ACD"); and anemia based on hepcidin levels (>20 ng/mL) vs. no anemia ("Hepcidin"). BMI, body mass index; OR, odds ratio; TB, tuberculosis
Estimates from logistic regression models (BMI, HIV, viral and helminth infection) and conditional regression models (TB). TB adjusted for BMI and HIV. ORs on log scale.

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Both low BMI (<18.5 mg/kg) and HIV infection, but not viral or helminth coinfections, were risk factors for anemia (defined by hemoglobin levels) and ACD (Figure 15). In multivariate analyses adjusted for BMI and HIV, TB was a risk factor for all types of anemia: aOR 3.1 (95% confidence interval [CI] 1.2-8.1, p=0.03) for anemia based on hemoglobin levels, aOR 3.1 (95% CI 1.2-8.0, p=0.02) for ACD, and aOR 7.0 (95% CI 0.9-57.0, p=0.07) for anemia based on hepcidin levels.

8.4.3 Associations between hepcidin concentration and coinfections

The overall median hepcidin concentration was 27.3 ng/mL (IQR 9.2-81.4). Hepcidin levels were significantly increased in cases compared to controls (median 63.7 ng/mL, IQR [22-121.9] vs. 14.2 ng/mL, IQR [4.5-27.4], p=0.003). Hepcidin levels were also higher in men and patients with moderate and severe anemia (Supplementary Table 3, Supplementary Table 4 and Supplementary Table 5). Associations between hepcidin levels and hematological, iron, and inflammatory parameters are shown in Supplementary Table 7. Ferritin, transferrin, and the inflammatory markers procalcitonin and CRP were all associated with hepcidin levels. The concentration of sTfR was negatively associated with hepcidin levels in controls, but to a lesser extent in cases.

There was no evidence for a cumulative effect of coinfections with HIV, helminths, and respiratory pathogens on hepcidin levels (Figure 16). However, hepcidin levels were increased in controls with helminth infection compared to controls without helminth infection, though this effect was not seen among TB cases (Figure 16B). *Stronglyloides stercoralis* infection was predominant driver of hepcidin levels among controls who had helminths infection (Supplementary Figure 8).

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Table 10. Etiology of anemia and iron deficiency based on single laboratory parameters among TB cases and controls

Classification	No. included [1]	n (%)		*P* value [2]
	Cases / Controls	Cases	Controls	
Cause-specific anemia [3]	97 / 88			<0.001
ACD		58 (59.8)	23 (26.1)	
IDA		1 (1.0)	9 (10.2)	
ACD+IDA		10 (10.3)	3 (3.4)	
No anemia		28 (28.9)	53 (60.2)	
Iron deficiency [4]				
CRP containing index <0	99 / 89	80 (80.8)	86 (96.6)	0.006
Ferritin <30 µg/L	101 / 89	2 (2.0)	13 (14.6)	0.02
MCV <80 f/L	102 / 98	67 (65.7)	44 (44.9)	0.009
MCH <27.5 g/dL	102 / 98	75 (73.5)	65 (66.3)	0.27
MCHC <32 g/dL	102 / 98	23 (22.6)	30 (30.6)	0.23
sTfR >2.5 mg/L	89 / 75	17 (19.1)	3 (4.0)	0.03
sTfR index >1.5	89 / 75	4 (4.5)	8 (10.7)	0.14
Hepcidin >20 ng/mL	81 / 65	62 (76.5)	23 (35.4)	0.002

ACD, anemia of chronic disease; ACD+IDA, multifactorial anemia (ACD+IDA); IDA, iron deficiency anemia;
CRP, C-reactive protein; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; sTfR, soluble transferrin receptor

[1] Participants with an available laboratory result
[2] Accounts for case-control matching
[3] Based on algorithm in Supplementary Figure 5
[4] Based on single laboratory parameters (e.g., independent from hemoglobin levels)

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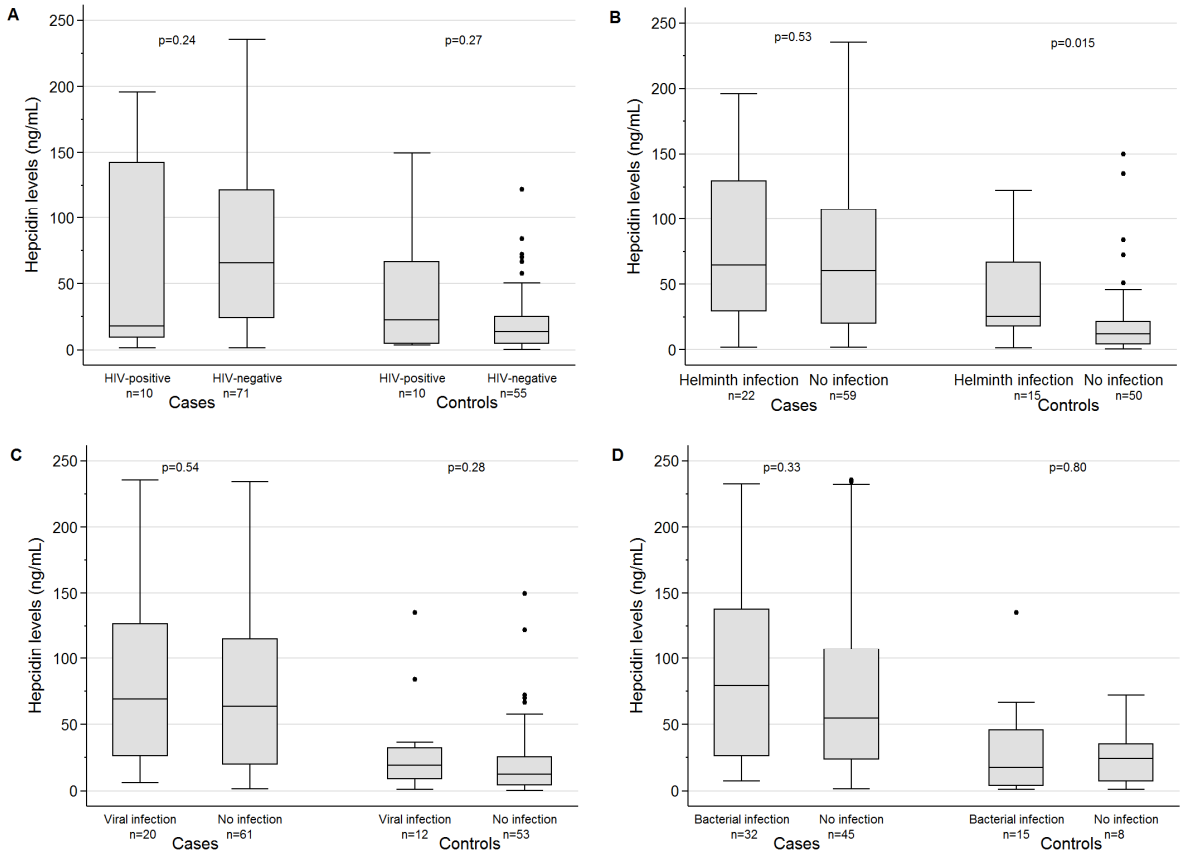

Figure 16. Box plots of hepcidin levels and coinfections in TB patients (cases) and household controls without TB (controls).
A: Coinfection with HIV; **B:** Coinfection with any helminth infection (soil-transmitted and intestinal helminths). **C:** Coinfection with viral respiratory pathogens. **D:** Coinfection with bacterial respiratory pathogens. P values for comparison between cases and controls: p<0.001. All study participants with available laboratory results were included.

8.4.4 Associations between hepcidin concentrations, progression to disease, and disease severity

Hepcidin levels were significantly higher in TB cases and controls who developed TB during follow-up compared to controls who did not develop TB (test for trend, p<0.001, Figure 17A), even when restricting the comparison to HIV-negative study participants (p<0.001) (Figure 17B). Furthermore, the inflammation markers procalcitonin and CRP were also increased in TB cases and controls who developed TB (Figure 17C and Figure 17D). During explorative analyses, the distribution of hematological, iron and inflammatory parameters differed between cases and controls and even when compared with controls who developed TB within 12 months (Supplementary Table 4 and Supplementary Table 6).

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The association of increased hepcidin levels and progression to active TB was further confirmed by the regression analysis (Table 11), which showed an association between increasing hepcidin levels and TB even when taking HIV infection into account (coefficient 0.65, 95% CI 0.45-0.85, p<0.001). Hepcidin concentrations were also positively associated with clinical severity of TB as defined by the mycobacterial load in the sputum and the TB symptom scoring. Similar associations were found for the inflammatory marker procalcitonin as well as hemoglobin.

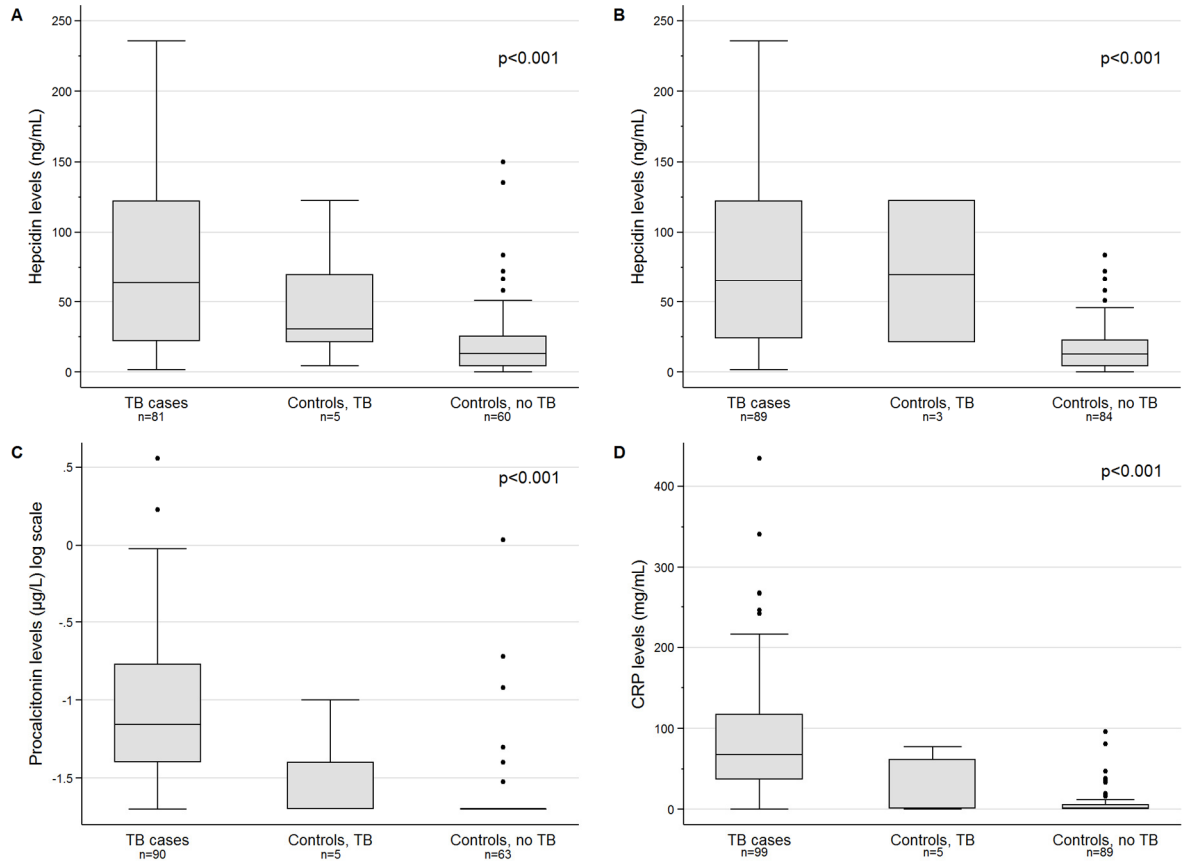

Figure 17. Box plots of hepcidin levels and inflammatory parameters in TB patients (cases), household controls who developed TB within 12 months after enrolment (controls, TB), and household controls who did not develop TB (controls, no TB), at the time of TB diagnosis or enrolment.
A: Hepcidin concentrations (ng/mL) among all study participants; **B:** Hepcidin concentrations among HIV-negative participants only. **C:** Procalcitonin concentrations (µg/L). **D:** C-reactive protein (CRP) concentrations (mg/L). All study participants with available laboratory results were included: hepcidin (n=146), procalcitonin (n=158), CRP (n=193). *P* values: tests for trend across groups.

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Table 11. Associations between log hepcidin, procalcitonin, and hemoglobin levels with progression from exposed individuals to TB and TB disease severity among TB cases (cases) and controls who did (controls, TB) or did not develop TB (controls, no TB) during follow-up.

	Hepcidin (ng/mL)		Procalcitonin (µg/L)		Hemoglobin (g/dL)	
	Coefficient (95% CI)	*P* value [1]	Coefficient (95% CI)	*P* value [1]	Coefficient (95% CI)	*P* value [1]
Progression to TB [2 3]		<0.001		<0.001		<0.001
Controls, no TB	Ref		Ref		Ref	
Controls, TB	0.36 (-0.47-1.18)		0.54 (-0.10-1.18)		-0.02 (-0.10 to 0.06)	
Cases	0.65 (0.45-0.85)		0.54 (0.40-0.69)		-0.04 (-0.06 to -0.02)	
TB symptoms scoring		<0.001		<0.001		<0.001
No TB	Ref		Ref		Ref	
Mild TB symptoms	0.47 (0.21-0.72)		0.47 (0.28-0.66)		-0.02 (-0.04 to 0.005)	
Severe TB symptoms	0.84 (0.51-1.17)		0.58 (0.36-0.81)		-0.07 (-0.10 to -0.04)	
TB bacterial load [4]		<0.001		<0.001		<0.001
No TB	Ref		Ref		Ref	
Low bacterial load	0.52 (0.22-0.83)		0.48 (0.28-0.69)		-0.04 (-0.06 to -0.008)	
High bacterial load	0.68 (0.40-0.97)		0.55 (0.34-0.77)		-0.04 (-0.07 to -0.02)	

95% CI, 95% confidence interval; Ref, reference group; TB, tuberculosis

Estimations derived from a regression model with hepcidin (log10) as the outcome. The model "TB and progression to TB" includes study participant group (TB cases, controls with TB, controls without TB) and HIV infection.

[1] Accounts for case-control matching
[2] Coefficients adjusted for HIV infection
[3] TB patients (cases), household contact controls (controls), household contact controls who developed TB within 12 months after enrolment (controls, TB), and household controls without TB (controls, no TB).
[4] Sputum smear microscopy (quantitative scoring): scant and 1+ (low bacterial load), 2+ and 3+ (high bacterial load)

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8.5 Discussion

In our matched case-control study of TB cases and household contact controls without TB, we demonstrated that ACD was the predominant cause of anemia among TB cases. This is in line with previous observations that anemia is mainly due to chronic disease (Minchella et al., 2015b; Weiss and Goodnough, 2005; World Health Organization, 2001), and ACD has been shown to be the predominant cause of anemia in HIV-positive TB patients (Andrew et al., 2014). This type of anemia is largely due to the underlying chronic inflammatory state existing in chronic diseases.

Diagnosis of ID in chronic inflammatory diseases such as TB is challenging. For example, using ferritin as a single parameter could underestimate ID because ferritin is upregulated in inflammation (Thurnham et al., 2010). Helminths infections especially from *Strongyloides stercoralis*, low dietary iron intake and absorption from monotonous, cereal-based diets contribute to the burden of anemia (Zimmermann et al., 2005), and may be the reason for the IDA observed in the control group. We did not find a significant association between TB disease and IDA, which is potentially due to tight regulation of iron metabolism by inflammation, existing iron recycling from senescent red blood cells, and absorption of iron by enterocytes (Drakesmith and Prentice, 2012).

The sTfR concentration was overall negatively associated with hepcidin levels, but to a lesser extent in cases, which suggests that chronic infection with TB interferes in the regulatory mechanisms. This is in agreement with previous reports on the influence of inflammation on sTfR levels (Rohner et al., 2017). The higher concentrations of sTfR observed in TB cases compared to controls suggest an erythropoetic stimulus and a demand of iron in TB patients. The two different signals, elevated hepcidin levels causing hypoferremia and the erythropoetic stimulus, indicate a delicate balancing act for iron that is required during chronic infection: both deficiency and excess of iron (Drakesmith and Prentice, 2012) can increase the risk of worse TB treatment outcomes (Isanaka et al., 2012a).

Hepcidin was associated with TB disease severity and progression to active TB, but not with HIV, helminth, and respiratory pathogen coinfections. Infections with viruses such as hepatitis B and C have been observed to have little effect on hepcidin homeostasis (Armitage et al., 2014). Yet at the

time of recruitment, we observed hepcidin levels that were already elevated well before active disease appeared in controls who developed TB during follow-up. This finding held even when excluding HIV-positive patients and adjusting the regression model to HIV infection as the most important risk factor for progression from infection to active TB (Corbett et al., 2003). This suggests that altered iron metabolism long precede active disease. Regarding the hormone as a participant in innate immune response, we noted that, other inflammatory markers procalcitonin and CRP were also increased before development of active TB. Elevated levels of all three of these markers suggest that the body is already in an inflammatory state as previously reported (Armitage et al., 2014; Kerkhoff et al., 2016b, 2016a; Minchella et al., 2015b), but the association of hepcidin with procalcitonin found in our study has not been reported so far.

Anemia resulting from chronic inflammation has a complex pathophysiology depending on the underlying disease process. Previous studies have found results similar to ours (Kerkhoff et al., 2016a; Minchella et al., 2014), but have not considered coinfections, inflammation parameters, follow-up of TB-exposed individuals (progression to disease) and clinical presentation, which are novel in our study. Our results are also in line with reports from South Africa, which focused only on HIV-positive TB patients (Kerkhoff et al., 2016b, 2016a). There are concerns of the clinical benefit from general supplementation of iron in anemic TB patients (McDermid et al., 2013). Moreover, a clinical trial on iron supplementation in TB patients showed a positive effect on hematological indices in the intervention group compared to the placebo group after one month, but these effects disappeared after two and six months (Devi et al., 2003). This indicates that with the resolution of infection (and the decrease in hepcidin levels), adequate concentrations of iron become available in the blood of the majority of TB patients by the release of sequestered iron and improved iron absorption. However, a small proportion of TB patients with underlying ID may still benefit from iron supplementation, but the accurate detection of ID in these patients remains an unresolved problem. Low hepcidin levels may help distinguish patients with IDA versus ACD (Girelli et al., 2016), and patients who may benefit most from iron supplementation. More work is needed to understand the clinical utility of hepcidin in the context of coinfections (Wang and Babitt, 2016).

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Our study had some limitations, notably among them no follow-up serum taken after completion of TB treatment to further evaluate the role of hepcidin and other inflammatory markers in TB. However, we were able to collect a wide range of clinical data that encompassed information on coinfections including HIV, helminths, and viral and bacterial respiratory pathogens, and laboratory parameters that included inflammatory and hematological markers. The study design allowed comparison of cases versus controls with sufficient power. We did not assess the β-thalassemia trait, which could have influenced the hematological parameters (Rathod et al., 2007). Finally, the sample size of household contact controls was relatively small.

In conclusion, hepcidin was marginally upregulated by coinfections other than *M. tuberculosis*, and could be a marker identifying more severe TB disease and high-risk individuals among persons exposed to TB. In the management of anemia in TB patients, a small proportion would benefit from iron supplementation (Isanaka et al., 2012a, 2012b). However, iron supplementation must be approached cautiously because the functional iron deficit that is observed in TB patients with anemia is mostly temporary due to sequestration of iron in the cells and reversible during TB treatment (Devi et al., 2003). Clinical management of anemia and nutritional interventions among TB patients to improve the clinical course and TB treatment outcomes in under-resourced settings need further investigation. Future studies with larger sample sizes and also including additional biomarkers could further explore and confirm our findings.

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Data availability

Datasets that were used for analysis and preparation of this manuscript are available upon request and completion of the data transfer agreement through the Medical Research Coordinating Committee (MRCC) by the concerned parties. To initiate request of data used in this study, please contact Dr. Fredrick Haraka (fharaka@ihi.or.tz, Ifakara Health Institute, P.O. Box 78 373, Tanzania) who is responsible for protection of data and materials at Tuberculosis Research Group. Once the data transfer agreement has been approved by the MRCC, data will then be sent electronically to concerned parties.

Author's contribution

Conceived and designed the study: JH, CIC, FM, MS, SG, KR, MBZ and LF. JH, CIC, FM, MZ, NS and LF analysed the data. JH, CIC, FM, and LF contributed clinical data. MS, TB, SG, NS and LR contributed laboratory data. JH, CIC and LF prepared the first draft of the manuscript. JH, CIC, FM, MZ, MBZ and LF contributed in the first major revision of the manuscript. All authors contributed in final manuscript revisions and approved the final version.

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Conflict of interest

All authors declared no conflict of interest. Authors TB and LR have a commercial affiliation to the "labormedizinisches zentrum". However this commercial affiliation did not alter the adherence to PLOS ONE policies on sharing data and materials, study design nor decision to publish.

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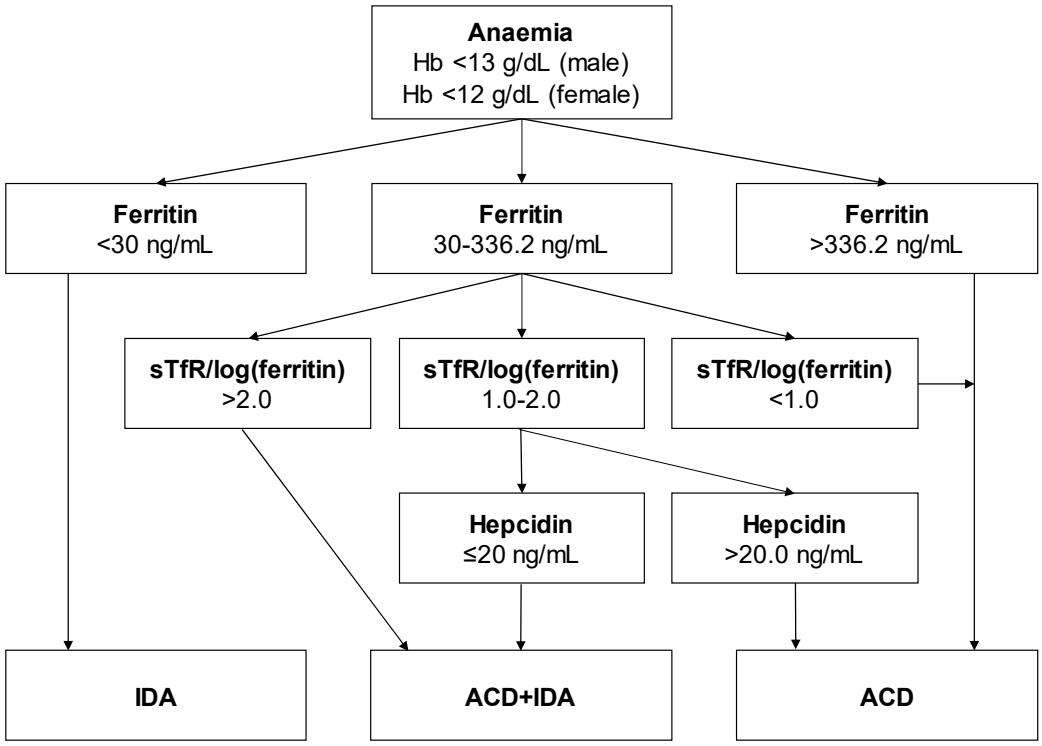
8.6 Supporting information

Supplementary Figure 5. Anemia case definitions according to iron deficiency and chronic disease. (Kerkhoff et al., 2016b)



ACD, anemia of chronic disease; Hb, hemoglobin; IDA, iron deficiency anemia; sTfR, soluble transferrin receptor

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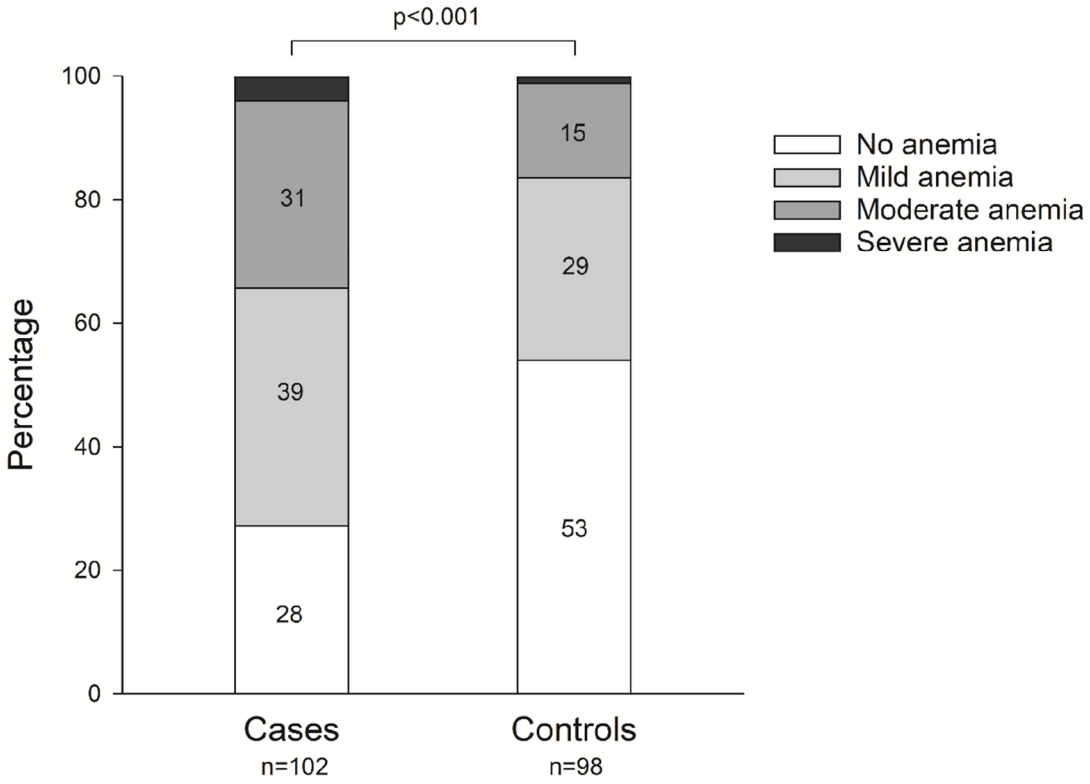
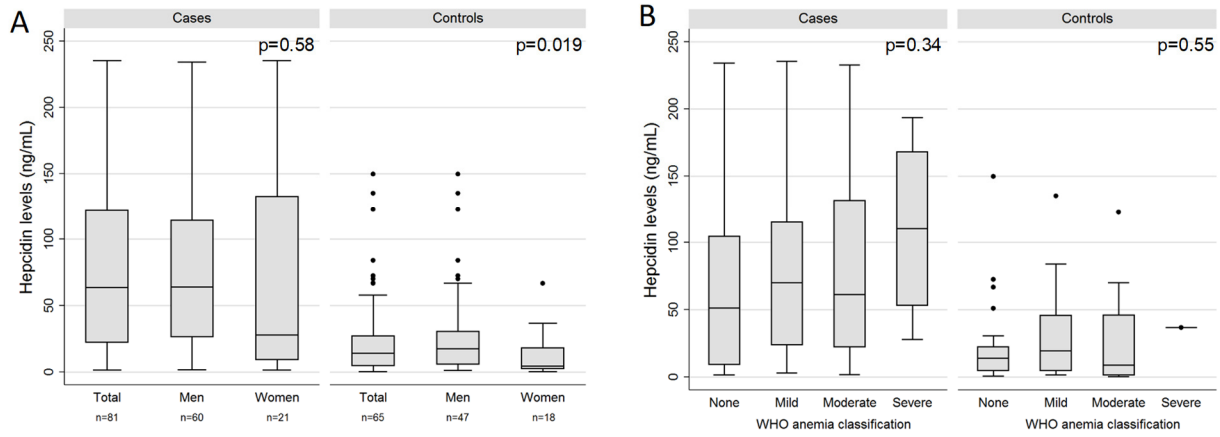
Supplementary Figure 6. WHO anemia classification in cases and controls.
Numbers on the bars indicate absolute numbers



P value was obtained using a chi-square test across the two groups

Supplementary Figure 7. Box plots of hepcidin levels (ng/mL) in TB patients (cases) and household controls without TB (controls), stratified by sex (A) and by WHO anemia severity classification (B)



P values were obtained using Kruskal-Wallis tests

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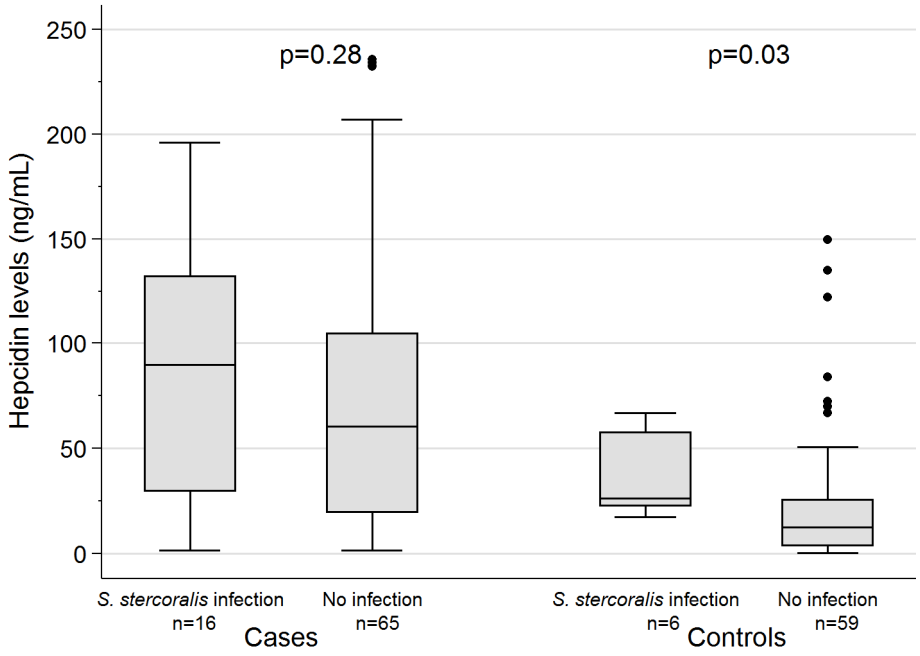
Supplementary Figure 8. Box plots of hepcidin levels (ng/mL) in TB patients (cases) and household controls, stratified by *Strongyloides stercolaris* infection.



P values were obtained using Kruskal-Wallis tests

Supplementary Table 2. Detection of respiratory viral and bacterial pathogens using a multiplex real-time PCR in nasopharyngeal swabs.

Viral species Anyplex II RV16 (panels A and B)	**Bacterial species** Allplex respiratory panel 4
Adenovirus	*Mycoplasma pneumonia*
Influenza A	*Chlamydophila pneumoniae*
Influenza B	*Legionella pneumophila*
Rhinovirus A/B/C	*Haemophilus influenzae*
Respiratory syncytial virus A	*Streptococcus pneumoniae*
Respiratory syncytial virus B	*Bordetella pertussis*
Parainfluenza virus 1	*Bordetella parapertussis*
Parainfluenza virus 2	
Parainfluenza virus 3	
Parainfluenza virus 4	
Bocavirus 1/2/3/4	
Metapneumovirus	
Coronavirus 229	
Coronavirus OC4	
Coronavirus NL63	
Enterovirus	

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Supplementary Table 3. Hematological, iron and inflammatory parameters according to anemia severity among cases and controls.

Parameter	Cases, median (IQR)				Controls, median (IQR)			
	None (n=28)	Mild (n=39)	Mod/severe (n=35)	*P* value	None (n=53)	Mild (n=29)	Mod/severe (n=16)	*P* value
Iron (µmol/L)	6.7 (3.7-12.8)	4.5 (3.6-6.1)	3.8 (3.0-6.1)	0.02	15.3 (10.8-19.1)	11.6 (8.9-14.3)	8.6 (5.5-13.9)	0.003
Ferritin (ng/mL)	222.2 (128.1-566.5)	384.5 (199.1-790.1)	333.7 (181.0-524.0)	0.14	112.1 (68.0-152.5)	88.8 (44.2-169.6)	70.9 (14.9-171.0)	0.47
Soluble transferrin receptor (mg/L)	1.4 (1.4-1.7)	1.9 (1.5-2.1)	2.1 (1.6-2.9)	0.006	1.4 (1.2-1.6)	1.4 (1.2-1.8)	1.9 (1.5-2.3)	0.044
Transferrin (g/L)	1.8 (1.5-2.2)	1.7 (1.4-1.9)	1.4 (1.3-1.8)	0.009	2.6 (2.4-2.8)	2.4 (2.0-2.7)	2.3 (2.1-2.)	0.1
Hepcidin (ng/mL)	51.0 (9.2-104.5)	69.9 (23.6-115.2)	70.1 (23.1-132.7)	0.53	13.9 (4.6-22.3)	19.5 (4.5-45.8)	12.7 (1.1-36.6)	0.59
CRP (mg/L)	54.4 (19.8-115.5)	80.5 (30-112.3)	89.2 (49.9-145.2)	0.16	0.9 (0.6-3.3)	3.3 (0.7-10.2)	4.1 (0.6-19.3)	0.024
Procalcitonin (µg/L)	0.05 (0.02-0.21)	0.07 (0.04-0.2)	0.08 (0.05-0.19)	0.31	0.020 (0.02-0.02)	0.02 (0.02-0.04)	0.02 (0.02-0.05)	0.25
Hemoglobin (g/dL)	13.7 (13.3-14.5)	12.3 (11.5-12.7)	9.7 (8.5-10.3)	0.001	14.1 (13.5-15.0)	12.1 (11.5-12.6)	9.5 (8.6-10.3)	0.001
MCV (f/L)	78.4 (71.4-83.5)	78.6 (71.6-84.3)	70.5 (63.4-76.9)	0.006	83.9 (78.7-87.2)	78.0 (72.3-83.5)	78.0 (65.7-85.6)	0.006
MCH (pg/cell)	26.4 (24.1-28.4)	26.1 (23.4-27.7)	22.4 (20.5-24.5)	<0.001	27.8 (25.8-29.8)	24.2 (23.0-26.8)	22.9 (20.5-26.1)	<0.001
MCHC (g/dL)	34.0 (33.6-34.8)	33.1 (32.3-34.2)	32.2 (31.4-32.9)	<0.001	33.4 (32.7-34.8)	32.2 (31.0-32.9)	31.1 (29.7-32.3)	<0.001
Red blood cell distribution width (f/L)	14.6 (13.1-16.2)	14.6 (13.6-15.9)	16.7 (14.9-19.4)	0.005	14.1 (13.1-14.9)	14.7 (13.9-16.3)	15.6 (14.7-19.3)	0.008

CRP, C-reactive protein; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; Mod/severe, moderate and severe anemia (WHO classification)

P values were obtained using Kruskal-Wallis tests

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Supplementary Table 4. Hematological, iron and inflammatory parameters among cases, controls and controls who developed tuberculosis.
(See also Table 2)

Parameter	**No. included** [1]	**Median (IQR)**		
(unit)	Cases / Controls, TB [2] / Controls	Cases	Controls, TB [2]	Controls
Iron (µmol/L)	99 / 5 / 86	4.4 (3.3-7.1)	11.6 (4.0-11.7)	13 (9.0-17.7)
Ferritin (ng/mL)	101 / 5 / 84	309.8 (162.2-601.2)	171 (107.4-263.6)	100 (59.1-144)
sTfR (mg/L)	89 / 5 / 70	1.8 (1.4-2.2)	1.5 (1.4-1.9)	1.4 (1.2-1.8)
Transferrin (g/L)	99 / 5 / 89	1.6 (1.4-2.0)	2.3 (1.8-2.3)	2.5 (2.3-2.8)
Hepcidin (ng/mL)	81 / 5 / 60	63.7 (22.0-121.9)	30.5 (21.1-69.9)	13.2 (4.2-25.5)
CRP (mg/L)	99 / 5 / 89	67.8 (36.5-116.9)	1.6 (0.9-61.2)	1.5 (0.6-5.8)
Procalcitonin (µg/L)	90 / 5 / 63	0.07 (0.04-0.17)	0.04 (0.02-0.04)	0.019 (0.019-0.02)
Hemoglobin (g/dL)	102 / 5 / 93	12.1 (10.3-12.9)	11.1 (10.6-11.3)	13.1 (11.8-14.3)
MCV (f/L)	102 / 5 / 93	75.5 (68.6-82.8)	85.3 (77.0-94.0)	81 (76.0-86.0)
MCH (pg/cell)	102 / 5 / 93	25.0 (22.4-27.5)	28.1 (25.5-28.7)	26.3 (23.8-29.1)
MCHC (g/dL)	102 / 5 / 93	33.1 (32.0-34.0)	32.9 (32.6-33.0)	32.7 (31.2-33.9)
Red blood cell distribution width (f/L)	63 / 5 / 77	14.9 (13.8-16.7S)	14.6 (14.1-15.8)	14.7 (13.5-15.6)

CRP, C-reactive protein; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; sTfR, soluble transferrin receptor; MCHC, mean corpuscular hemoglobin concentration

[1] Patients with an available laboratory result
[2] Controls who developed tuberculosis

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Supplementary Table 5. Hematological, iron and inflammatory parameters according to sex, among cases and controls.

Parameter	Cases median (IQR)			Controls median (IQR)		
	Male (n=78)	**Female** (n=24)	***P* value**	**Male** (n=74)	**Female** (n=24)	***P* value**
Iron (µmol/L)	4.4	4.0 (3.0-9.2)	0.9	14.3 (9.4-19.0)	9.8 (6.3-13.1)	0.008
Ferritin (ng/mL)	424.5 (212.3-68.2)	160.9 (117.6-309.8)	0.001	120.3 (76.2-180.8)	32.7 (14.9-72.8)	<0.001
Soluble transferrin receptor (mg/L)	1.7 (1.4-2.1)	1.9 (1.6-2.8)	0.1	1.3 (1.1-1.6)	1.9 (1.4-2.2)	<0.001
Transferrin (g/L)	1.6 (1.4-1.9)	1.6 (1.3-2.2)	0.9	2.4 (2.3-2.7)	2.7 (2.3-3.3)	0.015
Hepcidin (ng/mL)	64.0 (26.4-114.4)	27.8 (9.2-132.7)	0.58	17.5 (5.6-30.5)	4.6 (2.4-18.3)	0.019
CRP (mg/L)	76.3 (41.3-115.1)	58.8 (29.2-158)	0.7	1.5 (0.6-7.2)	1.7 (0.6-5.5)	0.9
Procalcitonin (µg/L)	0.09 (0.04-0.19)	0.06 (0.03-0.09)	0.3	0.02 (0.02-0.03)	0.02 (0.02-0.03)	0.7
Hemoglobin (g/dL)	12.4 (10.8-13.3)	10.1 (8.4-11.6)	<0.001	13.6 (12.5-14.7)	1.2 (10.1-12.1)	<0.001
MCV (f/L)	77.2 (686-83.4)	73.4 (65.5-79.5)	0.1	83.5 (77.2-87.0)	75.5 (67.5-81.6)	0.001
MCH (pg/cell)	25.7 (22.8-27.7)	23.5 (21.3-25.9)	0.03	26.9 (24.2-29.7)	23.9 (20.9-26.2)	<0.001
MCHC (g/dL)	33.3 (32.4-34.1)	31.8 (30.9-33.3)	0.004	33.0 (31.7-34.2)	32.2 (29.9-33.0)	0.01
Red blood cell distribution width (f/L)	14.6 (13.6-16.2)	16.8 (14.8-19.8)	0.004	14.5 (13.3-15.6)	14.8 (13.9-16.7)	0.1

CRP, C-reactive protein; IQR, interquartile range; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration

P values were obtained using Kruskal-Wallis tests

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Supplementary Table 6. Etiology of anemia and iron deficiency based on single laboratory parameters among TB cases, controls and controls who developed tuberculosis (also see Table 3).

Classification	**No. included** [1]	**n (%)**		
	Cases / Controls TB [2] / Controls	Cases	Controls, TB [2]	Controls
Cause-specific anemia [3]	97 / 5 / 83			
ACD		58 (59.8)	3 (60)	20 (24.1)
IDA		1 (1.0)	0	9 (10.8)
ACD+IDA		10 (10.3)	1 (20.0)	2 (2.4)
No anemia		28 (28.9)	1 (20.0)	52 (62.7)
Iron deficiency [4]				
CRP containing index <0	99 / 5 / 84	80 (80.8)	4 (80.0)	82 (97.6)
Ferritin <30 µg/L	101 / 5 / 84	2 (2.0)	0	13 (15.5)
MCV <80 f/L	102 / 5 / 93	67 (65.7)	2 (40.0)	42 (45.2)
MCH <27.5 g/dL	102 / 5 / 93	75 (73.5)	2 (40.0)	63 (67.7)
MCHC <32 g/dL	102 / 5 / 93	23 (22.6)	1 (20.0)	29 (31.2)
sTfR >2.5 mg/L	89 / 5 / 70	17 (19.1)	0	3 (4.3)
sTfR index >1.5	89 / 5 / 70	4 (4.5)	0	8 (11.4)
Hepcidin >20 ng/mL	81 / 5 / 60	62 (76.5)	4 (80.0)	19 (31.7)

ACD, anemia of chronic disease; ACD+IDA, multifactorial anemia (ACD+IDA); IDA, iron deficiency anemia; CRP, C-reactive protein; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; sTfR, soluble transferrin receptor

[1] Patients with an available laboratory result
[2] Controls who developed tuberculosis
[3] Based on algorithm in Supplementary Figure 1
[4] Based on single laboratory parameters (e.g., independent from hemoglobin levels)

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Supplementary Table 7. Associations between log hepcidin levels (ng/mL) and other hematological indices (log scale).

Parameter	All		Cases		Controls	
	Coefficient (95% CI)	*P* value	Coefficient (95% CI)	*P* value	Coefficient (95% CI)	*P* value
Iron (µmol/L)	-1.08 (-1.36 to -0.79)	<0.001	-1.32 (-1.60 to -1.04)	<0.001	0.33 (-0.31-0.98)	0.30
Ferritin (ng/mL)	0.96 (0.83-1.09)	<0.001	0.75 (0.51-1.00)	<0.001	0.98 (0.82-1.14)	<0.001
Soluble transferrin receptor (mg/L)	-0.05 (-0.068-0.57)	0.87	-0.04 (-0.060-0.69)	0.89	-1.62 (-2.69 to -0.55)	<0.004
Transferrin (g/L)	-2.75 (-3.33 to -2.18)	<0.001	-1.91 (-2.73 to -1.10)	<0.001	-3.92 (-5.46 to -2.37)	<0.001
CRP (mg/L)	0.54 (0.46-0.62)	<0.001	0.66 (0.51-0.80)	<0.001	0.52 (0.33-0.71)	<0.001
Procalcitonin (µg/L)	0.77 (0.60-0.95)	<0.001	0.58 (0.37-0.78)	<0.001	0.89 (0.42-1.36)	<0.001
Hemoglobin (g/dL)	-0.75 (-2.00-0.51)	0.24	-0.35 (-1.81-1.11)	0.64	0.86 (-1.03-2.75)	0.37
MCV (f/L)	-0.64 (-2.66-1.37)	0.53	0.10 (-2.28-2.47)	0.93	1.47 (-1.43-4.38)	0.32
MCH (pg/cell)	0.21 (-1.53-1.95)	0.81	-0.06 (-2.23-2.10)	0.95	1.97 (-0.29-4.23)	0.087
MCHC (g/dL)	3.80 (-0.32-7.93)	0.071	-1.58 (-7.95-4.78)	0.62	4.34 (-0.035-9.02)	0.069

95% CI, 95% confidence interval; CRP, C-reactive protein; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; sTfR, soluble transferrin receptor

Estimations derived from regression models (on log scale)

9. Discussion

9.1 General discussion

The main objective of this PhD project was to broaden our understanding of the epidemiology and transmission of TB in rural and urban Tanzania. The PhD project was embedded within the existing TB DAR cohort platform, which is a large prospective hospital-based cohort of adult TB cases and controls in urban and rural Tanzania. The cohort has had multiple funders, initially funded generously by the Rudolf Geigy Foundation of Basel, Switzerland. Currently the cohort is funded through sub-contracts from the Swiss Tropical and Public Health Institute through the Department of Medical Parasitology and Infectious Diseases. The cohort is jointly implemented in Tanzania by IHI and Swiss TPH with sample and data analyses done in both settings i.e., Bagamayo, Tanzania and Basel, Switzerland, respectively.

Specifically, this PhD project had the following objectives: (i) to study the infrastructure-related (locations of public importance) risk of TB transmission in Tanzania; (ii) to determine the diagnostic delay of TB and its associated risk factors among newly diagnosed smear positive TB patients in Dar es Salaam; (iii) to understand differences in the epidemiology of TB and comorbidities among adult TB patients from rural and urban Tanzania; and (iv) to investigate the associations of hepcidin levels with coinfections, TB disease severity and progression from TB infection to TB disease among adults in Tanzania. In the following sections, we will discuss the principal findings of this PhD project, potential innovations from the findings and highlight limitations.

Discussion

9.2 Summary discussion

9.2.1 Infrastructure-related risk of TB transmission in urban Tanzania

This was the first time in this setting where a TB transmission study was done using this novel approach of measuring CO_2 in shared spaces within different locations of public importance in Tanzania (Hella et al., 2017). Such studies were only limited in South Africa which in many parts is not representative of the sub-Saharan settings (Andrews et al., 2014, 2013; Issarow et al., 2015; Wood et al., 2014). Generally, the TB transmission risk varies by location due to differences in time spent at each location, ventilation conditions and social contacts. Our quantitative comparison of transmission risks has the potential to reveal why Dar es Salaam is a TB transmission hotspot in Tanzania. Briefly, we found that the highest annual risk of TB transmission was in prisons (41.6%), public transportation (20.3%), schools (4.02%) and night clubs (1.7%), while it was the least in markets (0.5%), religious halls (0.13%) and social halls (0.12%).

Prisons were by far the setting with the highest risk of TB transmission where we found the transmission to range all the way to 90%, a finding which had been seen in previous studies in South Africa and Brazil, which used traditional methods of studying TB transmission (Johnstone-Robertson et al., 2011a; Urrego et al., 2015). After our work, new studies came out highlighting this excess risk of TB transmission carried in incarceration. A study published from China, which investigated for active TB among two large prisons in China by clinical examination and Chest X-rays, found a higher risk of TB disease among prisoners than that seen in the general population (Tong et al., 2019). Compared to the Chinese general population, female prisoners had three times the risk of TB (OR 3.30, 95% CI 2.15, 5.09) while male prisoners had twice the risk of TB (OR 2.06, 95% CI 1.29, 3.3) (Tong et al., 2019). Likewise, another study in Brazil which investigated the risk of TB incidence in prison settings found the prison population to have a 22-fold risk as compared to the general population (Pelissari and Diaz-Quijano, 2019).

There are many factors that need to be considered in the control of the TB epidemic, good infrastructure setting (i.e., less crowded, good ventilation etc.,) is amongst the most important factor that can contribute in reducing the risk of TB transmission. Our finding highlights the risks of

transmission in prison and other settings of public importance, which share poor ventilation conditions and a large social mixing contact – which is highly conducive for TB transmission and driving the epidemic in high TB burden settings. For example, the 2019 Global Tuberculosis Report shows health care workers in Tanzania and other seven countries (Algeria, Burkina Faso, Colombia, Dominican Republic, Honduras, India and Lesotho) to have double the TB notification rate per 100,000 population as compared to the general population (World Health Organization, 2019). This highlights the importance of implementing TB infection prevention in particular infrastructure settings, which will contribute in the TB control efforts.

9.2.2 TB diagnostic delay and associated risk factors in urban Tanzania

In this second manuscript of the PhD project, we showed that 21% of patients delayed seeking care for TB symptoms (median delay of three weeks from first symptom until TB diagnosis is made). This diagnostic delay was shorter compared to previous studies in sub-Saharan Africa, Asia as well as Eastern Europe (Irani et al., 2007; Mfinanga et al., 2008; Rabin et al., 2013; Saifodine et al., 2013; Thakur and Murhekar, 2013; Yimer et al., 2014), illustrating the efforts in health promotion done by TB programs and other implementing partners. The diagnostic delay we measured (time from onset of symptoms until diagnosis) was also shorter than the treatment delay (time from onset of symptoms until treatment) reported in a study conducted in six rural and urban districts in Tanzania (median treatment delay time was 10 – 14.5 weeks) (Hinderaker et al., 2011).

We found that more than three quarters of participants had sought care from formal facilities prior to TB diagnosis in urban Tanzania. This indicates that a high proportion of TB patients is seen by several different health care providers before receiving TB diagnosis, suggesting a low suspicion index among health care providers. Parallel to this observation, the NTLP is currently implementing a quality improvement (QI) intervention which aims at improving TB case detection at the facilities and from the community (The Global Fund, 2018). This missed opportunity faced by patients was previously brought to light from the first national TB prevalence survey in Tanzania, which reported that one third of patients suspected of having TB sought care before the survey, which confirms that many infectious TB cases are missed by the health care system in Tanzania (Senkoro et al., 2015).

Discussion

We found that diagnostic delay was not reduced among HIV-positive patients despite the widely implemented TB/HIV collaborative activities by the National AIDS Control Programme and the NTLP in Tanzania (National Tuberculosis and Leprosy Program, 2013). Interestingly, chest pain was associated with a shorter diagnostic delay, in contrast to studies from Afghanistan and Brazil (Maciel et al., 2010; Sabawoon et al., 2012). Hemoptysis was associated with a prolonged diagnostic delay in our study due to the fact that this symptom is likely among patients with advanced disease (Kardjito and Grange, 1980). However, this finding was opposite to two systematic reviews, which argue that presence of hemoptysis may necessitate health care providers to investigate for TB early on (Cai et al., 2015; Storla et al., 2008).

Lastly, we found that the distance of patients houses to pharmacies could influence their health seeking behavior – a finding which could strategically be used in designing public health interventions by using GIS techniques (MacPherson et al., 2013; Sabde et al., 2011; Theron et al., 2015). We found that patients living closer to pharmacies tended to visit facilities more frequently. This suggests that the availability of health care services (public/private) affects use by the resident population. In line with our results, a study from Tanzania showed that residing at a distance of more than 5 km from a TB diagnostic center was associated with diagnostic delay (Mfinanga et al., 2008). In addition, it has been documented previously that longer walking distance to health care facilities was correlated with patient delay (Cai et al., 2015).

9.2.3 Differences in TB epidemiology and comorbidities in urban and rural Tanzania

In the third manuscript of the PhD project, we found striking differences in rural versus urban TB patients characteristics which could be used in customized public health interventions in TB control. TB patients from rural settings were likely to be older, with lower haemoglobin levels representing more features of advanced TB disease and significantly reported prior history of TB contact, which was not the case in the urban setting. TB patients from rural setting were more likely to visit traditional healers prior to TB diagnosis a factor which propagates TB diagnosis delay and contribute to ongoing TB transmission (Ngadaya et al., 2009; Verhagen et al., 2010; Wandwalo and Mørkve, 2000).

Discussion

Previous studies elsewhere have associated helminth co-infections with rural residence (Alemu and Mama, 2017; Walson et al., 2010), while from the TB DAR cohort, we had previously shown a positive association between TB and helminth co-infection from Dar es Salaam (Mhimbira et al., 2017). In this PhD project, we found *S. stercoralis* to be the principal driver of helminthiasis in the urban setting; a finding which was comparable to previous investigations (Mhimbira et al., 2017; Yelifari et al., 2005). On the contrary, *Schistosoma spp*., were the principal driver of helminthiasis in the rural setting as similarly seen in the Democratic Republic of Congo and elsewhere in Tanzania (Madinga et al., 2015; Mazigo et al., 2012).

9.2.4 Hepcidin and anaemia in TB cases and controls in urban Tanzania

In the fourth manuscript of the PhD project, we found that hepcidin levels could be increased long before an individual developed active TB disease, paving a way for strategies to use this as a biomarker to identify incipient and sub-clinical TB cases and start TB treatment early before overt disease. We found anaemia of chronic disease (ACD) to be the predominant cause of anaemia among TB cases as compared to controls which is explained by the TB disease status (Minchella et al., 2015b; Theurl et al., 2008; Weiss and Goodnough, 2005). This mechanism is perhaps adaptive to the host by depriving iron needed by the parasite which in a chronic state results in ACD (Drakesmith and Prentice, 2012).

We found the iron homeostasis regulator hepcidin to be associated with TB disease severity and progression to active TB among controls who initially were without TB. It is known that infections such as viruses could have effects on hepcidin homeostasis (Armitage et al., 2014). We also found other inflammatory markers (procalcitonin and C-reactive protein) to be elevated prior to development of active TB suggesting early inflammatory responses prior to overt disease (Kerkhoff et al., 2016b; Minchella et al., 2015a, 2014). To our knowledge, this is the first study to report the association of elevated hepcidin together with procalcitonin prior to TB disease progression.

9.3 Conclusions

We draw the following conclusions from this PhD project;

- We demonstrated that using CO_2 measurements, we could identify locations with poor ventilation to be associated with higher risk of TB transmission. This novel approach can guide targeted infection control interventions for TB control.
- Due to passive TB case detection, there is still significant TB diagnosis delay in urban Tanzania and that the use of medication prior to TB diagnosis was common, providing an opportunity to use pharmacies in the formal referral systems for TB diagnosis.
- TB patients from rural Tanzania are likely to be older, with recurrent TB, with features of advanced TB disease due to a longer TB diagnosis delay and seek more frequently care from traditional healers. The overall prevalence of helminth co-infections among TB patients was higher in urban Tanzania, predominantly driven by *S. stercoralis* infection, but the prevalence of *S. mansoni* was higher in the rural Tanzania.
- Hepcidin was marginally upregulated by coinfections other than Mtb, and thus this could be used as a marker for identifying patient with severe TB disease and high-risk persons exposed to TB.

9.4 Recommendations

9.4.1 Policy contribution

Below are our policy recommendations drawn from this PhD project;

(i) In efforts to control the TB epidemic, National TB Programs (NTPs) in the sub-Saharan settings could identify likely TB transmission hotspots by similar modelling approaches using environmental CO_2 measurements, so as to institute targeted interventions aiming to prevent TB transmission among certain high-risk individuals/groups.

(ii) NTPs should improve TB diagnostic capacities by improving access to diagnostics and consider to formally include pharmacies within the health system to screen and improve linkages to TB diagnostics located in health care facilities.

(iii) NTPs should strive to understand fundamental differences in the TB epidemiology in different settings and translate that into specific interventions based on such differences. For example, the NTLP should build the capacity of traditional healers in rural Tanzania, for improving early detection of TB cases and referral for TB treatment.

9.4.2 Research outlook

Below are potential research questions brought up from this PhD project;

(i) Quantifying the role of improved ventilation on TB incidence and prevalence among prisoner settings in Tanzania.

(ii) Can a sputum referral system from pharmacies within the catchment area of the TB diagnostic facilities result into improved case detection?

(iii) Is recurrent TB in rural Tanzania due to TB reactivation or re-infection?

(iv) Is there a role in a nutritional intervention in controlling TB incidence among the aging rural population in Tanzania?

(v) Is there a role in reducing TB incidence if we are to strengthen targeted mass drug administration against helminth infections?

(vi) Can hepcidin levels (alone or in combination with procalcitonin and CRP) predict incident TB among people with documented TB contact?

Discussion

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10. References

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11. Carriculum vitae

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Career summary

Dr. Jerry Hella is an epidemiologist and a public health specialist working at the Tuberculosis Research group at the Ifakara Health Institute. He has a background in clinical medicine (MD) and holds a MSc. in Epidemiology from the University of Basel. He began his career in research in 2012 as a study doctor at IHI and since then he has participated in different clinical research activities in Tanzania. He has extensive experience on data management ranging from database creation data management and analyses using different platforms such as Stata and R. He is passionate in geo-spatial analysis, data visualization and modelling of infectious disease.

Education

PhD in Epidemiology	Swiss Tropical and Public Health Institute/University of Basel, Basel, Switzerland (September 2016 -January 2020)
MSc in Epidemiology	Swiss Tropical and Public Health Institute/University of Basel, Basel, Switzerland (September 2014 -January 2016)
Doctor of Medicine	Kilimanjaro Christian Medical College, Tumaini University, Kilimanjaro, Tanzania (November 2005-August 2010)

Scientific achievements

The Bill and Melinda Gates Foundation October 2019
Award – Rising star scientist in Global Challenges

Swiss School of Public Health November 2018
Award – Best published PhD article in Public Health in 2018 (Award: 2'000 CHF)

Royal Society of Tropical Medicine and Hygiene June 2018
Small grant award (Fund: 5'000 GBP)

Clinic on Meaningful Modelling of Epidemiological Data May 2018
Scholarship award for travel and accommodation

Swiss Embassy Excellence Scholarship September 2016
PhD programme

The Union – Oral abstract presentation October 2014
Scholarship award for travel and accommodation

Tumaini University, Kilimanjaro Tanzania November 2010
Award – Overall best MD student

Carriculum vitae

Work experience

Ifakara Health Institute, Dar es Salaam, Tanzania — August 2012 - Present
Deputy Head of Department (Intervention and Clinical trials)
Research scientist

Mbeya Referral Hospital, Mbeya, Tanzania — November 2010- June 2012
Medical internship
Surgical Registrar

Ndolage Mission Hospital, Kagera, Tanzania — April 2009
Elective student

Technical skills

Statistical software: Stata, R, Ms. Excel
Mapping software: R, ArcGIS, qGIS
Database tools: ODK, MySQL, Ms. Access, EpiData, EpiInfo, Ms. Excel
Graphics software: GIMP
Other software: Latex

Publications

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2. Hiza H, **Hella J**, Arbués A, Magani B, Sasamalo M, Gagneux S, Reither K, Portevin D. **Case-control diagnostic accuracy study of a non-sputum CD38-based TAM-TB test from a single milliliter of blood.** Sci Rep. 2021; doi: 10.1038/s41598-021-92596-z
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6. Ndege R, Ngome O, Bani F, Temba Y, Wilson H, Vanobberghen F, **Hella J**, Gingo W, Sasamalo M, Mnzava D, Kimera N, Hiza H, Wigayi J, Mapesi H, Kato IB, Mhimbira F, Reither K, Battegay M, Paris DH, Weisser M, Rohacek M. **Ultrasound in managing extrapulmonary tuberculosis: a randomized controlled two-center study.** BMC Infect Dis. 2020; doi: 10.1186/s12879-020-05073-9
7. Arpagaus A, Franzeck FC, Sikalengo G, Ndege R, Mnzava D, Rohacek M, **Hella J**, Reither K, Battegay M, Glass TR, Paris DH, Bani F, Rajab ON, Weisser M; KIULARCO Study Group. **Extrapulmonary tuberculosis in HIV-infected patients in rural Tanzania: The prospective**

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18. Hiza H, Doulla B, Sasamalo M, **Hella J**, Kamwela L, Mhimbira F, Reither K, Gagneux S, Jugheli L, Fenner L. **Preservation of sputum samples with cetylpyridinium chloride (CPC) for**

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Membership

1. Tanganyika Medical Association (2010 to date)
2. Medical Association of Tanzania (2011 to date)
3. Reviewer British Medical Journal of Case Reports (2013 to date)
4. National Tuberculosis Operational Research Committee (2018 to date)
5. National Tuberculosis Laboratory Technical Working Group (2016 to date)