Epidemiology of co-infections in Tuberculosis patients in Tanzania: HIV, helminth infection and respiratory pathogens.

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List of Abbreviations

AFB	Acid Fast Bacilli
AIDS	Acquired Immune Deficiency Syndrome
aOR	adjusted Odds Ratio
ART	Anti-Retroviral Therapy
BD	Becton Dickinson
BMI	Body Mass Index
CCA	Circulating Anodic Antigen
CCA	Circulating Cathodic Antigen
CDC	Centers for Disease Control and Prevention
CD4+	Cluster of Differentiation 4+
CI	Confidence Interval
COPD	Chronic Obstructive Pulmonary Diseases
CTRL	Central Tuberculosis Reference Laboratory
DNA	Deoxyribonucleic acid
DPO	Dual Priming Oligonucleotide
DOT	Directly Observed Therapy
DOTS	Directly Observed Therapy Short course
ELISA	ELISA, Enzyme-Linked Immunosorbent Assay
EPG	Egg per gram
EPTB	Extrapulmonary TB
ETR	Electronic Tuberculosis Register
FBC	Full Blood Count
GIS	Geographical Information System
GPS	Geographical Positioning System
HAART	Highly Active Anti-Retroviral Therapy
Hb	Hemoglobin
HIV	Human Immunodeficiency Virus
HRV	Human Rhino Virus
IFN	Interferon
IHI	Ifakara Health Institute
IQR	Inter Quartile Range
IRB	Institutional Review Board
IRIS	Immune Reconstitution Inflammatory Syndrome
IUATLD	International Union Against TB and Lung Diseases
LJ	Löwenstein Jensen
MDA	Mass Drug Administration
MHC II	Major Histocompatibility Complex II
MoHCDGEC	Ministry of Health, Community Development, Gender, Elderly and Children
MTB	*Mycobacterium tuberculosis*
MUAC	Mid Upper Arm Circumference
NACP	National AIDS Control Programme
NIMR	National Institute for Medical Research
NSHP	National School Health Program

NTDCP	Neglected Tropical Diseases Control Programme
NTD	Neglected Tropical Diseases
NTLP	National Tuberculosis and Leprosy Programme
ODK	Open Data Kit
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PCT	Patient-Centered Treatment
PTB	Pulmonary Tuberculosis
RIF	Rifampicin
RNA	Ribonucleic acid
RR	Risk Ratio
RSTMH	Royal Society of Tropical Medicine and Hygiene
RV	Respiratory Virus
STH	Soil Transmitted Helminth
Swiss TPH	Swiss Tropical and Public Health Institute
TB	Tuberculosis
TOCE	Tagging Oligonucleotide Cleavage and Extension
USA	United States of America
WB	Whole Blood
WHO	World Health Organization
ZN	Ziehl-Nielsen

Acknowledgements

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Summary

Background: Tuberculosis (TB) caused by *Mycobacterium tuberculosis* complex is a global public health concern, causing significant morbidity and mortality. The resource limited settings are the worst hit, especially where there is also high burden of co-infections which increase the risk of developing TB and negatively affect TB treatment outcomes. HIV, helminth and respiratory pathogens are prevalent in Tanzania, a country that is in among the top 30 high burden countries as categorized by World Health Organization (WHO). There is epidemiological evidence of increased risk of developing TB with HIV, and little evidence on the association of TB and helminth infection and respiratory pathogens. It is therefore important to understand the epidemiology of TB and co-infections, so that we can design interventions that will address the burden of TB and relevant co-infections.

The objectives: The main objective of this PhD thesis was to determine the burden and the association of HIV, helminth and respiratory pathogens (viruses and bacteria) co-infections and TB at Temeke district, Dar es Salaam, Tanzania. The specific objectives were: i) to determine the treatment outcome of TB and HIV co-infected patients routinely diagnosed at Temeke district treated under home- and facility-based direct observed therapy (DOT), ii) to determine the prevalence of helminth infection and respiratory pathogens among smear positive TB cases and household contact controls without TB, iii) to investigate the risk factors for helminth infection and respiratory pathogens among smear-positive TB cases and household contact controls without TB, and iv) to determine the clinical effects of helminth infection and respiratory pathogens on clinical phenotypes and clinical outcomes among smear-positive TB patients.

Methods: This PhD is nested within a large TB cohort in Dar es Salaam region (TB-DAR): a prospective collection of clinical data and biological specimens to study the epidemiology of tuberculosis, including molecular epidemiology and the evaluation of new diagnostics and biomarkers). There are two distinctive methodological parts as described below:
Objective 1: We obtained anonymized electronic data from the TB district register of all adult TB patients (aged ≥15 years) who were notified between 2010 and 2013 in a single geographical area of two TB sub-districts (Wailes I and Wailes II) in the Temeke district, Dar es Salaam, Tanzania.

Objective 2-4: We consecutively enrolled ≥18 years TB patients and household contact controls between November 2013 until October 2015 to reach the required sample size. Any individual living in the same household as the index TB patients enrolled in the study is referred to as a household contact control. Controls at recruitment were free of symptoms and signs suggestive of TB, healthy on physical examination, and had a negative Xpert MTB/RIF result (Cepheid; California, USA). We collected sputum, nasopharyngeal swabs, stool and urine samples from TB patients and household controls at recruitment. Löwenstein-Jensen mycobacterial culture was used to confirm TB. Kato-katz and Bearman methods for detection of soil transmitted helminths infections from stool. Urine filtration and Circulating Cathodic Antigen (CCA) assay for detection of Schistosomiasis. Nasopharyngeal swabs samples were analyzed using Allplex™ Respiratory full panel assay and PCR Anyplex™II RV16 for detection of respiratory bacterial and viral pathogens respectively.

Principle findings: In a study to determine the treatment outcome of TB and HIV co-infected patients routinely diagnosed at Temeke district treated under home- and facility-based DOT: data of 4,835 adult TB patients were analyzed, with a median age of 35 years, 2,943 (60.9%) were men and TB/HIV co-infection prevalence of 39.9%. A total of 3,593 (74.3%) patients were treated under home-based DOT. Patients on home-based DOT were more likely to die compared to patients on facility-based DOT (RR 2.04, 95% Confidence Interval [95% CI]: 1.52-2.73), and more likely to complete TB treatment (RR 1.14, 95% CI: 1.06-1.23), but less likely to have a successful treatment outcome (RR 0.94, 95% CI: 0.92-0.97). Home-based DOT was preferred by women (adjusted Odds Ratio [aOR] 1.55, 95% CI: 1.34-1.80, p<0.001), older people (aOR 1.01 for each year increase, 95% CI: 1.00-1.02, p=0.001) and patients with extra-pulmonary TB (aOR 1.45, 95% CI: 1.16-1.81, p=0.001), but less frequently by patients on a retreatment regimen (aOR 0.12, 95% CI: 0.08-0.19, p<0.001).

In a study to assess the association of TB and helminth infection: a total of 597 TB patients and 375 household contact controls were included. The median age was 33 years and 60.2% (585/972) were men. The prevalence of any helminth infection among TB patients was 31.8% (190/597) and 25.9% (97/375) among controls. *Strongyloides stercoralis* was the predominant helminth species (16.6%, 161), followed by hookworm (9.0%, 87) and *Schistosoma mansoni* (5.7%, 55). An infection with any helminth was not associated with TB (aOR 1.26, 95% CI: 0.88-1.80, p=0.22), but *S. mansoni* infection was (aOR 2.15, 95% CI: 1.03-4.45, p=0.040). Moreover, *S. mansoni* infection was associated with lower sputum bacterial load (aOR 2.63, 95% CI: 1.38-5.26, p=0.004) and tended to have fewer lung cavitations (aOR 0.41, 95% CI: 0.12-1.16, p=0.088).

When assessing the interaction between TB and respiratory pathogens: we analyzed 794 study participants, of which 489 (61.6%) were TB patients and 305 (38.4%) were household contact controls. The median age of the study participants was 33 years; 61% (484/794) were men, and 21% (168/ 794) were HIV-positive. TB patients had a higher prevalence of HIV (28.6%; 140/489) than controls (9.2%; 28/305). Overall prevalence of respiratory viral pathogens was 20.4% (160/794; 95%CI 17.7-23.3%) and of bacterial pathogens 38.2% (303/794; 95%CI 34.9-41.6%). TB patients and controls did not differ in the prevalence of respiratory viruses (Odds Ratio [OR] 1.00, 95%CI 0.71-1.44), but respiratory bacteria were less frequently detected in TB patients (OR 0.70, 95%CI 0.53-0.94). TB patients with both respiratory viruses and respiratory bacteria were likely to have more severe disease (adjusted OR [aOR] 1.6, 95%CI 1.1-2.4; p=0.011). TB patients with respiratory viruses tended to have more frequent lung cavitations (aOR 1.6, 95%CI 0.93-2.7; p=0.089).

Conclusion: TB patients under home-based DOT had more risk factors for death such as older age, HIV infection and sputum smear-negative TB, and had higher TB mortality compared to patients under facility-based DOT. Assessment of TB mortality risk factors and offering additional clinical care could be beneficial in reducing TB mortality. Further operational research is warranted to monitor implementation of DOT and discern other risk factors for deaths. *S. mansoni* infection was an independent risk factor for active TB and altered the clinical presentation in TB patients. *S. mansoni* infection may play a role in TB pathogenesis in humans. Bidirectional screening of TB and helminth including treatment for both diseases should be considered in a clinical management of patients. Respiratory viruses are common

for both TB patients and household controls. TB patients may present with more severe TB disease, particularly when they are co- infected with both bacteria and viruses.

1. Introduction

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* complex (MTBC) (WHO, 2016). The MTBC comprises of closely related bacterial species and sub-species, including *M. tuberculosis M. bovis* and *M. africanum* which are adapted to humans (Coscolla and Gagneux, 2014). *M. tuberculosis* is the commonest cause of TB in humans. TB presents as pulmonary (PTB) and extrapulmonary TB (EPTB). Transmission of TB occurs when an individual inhales infectious droplets containing *M. tuberculosis* produced by an infectious TB patients during coughing, talking, sneezing or singing (Pai et al., 2016; Rieder, 1999). Individuals with *M. tuberculosis infection* (tuberculosis infection) do not show any symptoms. Whereas PTB manifest with symptoms such as cough, prolonged low grade fevers, excessive night sweats, unintentional weight loss and hemoptysis (WHO, 2016).

The risk of infection to *M. tuberculosis* is determined by the number of infectious TB patients in the community, duration infectiousness of those TB patients in the community, and close contact between a TB patient and susceptible contact per unit of time of infectiousness (Rieder, 1999). It is estimated around 2-3 billion people have tuberculous infection (WHO, 2016). However, only 5-10% of individuals with tuberculosis infection will end up developing TB in their lifetime (Rieder, 1999; WHO, 2016). Diseases and conditions like human immunodeficiency virus (HIV) infections, malnutrition, smoking, diabetes mellitus, helminth infections are known to increase the risk of developing TB from tuberculous infection (Marais et al., 2013). However, HIV is by far the most significant risk factor for developing TB especially in sub-Saharan Africa where HIV and TB are most prevalent (WHO, 2016). Furthermore, helminths and respiratory bacterial and viruses could also potentially play a pathogenic role in TB development. TB clinical course and treatment outcome can also be negatively affected by comorbidities such as HIV and diabetes mellitus (Marais et al., 2013; Waitt and Squire, 2011).

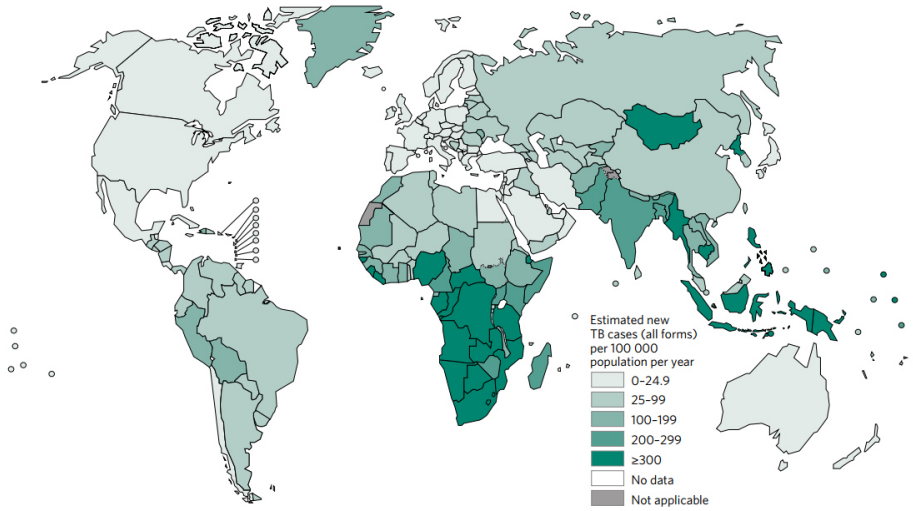
It is worth to mention, the emergence of drug resistance TB across the globe which is both alarming and threatens to halt and even reverse the achievements so far gained in TB control. *M. tuberculosis* strains become resistant to either first line or second lines anti-TB drugs and are known as multi-drug resistance TB (MDR-TB) or extensively drug resistance TB respectively (XDR-TB). MDR-TB are resistant to at least isoniazid, rifampicin which are the first line TB drugs. Meanwhile XDR-TB is a type of multidrug-resistant tuberculosis (MDR TB) that is also resistant to any fluoroquinolone and at least one of three injectable second-line drugs (i.e., amikacin, kanamycin, or capreomycin) (WHO, 2016).

This PhD focuses on HIV, helminth infections and respiratory pathogens co-infections. Noteworthy, the distribution HIV and helminth are disproportionately high in high TB burden settings like Tanzania. The geographical overlap of TB and these risk factors warrant further understanding of their epidemiology. The subsequent sections describe the epidemiology of TB and co-infections and the immunological interplay.

1.1. Tuberculosis

1.1.1. Global tuberculosis epidemiology

MTBC accounted for an estimated 10.4 million new (incident) TB cases worldwide and 1.4 million TB related deaths in the year 2015 (WHO, 2016). TB disproportionately affects more men than women (5.9 million vs. 3.5 million) (WHO, 2016). Over 60% of the TB cases notified in 2015, came from six countries namely India, Indonesia, China, Nigeria, Pakistan and South Africa. The World Health Organization (WHO) has reclassified the countries with high TB burden to 30 from the previous 22. These 30 high TB burden countries accounted for 87% of all estimated incident TB cases in the world in 2015 (WHO, 2016). Figure 1 shows the estimated number of new TB cases (all forms) per 100,000 populations per year in 2015. It is evident from Figure 1, that the burden of TB is high in the sub-Saharan Africa, and most countries are having incidence rates of above 200 TB patients per 100,000 population per year.

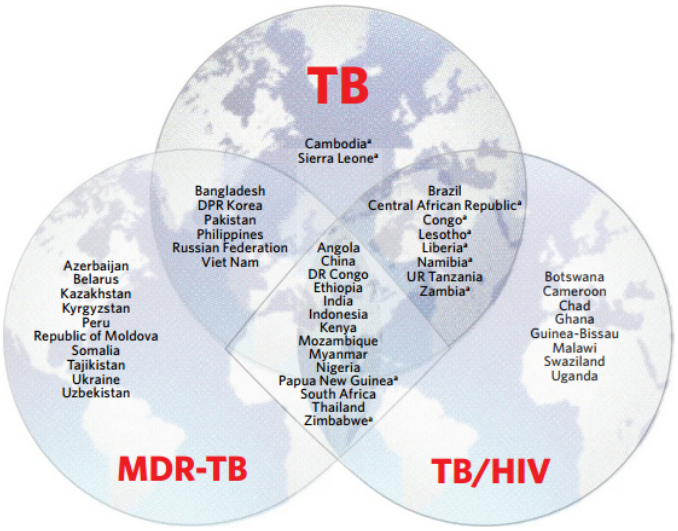


(Source: Global tuberculosis report 2016)

Figure 1. Estimated TB incidence rates, 2015

1.1.1. Tuberculosis in Tanzania

Tanzania is among the 30 high TB burden countries as categorized by WHO in the sub-categories of high TB and TB/HIV burden as shown in Figure 2 (WHO, 2016). The prevalence survey that was done in 2013, estimates the prevalence of TB to be around 295 per 100,000 population (NTLP, 2013). The total notification of all forms of TB cases in 2014 was 63,151 (NTLP, MoHSW, 2015). About 25% of these 63,151 notified TB patients came from Dar es Salaam, a densely-populated city. Tanzania is one of the many countries in sub-Saharan Africa that has seen a six-fold increase in number of TB patients since early 1980's (Egwaga, 2003). The remarkable rise and sustained TB burden in Tanzania is attributed to the concurrent HIV epidemic (Chum et al., 1996; Egwaga, 2003).

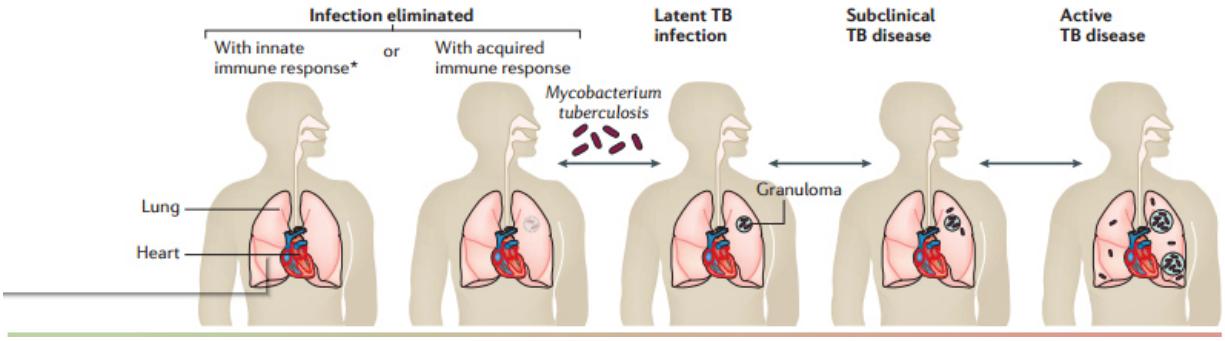


(Source: Global tuberculosis report 2016)

Figure 2. List of countries with high TB burden that will be used by WHO during the period 2016–2020, and their areas of overlap.

1.1.2. TB spectrum from tuberculous infection to TB disease

Approximately 2-3 billion people in the world are estimated to be infected with *M. tuberculosis* (WHO, 2016). Tuberculous infection can either be eliminated from the host by the host innate or persists to attain latency lifelong or later on develop TB (Pai et al., 2016; Yates et al., 2016). In response to infection, the host immunity forms a granuloma that limits the mycobacterial growth in the lungs. TB develops when an individual acquires or develops a TB related risk factors such as HIV, helminth infection and diabetes mellitus (Marais et al., 2013; Pai et al., 2016). These co-infections impair TB specific immunity rendering the body unable to contain the bacteria within the granuloma, and thus causing TB disease (Pai et al., 2016). Figure 3 illustrates the spectrum of TB from tuberculous infection to TB (Pai et al., 2016).



(Source: Tuberculosis, Pai et al., 2016)

Figure 3. The spectrum of TB – from *M. tuberculosis* infection to active (pulmonary) TB diseases.

1.1.3. Diagnosis and management of tuberculosis

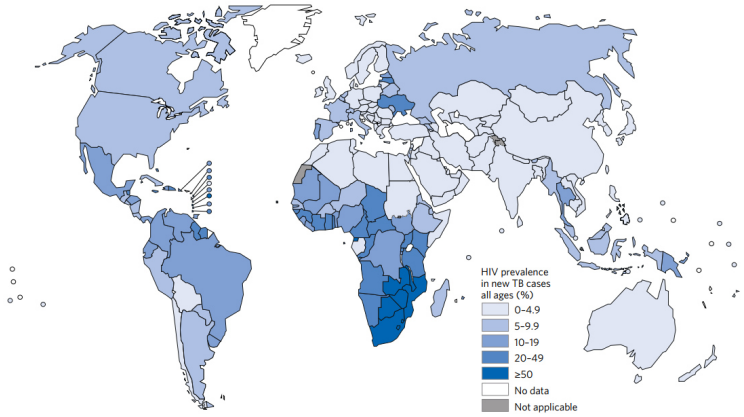
Early identification of infectious TB patients and appropriate treatment are the mainstay of TB control. Smear microscopy, which has low sensitivity, is the most common TB diagnostic tool at a primary health care level in resource limited settings (Pai et al., 2012). Gene Xpert MTB/RIF, a molecular TB diagnostic tool which is more sensitive than smear microscopy, has improved TB diagnosis and detection of rifampicin resistant TB especially in high HIV burden setting (Steingart et al., 2014).

Directly Observed Treatment Short course (DOTS) strategy recommended by the World Health Organization in 1994 (De Cock and Chaisson, 1999; Van Deun and Rieder, 2012; WHO, 2006), has proved to be one of the most effective public health interventions (Van Deun and Rieder, 2012). TB treatment using Directly Observed Therapy (DOT) TB is the mainstay of TB treatment which is based on the standardized regimen by WHO. Tanzania has adopted these guidelines and treats susceptible new TB patients for six months and eight months for the retreatment patients (NTLP, MoHSW, 2013).

1.2. Tuberculosis and human immunodeficiency virus

1.2.1. Global epidemiology of TB and human Immunodeficiency virus

HIV remains the most important known risk factor for developing TB from tuberculous infection (Lönnroth et al., 2009; Pai et al., 2016; WHO, 2016). HIV/AIDS infected individuals have 5-15% increased risk of developing TB in a year as compared to 5-15% in lifetime risk in immunocompetent individuals (Antonucci et al., 1995). The hallmark of HIV infection is the depletion of CD4+ T cells which are essential for control of *M. tuberculosis* infection in the human body (Pawlowski et al., 2012). Globally in the year 2015, TB/HIV co-infection is estimated to be around 11%, translating to about 1.2 million new TB patients (WHO, 2016). WHO Africa region had 31% of the total burden of TB/HIV co-infection. The majority of the countries in the sub-Saharan Africa have TB/HIV co-infection prevalence of ≥20% as shown in Figure 4 (WHO, 2016).



(Source: Global tuberculosis report 2016)

Figure 4: Estimated HIV prevalence in new TB cases in 2015.

1.2.2. TB and HIV in Tanzania

Tanzania is among the 30 high TB burden countries for the sub-categories of TB and TB/HIV (WHO, 2016). The rising and sustained TB epidemic since early 1980s is due to the concurrent HIV epidemic (Chum et al., 1996; Egwaga, 2003). TB caused a fourfold increase in mortality among HIV-positive patients (Kabali et al., 2013). HIV patients with low body mass index (BMI) are at increased risk of dying from TB (Maro et al., 2010).

Tanzania implements the collaborative TB/HIV services which addresses the burden of TB and HIV for patients affected by both diseases (NTLP, MoHSW, 2013). The burden of TB and HIV co-infection is slightly above the WHO Afro region TB/HIV co-infection rate. For instance, from the annual TB report of 2014, 63,151 TB cases were notified, of which 55,686 (88%) were counseled and tested for HIV status, and 19,890 (36%) were co-infected with HIV (NTLP, MoHSW, 2015). Thus, still yet HIV drives the TB epidemic in Tanzania.

1.2.3. The clinical effects of HIV on TB patients

HIV has significantly changed the clinical phenotypes and negatively influences the TB treatment outcomes favouring poor treatment outcomes (Fenner et al., 2012; Mhimbira et al., 2016; Sharma et al., 2004). In both HIV-negative and early HIV infection, TB predominantly presents with productive cough with upper lobe involvement, cavitation and smear-positive AFB results from smear microscopy (Sharma et al., 2004). On the other hand for severely immunosuppressed TB and HIV co-infected patients, they present with non-severe cough, extrapulmonary TB, atypical radiological presentation with lobar infiltration and severe disease (Sharma et al., 2004).

High TB mortality is observed in many TB patients who are also co-infected with HIV (Waitt and Squire, 2011). In 2015, there were a total of 0.4 million deaths resulting from TB diseases among people living with HIV (WHO, 2016). Severely immunosuppressed HIV-positive patients are likely to have life threatening infections (Pawlowski et al., 2012) such as *Pneumocystis jirovecii* pneumonia (PCP) (Field et al., 2014), develop Immune Reconstitution Inflammatory Syndrome (IRIS), which is a consequence of the immune recovery after initiation of ART among TB patients, that can result to death (Cohen and Meintjes, 2010; Lawn et al., 2005; Leone et al., 2010; Naidoo et al., 2012).

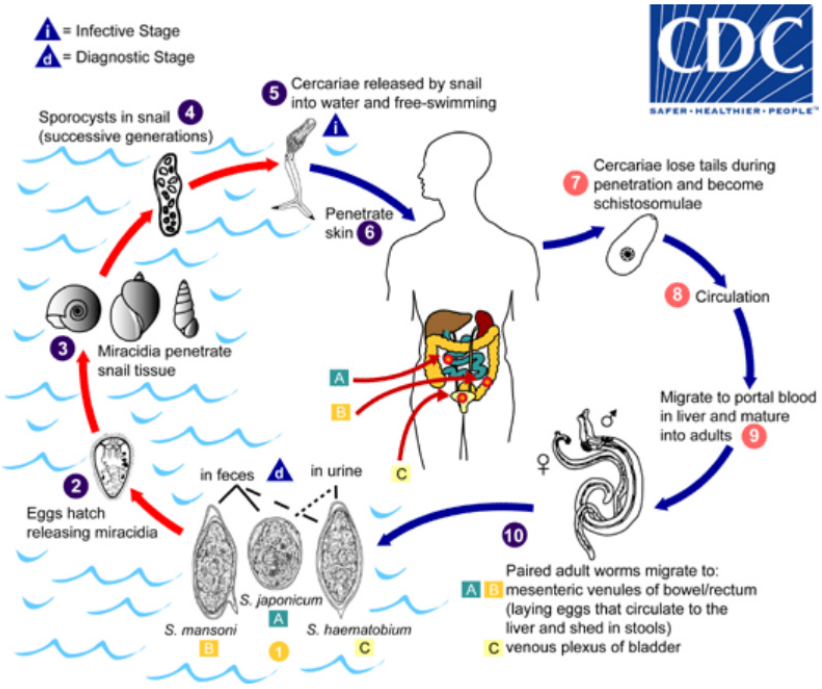
1.3. Helminth infection

1.3.1. Global epidemiology of helminth

Helminth infections are neglected tropical diseases (NTD) affecting both pediatric and adults in developing countries (McCarty et al., 2014). Helminth infections by soil-transmitted helminths, schistosomiasis, filariasis and/or food-borne trematode are common and affect over one billion people (Knopp et al., 2012; Pullan et al., 2014; Utzinger et al., 2012). Globally, the most prevalent helminth species are *Ascaris lumbricoides* followed by *Trichuris trichiura, Necator americanus and Strongyloides stercoralis* (Bethony et al., 2006; McCarty et al., 2014). Soil transmitted helminth (STH) (*A. lumbricoides*, *T. trichiura* and hookworms) are known to cause significant physical and intellectual growth retardation especially in children, but are largely neglected in resources to control them (Bethony et al., 2006). An estimated 819 million

people in the world are infected by *A. lumbricoides*, 465 million by *T. trichiura*, 439 million by hookworm and 252 million by *Schistosoma mansoni* (Babu and Nutman, 2016).

Poor hygiene and sanitation practices sustain the high transmission of helminth in high burden settings (Bethony et al., 2006). For instance, the life cycle of Schistosoma species shown in Figure 5, shows source of infection is from contaminated water by feaces and urine from an infected person.



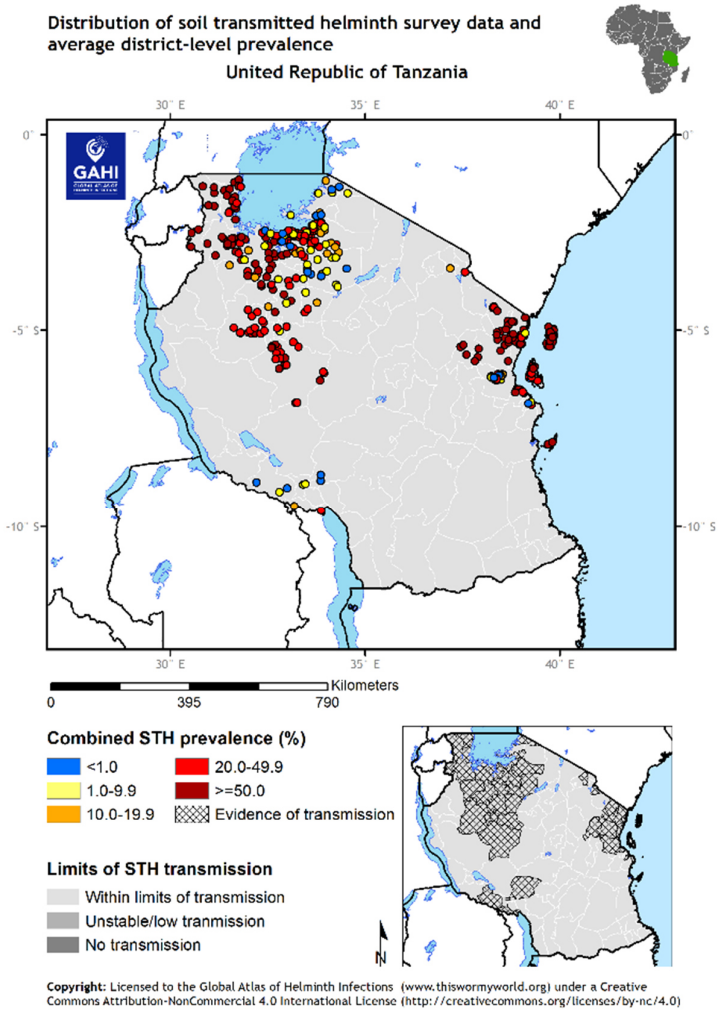
(Source: https://www.cdc.gov/parasites/schistosomiasis.html): accessed 04.01.2017)

Figure 5. life cycle of *S. haematobium, S. japonicum*, and *S. mansoni* species

Eggs are eliminated with feces or urine 1. Under optimal conditions the eggs hatch and release miracidia 2, which swim and penetrate specific snail intermediate hosts 3. The stages in the snail include 2 generations of sporocysts 4 and the production of cercariae 5. Upon release from the snail, the infective cercariae swim, penetrate the skin of the human host 6, and shed their forked tail, becoming schistosomulae 7. The schistosomulae migrate through several tissues and stages to their residence in the veins (8, 9). Adult worms in humans reside in the mesenteric venules in various locations, which at times seem to be specific for each species 10. For instance, *S. japonicum* is more frequently found in the superior mesenteric veins draining the small intestine A, and *S. mansoni* occurs more often in the superior mesenteric veins draining the large intestine B. However, both species can occupy either location, and they are capable of moving between sites, so it is not possible to state unequivocally that one species only occurs in one location. *S. haematobium* most often occurs in the venous plexus of bladder C, but it can also be found in the rectal venules. The females (size 7 to 20 mm; males slightly smaller) deposit eggs in the small venules of the portal and perivesical systems. The eggs are moved progressively toward the lumen of the intestine (*S. mansoni* and *S. japonicum*) and of the bladder and ureters (*S. haematobium*), and are eliminated with feces or urine, respectively 1.

1.3.2. Epidemiology of helminth infection in Tanzania

Tanzania is a high NTD burden setting. The prevalence of STH is estimated to be between 57-85% (Bundy et al., 2000). The burden of helminth infection depends on the area and the species (Brooker et al., 2000). For instance, prevalence of *S. mansoni* was 54% among women of reproductive age near Lake Victoria. Figure 6 shows prevalence variation of STH in Tanzania in the survey areas.



(Source: http://www.thiswormyworld.org/maps/distribution-of-soil-transmitted-helminth-survey-data-in-tanzania, accessed on 04.01.2017)

Figure 6. distribution of soil transmitted helminth survey data and average district-level prevalence.

1.3.3. The helminth-induced immune regulation on *M. tuberculosis*

The burden of TB and helminth infection overlap geographically. The burden of TB and helminth is high in developing countries affecting the communities with poor socio-economic status (Simon, 2016; WHO, 2015a). The distribution of TB and helminth co-infection globally is shown Figure 7. To note, Africa has overall higher prevalence of co-infections of TB, HIV, malaria and helminth as compared to the rest of the world (Salgame et al., 2013).

(Source: Salgame et al, 2013)

Figure 7. World map showing the geographic distribution of coinfection together with tuberculosis, malaria and/or HIV infection of adults.

Evidence from experimental mice models shows that immune dysregulations caused by helminth infections can negatively affect the prognosis of HIV, TB and malaria (Salgame et al., 2013; Simon, 2016). In TB, the immune response to helminth infections is characterized by the induction of $CD4^+$ T-helper 2 (Th2) that down-regulates $CD4^+$ T-helper 1 (Th1) cells (Babu and Nutman, 2016; Mishra et al., 2014; Monin et al., 2015; Salgame et al., 2013). Figure 8 summarizes the immunological pathways induced by helminth that immunomodulate the responses to TB (Babu and Nutman, 2016).

The clinical significance of helminth infection immunomodulation include increasing the human body susceptibility to infection, developing TB and possibly causing a protracted clinical course of the TB disease (Borkow and Bentwich, 2000; Resende Co et al., 2006). Helminth infection also reduces the protective effect of TB vaccines such as Bacillus Calmette–Guérin (BCG) (Elias et al., 2005). Therefore, where TB and helminthiases co-exist, helminth infections could potentially be a risk factor that sustain the TB epidemic.

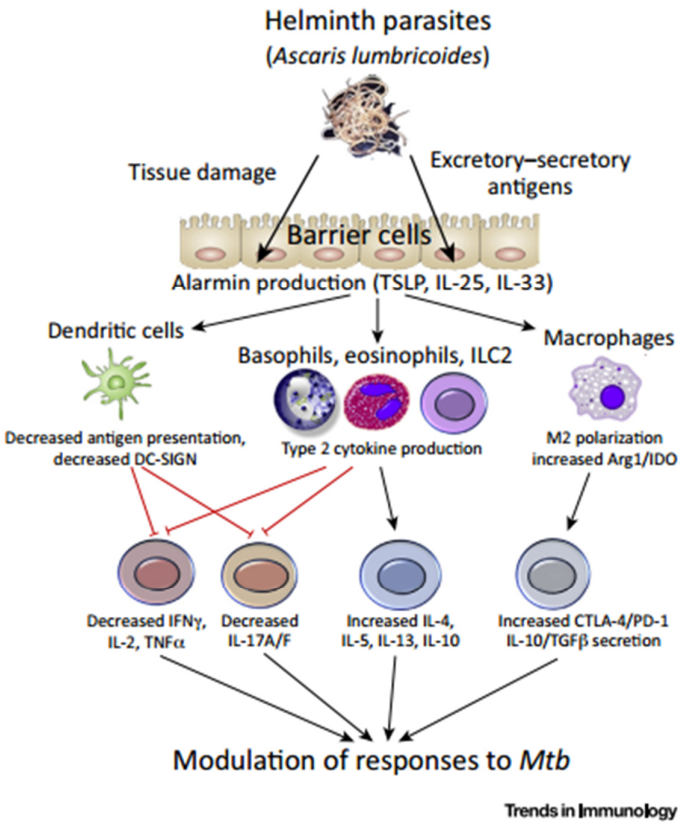


Figure 2. Modulation of Immune Response to Tuberculosis Infection by Helminths Based on Murine Models. Helminth parasites (for example, *Ascaris lumbricoides*) induce the release of alarmin cytokines, including thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 from barrier cells including epithelial cells. These cytokines then activate a variety of secondary cell types. In addition, helminth parasites or their excretory–secretory products can directly interact with dendritic cells, macrophages, basophils, and eosinophils to induce activation and initiation of the type 2 immune response. This subsequently modulates the T cell response to effect influence on *Mycobacterium tuberculosis* (Mtb) infection and disease through a variety of mechanisms described. Abbreviations: arg-1, arginase-1; CTLA-4, cytotoxic T lymphocyte associated protein-4; DC-SIGN, dendritic cell specific intracellular adhesion molecule-3 grabbing non-integrin; IDO, indoleamine 2,3 dioxygenase; IFN, interferon; IL, interleukin; ILC-2, type 2 innate lymphoid cells; PD-1, programmed cell death protein -1; TGF, transforming growth factor, TNF, tumor necrosis factor; TSLP, thymic stromal lymphopoietin.

(Source: Babu * Nutman 2016)

Figure 8. Modulation of immune response to TB infection by helminths based on murine models.

1.3.4. Control of intestinal helminth infection

The global health policies have been produced to address the challenges of NTD control. One of the global health policies is the 2012 London declaration on NTD which has several commitments which includes: i) to expand and extend programmes that ensures supply of drugs, ii) advance research, and iii) development, strengthen national and international collaborations and adequate funding ("The London Declaration | Uniting to Combat NTDs," n.d.). Chemotherapy through mass drug administration (MDA) is cost effective and is recommended in endemic area to reduce morbidity associated with helminth infection (Bethony et al., 2006; McCarty et al., 2014). The frequency of administration of MDA is dependent on the burden of helminth infection (McCarty et al., 2014). In addition, improving supply of clean and safe water, and strengthening sanitation and hygiene practices, can help to reduce the burden of helminth infection in high burden settings (Dangour et al., 2013).

1.4. Respiratory viral pathogens

1.4.1. Global epidemiology and impact of respiratory viral pathogens

Respiratory viral pathogens are of increasing public health importance especially the human influenza viruses. Respiratory virus pathogens cause varying degrees of morbidity and mortality in different settings depending on the level of co-infections. In the pediatric population from the multicounty study involving 17 centers in southern hemisphere, showed the most prevalent viruses to be Rhinovirus (RSV)/enterovirus (41.5%), followed by influenza (15.8%), adenovirus (9.8%), parainfluenza and respiratory syncytial virus (RSV) (both 9.7%), coronavirus (5.6%), human metapneumovirus (5.5%) and human bocavirus (HBov) (2.0%) (Taylor et al., 2017). In sub-Saharan Africa, influenza virus is responsible in causing 1-25% of outpatient acute respiratory illness (Gessner et al., 2011). In East Africa, RSV was detected from 12.5% nasopharyngeal swabs from children and adults from rural sites and 11.7% form urban site (Bigogo et al., 2013). The population survey reported detecting influenza virus 10.4% of the specimens, and showed seasonality between September to November and March to June (Wabwire-Mangen et al., 2016).

Evidence from industrialized countries show, viral respiratory pathogens may cause acute exacerbation of Chronic Obstructive Pulmonary Diseases (COPD) leading to hospitalization (O'Brien et al., 2000; Rohde et al., 2003). Also, respiratory viral infections are important preceding infection prior to bacterial pneumonia among adults and children and especially those with chronic underlying conditions (Glezen et al., 2000). The most notable effect of respiratory viruses in humans is, the influenza virus in particular, tend to selectively cause higher mortality among TB patients (Noymer, 2009; Walaza et al., 2015; Zürcher et al., 2016).

1.4.2. The immunological interaction between respiratory viral pathogens and TB

The immunological pathways which respiratory viruses, especially influenza A virus, affect the human host and increase TB progression from tuberculous infection is still unclear. However, two immunological mechanism have been proposed. First, experimental mouse models suggest that prior infection with Influenza A virus, enhance type I Interferon (IFN) signaling pathway which in turn enhances mycobacterial growth (Redford et al., 2013). Second, Influenza A virus decrease the Major Histocompatibility Complex II (MHC II) expression on dendritic cells, resulting to reduced activation of BCG-specific CD4 and CD8 cells, and impaired clearance of mycobacteria (Flórido et al., 2013). These two mechanisms both increase mycobacterial growth, suggesting TB patients co-infected with respiratory viral pathogens may have different clinical phenotypes compared to TB only patients.

1.4.3. Tuberculosis and respiratory bacteria pathogens

The burden and relationship between TB and respiratory bacteria are still not known. However, TB patients and controls with no TB, appear to have differences in composition and diversity of sputum microbiota (Cheung et al., 2013). Also new, recurrent and treatment failure TB patient categories seem to have different lung microbiota (Wu et al., 2013). The findings from these two studies may suggest the pathogenic role of respiratory bacteria on TB pathogenesis. However, the actual immunological mechanism underpinning that assumption and clinical significance of respiratory pathogens on TB are still unknown.

1.5. Global TB control strategy

The End TB Strategy set the global strategy and targets for TB prevention, care and control after 2015 (WHO, 2015b) The End TB Strategy has put up ambitious targets to reduce TB incidence by 90% (<10 TB cases per 100,000 population) and TB deaths by 95% by the year 2035. The pillars and components of the strategy are: i) integrated, patient-centred care and prevention, ii) bold policies and supportive systems and iii) intensified research and innovation (WHO, 2015b).

The targets of End TB Strategy can only be attained if the annual decline of TB incidence is accelerated from 2% per year in 2015 to 10% per year in 2015 (WHO, 2015b). To achieve such a decline; low cost sensitive screening test, efficient vaccines and more effective preventive therapy are needed to eradicate TB, coupled with investment in public health to control and reduce the drivers TB such as HIV, diabetes mellitus, smoking and undernutrition (Dheda et al., 2016).

2. Rationale and research questions

TB remains a disease of public health concern in the world. TB affects communities that have low socio-economic status, with underfunded health systems and have high burden of risk factors for developing TB. Tanzania is among the 30 high burden countries with a significant overlap of high burden of HIV and helminth infections. HIV has increased and sustained the TB burden in Tanzania since early 1980s. TB mortality is also high among TB patients co-infected with HIV. The high burden of helminth and possible association as a risk factor for TB, warrants understanding of the co-infection epidemiology and the clinical significance of helminth infection. The burden or respiratory pathogen is unknown and what clinically significant effects do they have on the TB epidemiology.

The epidemiological evidence of the association between TB and helminth and respiratory pathogens will pave the way to designing interventions to address the burden of TB and co-infections. The opportunities of programme integration and resource mobilization to fight the burden of TB and NTD are justifiable once we understand their interaction. The knowledge gained may influence policy change and clinical practice in the clinical care of TB patients and those with helminth infection.

This PhD thesis contributes to the knowledge on ways to achieve End TB Strategy targets. First, identifying possible risk factors for TB may help to halt and reverse the TB incidence. Controlling risk factors for TB, will help to attaining the target of reducing TB incidence by 90% by the year 2015. Second, we need to identify the factors associated with mortality in a programmatic setting to design interventions that will help to reduce TB mortality and attain the End TB Strategy of decrease the number of TB deaths by 90% by the year 2035.

The following were the research questions:

i. What are the TB treatment outcomes in high TB and HIV settings who are treated under home- or facility-based DOT?
ii. Are there differences in the burden of helminth infections and respiratory pathogens among TB patients and household contact controls with no TB?
iii. Are there differences in clinical phenotypes among TB patients co-infected with helminth infection or respiratory pathogens?

3. Objectives and aims

3.1. General objective

To determine the burden and the association of HIV, helminth and respiratory pathogens co-infections and TB at Temeke district, Dar es Salaam, Tanzania.

3.2. Specific objectives

1. To determine the treatment outcome of TB and HIV co-infected patients routinely diagnosed at Temeke district treated under home- and facility-based DOT.
2. To determine the prevalence of helminth infection and respiratory pathogens among smear positive TB cases and household contact controls without TB.
3. To investigate the risk factors associated for helminth infection and respiratory pathogens among smear-positive TB cases and household contact controls without TB.
4. To determine the clinical effects on helminth infection and respiratory pathogens on clinical phenotypes and clinical outcomes among smear-positive TB patients.

3.3. Specific aims

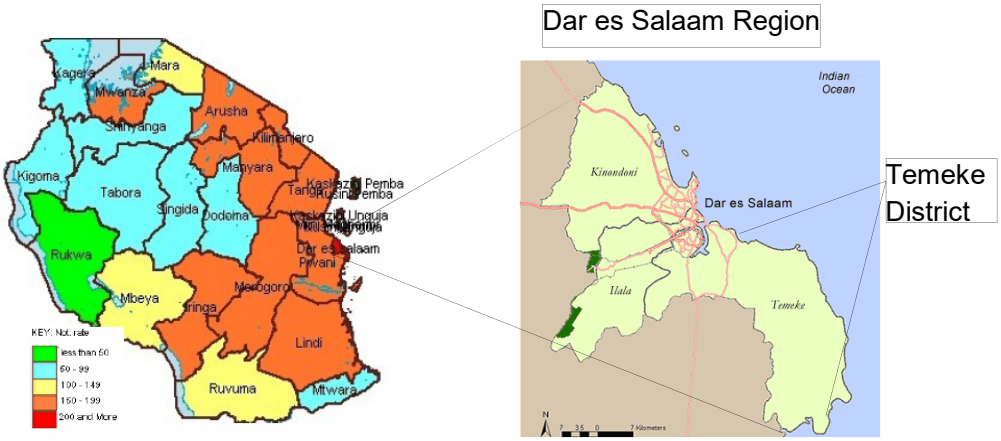
i. To collect clinico-epidemiological data of smear positive TB patients and describe their relationship with HIV, respiratory viral and intestinal helminth co-infection.
ii. To analyze routinely collected data from TB program and assess the TB and HIV co-infection and treatment outcome.
iii. To evaluate the effects of respiratory viral and intestinal helminth co-infection on the clinical phenotypes and TB treatment outcome of smear positive TB patients.

4. Methods

4.1. Study setting

The study was conducted at Temeke district, Dar es Salaam region in Tanzania. Temeke is the largest district of the three districts of Dar es Salaam region, occupying 48.3% of the total surface area, (Figure 9). Temeke has a total population of 1,368,881 (National Bureau of Statistics. Ministry of Finance, Dar es Salaam, 2013; PMORALG, 2014). Temeke district notified 4,373 TB patients in 2014 (NTLP, MoHSW, 2015) and has HIV prevalence of 5.2% in the general adult population (PMORALG, 2014). We further selected the two sub-districts of Wailes I and Wailes II, as categorized by National TB and Leprosy Programme, which are densely populated areas surrounding the Temeke district hospital. Temeke district hospital has the largest TB clinic serving the two sub-districts of Wailes I and II.

This PhD project is nested within a larger TB cohort study entitled; Tuberculosis Cohort Study in the Dar es Salaam region (TB-DAR): a prospective collection of clinical data and biological specimens to study the epidemiology of tuberculosis, including molecular epidemiology and the evaluation of new diagnostics and biomarkers). TB-DAR study is done within the IHI research platform in collaboration with Swiss TPH of Switzerland.



(Source: National Tuberculosis and Leprosy Programme Annual Report 2012)

Figure 9. Map of Tanzania with TB notification rate per 100,000 population notified in 2011.

4.2. Study population

Objective 1: We obtained anonymized electronic data from the TB district register of all adult TB patients (aged ≥15 years) who were notified between 2010 and 2013 in a single geographical area of two TB sub-districts (Wailes I and Wailes II) in the Temeke district, Dar es Salaam, Tanzania.

Objective 2-4: We consecutively enrolled study participants starting in November 2013 until October 2015 to reach the required sample size. Over this period, we included adult TB patients (≥18 years of age and sputum-smear positive) and household contact controls. Any individual living in the same household as the index TB patients enrolled in the study is referred

to as a household contact control. Controls at recruitment were free of symptoms and signs suggestive of TB, healthy on physical examination, and had a negative GeneXpert MTB/RIF result (Cepheid; California, USA). Table 1 summarizes the inclusion and exclusion criteria of the study population for objective 3-4.

Table 1. Inclusion and exclusion criteria

Study group	Inclusion criteria	Exclusion criteria
Pulmonary TB cases	Smear-positive TB patient by AFB smear microscopy and confirmed by LJ or Gene Xpert MTB/RIF positive patient Age above 18 years	Inability to give consent. Less than 18 years of age.
Contact controls	Age above 18 years. No symptoms and signs of TB at recruitment. Age and sex matched to an index case.	TB confirmed by Xpert MTB Rif positive. Age less than 18 years of age. History of TB in the last two years.

4.3. Study design

Objective 1: A retrospective cohort study of routinely collected data by NTLP and National AIDS Control Programme (NACP) at Temeke hospital TB/HIV co-infection and treatment outcomes.

Objective 2-4: Mixed study design was used whereby case-control design was used to assess the association between TB and co-infections. A prospective cohort study was used to assess the clinical effects of helminth and respiratory viruses on clinical outcome of TB patients followed for 6 months of TB treatment.

4.4. Sample size

Case control study: The sample size was calculated based on the assumptions from previous publications of TB helminth co-infection rate (Elias et al., 2006). The estimated sample size using the “sampsi” command in Stata (significance level of test 0.05, two-sided, and a power of 80% to detect relevant differences) 332 for pulmonary TB cases and 332 household contact controls.

Longitudinal study: Assuming the co-infection rate of HIV, intestinal helminth infection and respiratory viral pathogens of 50% among TB patients and assuming treatment failure of patient with co-infection of 20% and 10% for TB only; the calculated sample size using the alpha=0.05 to detect 80% power, then the sample size needed was 424. Considering the 20% loss to follow-up, the sample size needed was 500 TB patients.

4.5. Laboratory samples and procedures:

Study participants’ samples were analyzed at Bagamoyo and Research Training Center (BRTC) laboratory of IHI. BRTC has TB biosafety laboratory level 2 & 3, hematology and

helminth laboratory. Table 2 below summarizes the specimens collected from the study participants and the analysis done. The detailed laboratory procedures are covered in the respective chapters of this thesis.

Table 2: Specimen type and tests

Sample	Laboratory tests
Sputum	Confirmation of *M. tuberculosis* on the Löwenstein-Jensen medium Gene Xpert MTB/RIF (Controls)
Nasopharyngeal swabs	Allplex™ Respiratory Full Panel assay (respiratory bacterial pathogens) and PCR Anyplex™II RV16[1] Detection (respiratory viral pathogens).
Stool	Kato-katz and Baermann methods for detection of helminth infections.
Urine	Circulating Cathodic Antigen for detection of *S. mansoni*. Urine filtration for detection of *S. haematobium.*

4.6. Statistical analysis

Objective 1: Descriptive statistics were used for patient characteristics, and groups were compared using Chi-Square and Wilcox rank-sum test as appropriate. We estimated treatment outcomes risk ratios (RR) comparing patients under home-based with patients under health facility-based DOT. We used logistic regression models to assess the association of home-based DOT on mortality and treatment success.

Objective 2-4: We used descriptive statistics to compare study participants' characteristics. The prevalence of helminth infections and respiratory pathogens were determined using generalized estimations equation adjusting for clustering at the household level. Multilevel mixed-effects logistic regression with random intercepts at the level of household models were used to assess the risk factors for helminth infection and respiratory pathogens. Logistic regression models were used to determine the association between TB and helminth infection or respiratory pathogens. All analyses were performed using Stata version 14.0 (Stata Corp; Texas, USA). For TB and helminth co-infection we produced maps describing the prevalence of helminth by wards (administrative units) using software package ArcGIS Desktop version 10.2 (Esri; California, USA).

[1] http://www.seegene.com/en/any/RV16_010.php

5. Home-based and facility-based Directly Observed Therapy of tuberculosis treatment under programmatic conditions in urban Tanzania

Short title: Home-based and facility-based DOT in tuberculosis control

Authors: Francis Mhimbira[1,2,3*], Jerry Hella[1,2,3], Thomas Maroa[1], Shadrack Kisandu[1], Magreth Chiryamkubi[4], Khadija Said[1,2,3], Grace Mhalu[1,2,3], Abdallah Mkopi[1], Beatrice Mutayoba[4], Klaus Reither[1,2,3], Sébastien Gagneux[2,3], Lukas Fenner [1,2,3,5]

Affiliations:
[1] Ifakara Health Institute, Dar es Salaam, Tanzania
[2] Swiss Tropical and Public Health Institute, Basel, Switzerland
[3] University of Basel, Basel, Switzerland
[4] National TB and Leprosy Programme, Ministry of Health, Community Development, Gender, Elderly and Children, Dar es Salaam, Tanzania
[5] Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland

*** Corresponding authors**
E-mail: fmhimbira@ihi.or.tz (FM)

Competing interest
None of the authors have any competing interests to declare.

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5.1. Abstract

Introduction: Decentralization of Directly Observed Treatment (DOT) for tuberculosis (TB) to the community (home-based DOT) has improved the coverage of TB treatment and reduced the burden to the health care facilities (facility-based DOT). We aimed to compare TB treatment outcomes in home-based and facility-based DOT under programmatic conditions in an urban setting with a high TB burden.

Methodology: A retrospective analysis of a cohort of adult TB patients (≥15 years) routinely notified between 2010 and 2013 in two representative TB sub-districts in the Temeke district, Dar es Salaam, Tanzania. We assessed differences in treatment outcomes by calculating Risk Ratios (RRs). We used logistic regression to assess the association between DOT and treatment outcomes.

Results: Data of 4,835 adult TB patients were analyzed, with a median age of 35 years, 2,943 (60.9%) were men and TB/HIV co-infection prevalence of 39.9%. A total of 3,593 (74.3%) patients were treated under home-based DOT. Patients on home-based DOT were more likely to die compared to patients on facility-based DOT (RR 2.04, 95% Confidence Interval [95% CI]: 1.52-2.73), and more likely to complete TB treatment (RR 1.14, 95% CI: 1.06-1.23), but less likely to have a successful treatment outcome (RR 0.94, 95% CI: 0.92-0.97). Home-based DOT was preferred by women (adjusted Odds Ratio [aOR] 1.55, 95% CI: 1.34-1.80, p<0.001), older people (aOR 1.01 for each year increase, 95% CI: 1.00-1.02, p=0.001) and patients with extra-pulmonary TB (aOR 1.45, 95% CI: 1.16-1.81, p=0.001), but less frequently by patients on a retreatment regimen (aOR 0.12, 95% CI: 0.08-0.19, p<0.001).

Conclusions/significance: TB patients under home-based DOT had more frequently risk factors of death such as older age, HIV infection and sputum smear-negative TB, and had higher mortality compared to patients under facility-based DOT. Further operational research is needed to monitor the implementation of DOT under programmatic conditions.

5.2. Introduction

In 2014 globally, almost 1.5 million people died from tuberculosis (TB) from an estimated 9.6 million who developed TB (WHO, 2015a). TB is now the leading cause of death from an infectious disease worldwide, surpassing those caused by Human Immunodefiency Virus (WHO, 2015b). Globally, TB mortality trends are on the decline, but remain high despite effective short-course treatment regimens (WHO, 2015a). In Africa, however, the decline did not meet the 2015 Stop TB Partnership goal of a 50% decline from 1990 to 2015 (WHO, 2015a).

Early diagnosis and effective treatment of TB are critical to reduce TB mortality and control the spread of TB [1]. The Directly Observed Treatment Short course (DOTS) strategy recommended by the World Health Organization in 1994 (De Cock and Chaisson, 1999; Van Deun and Rieder, 2012; WHO, 2006), has proved to be one of the most effective public health interventions (Van Deun and Rieder, 2012). The DOTS strategy provides a comprehensive package to control TB which consists of six components, and one of them addresses the use of standardized treatment with supervision and patients supporters (directly observed therapy, DOT) (WHO, 2006). DOT was pioneered in Tanzania in the 1980s, and resulted in improved cure rates from 60% to 80% (Van Deun and Rieder, 2012). TB treatment under DOT can be given at the health facility (facility-based DOT) or in the community (home-based DOT). The facility-based DOT approach requires that patients visit daily the health facility for supervised drug intake by health workers, with continuous assessment of adherence to TB medication (NTLP, MoHSW, 2013). However, this delivery system places a burden on the health care system and the patient (Maher, 2003; Wandwalo et al., 2006). This made it necessary to decentralize TB treatment to the community (home-based DOT) (Lwilla et al., 2003; Maher, 2003).

Although systematic reviews showed that patients under home-based DOT can achieve similar or better treatment outcomes compared to facility-based DOT (Volmink and Garner, 2003; Wright et al., 2015), the implementation of treatment under home-based DOT has also raised concerns (Frieden and Sbarbaro, 2007). Health care workers have expressed concerns about treatment adherence, storage of drugs and lack of supervision under home-based DOT which may contribute to unfavorable TB treatment outcomes (Egwaga et al., 2008). We therefore aimed to assess TB treatment outcomes in home-based and facility-based DOT under programmatic conditions in the high TB incidence country Tanzania.

5.3. Methods

5.3.1. Ethics statement

We used anonymized population-level data of notified TB patients. Hence, ethical approval and informed consent were not required for this analysis. The permission to use the data was authorized by the Program Manager of the National TB and Leprosy Programme (NTLP) in the Ministry of Health, Community Development, Gender, Elderly and Children (MoHCDGEC). All TB patients received the standardized TB treatment regimen in line with national TB treatment guideline (NTLP, MoHSW, 2013). HIV-positive TB patients were managed in accordance to the National Guidelines for the Management of HIV in Tanzania (NACP, 2015).

5.3.2. Study setting

Temeke is one of the three districts in Dar es Salaam region in Tanzania. Temeke is the largest district that occupies 48.3% of the total surface area of Dar es Salaam and has a total population of 1,368,881 (National Bureau of Statistics. Ministry of Finance, Dar es Salaam, 2013; PMORALG, 2014). Temeke district notified 4,373 TB patients in 2014 (NTLP, MoHSW, 2015) and has HIV prevalence of 5.2% in the general adult population (PMORALG, 2014). We selected Temeke district because it represents an urban setting with a high TB notification rate, and because it was one of the few districts in Tanzania where the acceptability study of home-based and facility-based DOT was done in 2006 (Wandwalo et al., 2006). The two sub-districts of Wailes I and Wailes II, as categorized by National TB and Leprosy Programme, are densely populated areas surrounding the Temeke district hospital which has the largest TB clinic serving these two sub-districts.

5.3.3. Study population and study definitions

We obtained anonymized electronic data from the TB district register of all adult TB patients (aged ≥15 years) who were notified between 2010 and 2013 in a single geographical area of two TB sub-districts (Wailes I and Wailes II) in the Temeke district, Dar es Salaam, Tanzania. The patient selection is presented in Figure 10. We excluded 31 patients with unknown DOT preference and 363 patients with treatment outcome “not evaluated” (“unknown” or “transferred out”). The included and excluded (patients with unknown DOT and “not evaluated” outcome) patients did not differ in baseline characteristics (Supplementary Table 1).

We used the study definitions according to the WHO updated guidelines (World Health Organization (WHO), 2013). A “new” patient was defined as never had treatment for TB, or has taken anti-TB drugs for less than one month; a “retreatment” patient was defined as previously treated patient who has received one month or more of anti-TB drugs in the past; TB death as any death that occurred during TB treatment. “Treatment success” combines cured and treatment completed outcomes (World Health Organization (WHO), 2013). “Unsuccessful” treatment combines failed treatment, loss to follow-up and died outcomes. Not evaluated combines the transferred-out and unknown treatment outcomes.

5.3.4. Facility-based and home-based DOT

According to the national guidelines, facility-based DOT requires health care workers to supervise daily intake of anti-TB medication during the intensive and continuation phases (NTLP, MoHSW, 2013). While at the clinic, TB patients will also receive health education and clinical evaluation by the clinicians and nurses. Under home-based DOT, a patient takes medication at home and is supervised by a treatment supporter who is chosen by the TB

patient (NTLP, MoHSW, 2013). The treatment supporter, who can be either a family member or a neighbor, supervises anti-TB medication intake at home on daily basis during the entire TB treatment phase (Egwaga et al., 2008; NTLP, MoHSW, 2013). Treatment supporters would also accompany the TB patients to the health facility for anti-TB medication refill weekly during the intensive phase and fortnightly during the intensive phase. TB patients with their treatment supporters while at the health facility will receive health education from health care workers; and TB patients will undergo a clinical evaluation (NTLP, MoHSW, 2013). Under the current Patient-Centered Treatment (PCT) approach, the patient can indicate the choice of DOT at the time of TB diagnosis (hereafter referred as “preference of DOT”).

5.3.5. Laboratory investigations

TB diagnosis was made either by microbiological confirmation of acid fast bacilli (AFB) in the sputum smear examination by Ziehl-Nielsen method or based on clinical grounds or radiological findings as determined by the attending clinician at the TB clinic. The quality control of smear microscopy was done by the Central Tuberculosis Reference Laboratory (CTRL) in Dar es Salaam. AFB smear positive results was as per World Health Organization/International Union Against Tuberculosis and Lung Disease grading: “scanty” with of 1–9 AFB per 100 oil immersion fields; 1+” with 10–99 AFB per 100 immersion fields; “2+” with 1–10 AFB per 1 immersion field and “3+” with >10 AFB per immersion field (NTLP, MoHSW, 2013; WHO, 2013).Tanzania also implements collaborative TB/HIV services, and all TB patients were offered rapid HIV testing as per National guidelines for the management of HIV and AIDS of Tanzania (NACP, 2015).

5.3.6. Statistical analyses

Descriptive statistics were used for patient characteristics, and groups were compared using Chi-Square and Wilcox rank-sum test as appropriate. We estimated treatment outcomes risk ratios (RR) comparing patients under home-based with patients under health facility-based DOT. We used logistic regression models to estimate the effect of home-based DOT on mortality and treatment success. The results are presented as crude and adjusted odds ratio (OR) after adjusting for age, sex, HIV status, site of disease, AFB sputum smear results at diagnosis, and patient category (new or retreatment patient). Point estimates are presented with their corresponding 95% Confidence Intervals (95% CIs). We also performed a complete case analysis excluding all patients with any missing value. All analyses were performed using Stata version 14.0 (Stata Corp, College Station, TX, USA).

5.4. Results

5.4.1. Patient characteristics

We analyzed 4,835 adult TB patients from the Temeke District, Dar es Salaam, notified between 2010 and 2013. The median age was 35 years (Interquartile Range [IQR]: 27-44 years); 2,943 (60.9%) were men. The HIV prevalence was 39.9 % (95% Confidence Interval [95% CI]: 38.5-41.3%). Home-based DOT was the most preferred (3,593 patients, 74.3%) compared to facility-based DOT. Patients on home-based DOT were more likely to be HIV-positive, women, with EPTB, smear-negative at diagnosis and older (Table 3). TB/HIV co-infected patients were more frequently men, of older age, and patients with EPTB and smear-negative TB, and opted more often for home-based DOT (Supplementary Table 2).

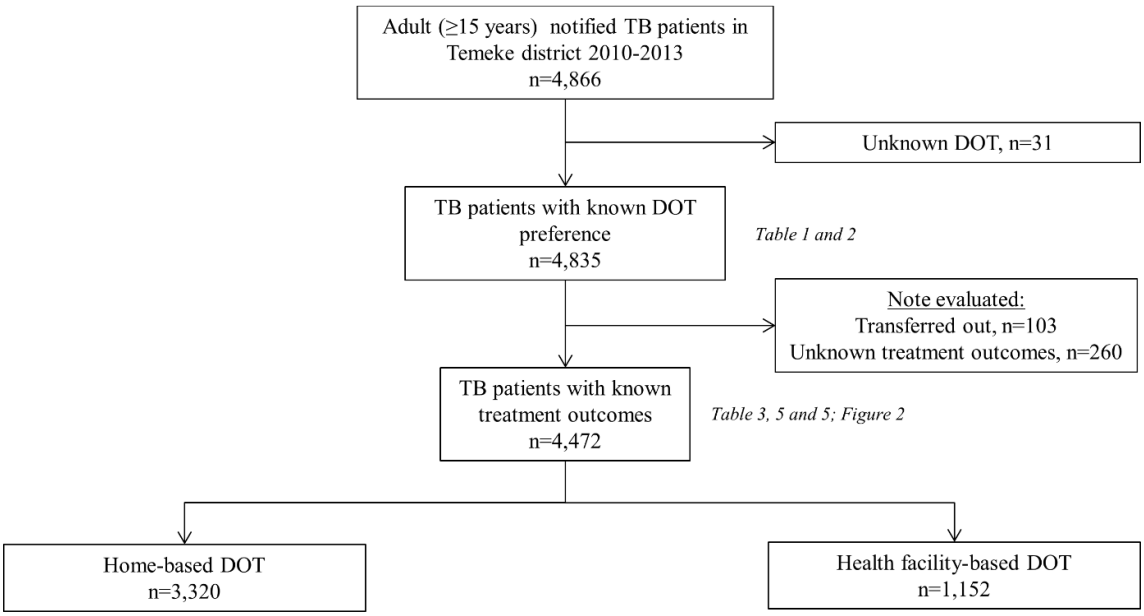

Figure 10. Selection of patients included in the study.

Table 3: Baseline characteristics of TB patients at Temeke district by choice of DOT.

Characteristics	All (n=4,835) n (%)	Home-based (n=3,593) n (%)	Facility-based (n=1,242) n (%)	p-value
Proportion of diagnosis, n (%)	4,835 (100)	3,593 (100)	1,242 (100)	
Sex				<0.001
Male	2,943 (60.9)	2,082 (57.9)	861 (69.3)	
Female	1,892 (39.1)	1,511 (42.1)	381 (30.7)	
Age in years, median (IQR)	35 (27-44)	35 (29-45)	34 (28-41)	0.007[a]
Age groups (years)				<0.001
15-19	285 (5.9)	238 (6.6)	47 (3.8)	
20-24	544 (11.3)	390 (10.9)	154 (12.4)	
25-29	724 (15.0)	521 (14.5)	203 (16.3)	
30-34	820 (17.0)	577 (16.1)	243 (19.6)	
35-39	706 (14.6)	521 (14.5)	185 (14.9)	
40-44	570 (11.8)	405 (11.3)	165 (13.3)	
45-49	428 (8.9)	319 (8.9)	109 (8.8)	
50-54	279 (5.8)	218 (6.1)	61 (4.9)	
≥55	479 (9.9)	404 (11.2)	75 (6.0)	
HIV status				0.002
Positive	1,927 (39.9)	1,485 (41.3)	442 (35.6)	
Negative	2,582 (53.4)	1,874 (52.2)	708 (57.0)	
Unknown status	326 (6.7)	234 (6.5)	92 (7.4)	
Site of disease				<0.001
PTB	3,977 (82.3)	2,884 (80.3)	1,093 (88.0)	
EPTB	858 (17.7)	709 (19.7)	149 (12.0)	
Category				<0.001
New	4,706 (97.3)	3,557 (99.0)	1,149 (92.5)	
Retreatment	129 (2.7)	36 (1.0)	93 (7.5)	
AFB smear result at diagnosis				<0.001
Smear-positive	2,438 (50.4)	1,706 (47.5)	732 (58.9)	
Smear-negative	2,352 (48.6)	1,845 (51.3)	507 (40.8)	
Unknown	47 (0.9)	42 (1.2)	3 (0.2)	

[a]Wilcox-ranksum test; n (%), absolute number and column percentage

Patients with unknown DOT preference were excluded (n=31, 0.6%).

AFB, acid-fast bacilli; IQR, Interquartile Range; HIV, Human Immunodeficieny Virus; TB, Tuberculosis; PTB, Pulmonary tuberculosis; EPTB, Extrapulmonary tuberculosis; DOT, Directly Observed Treatment

5.4.2. TB treatment outcomes by DOT preference

Overall, treatment success which combines cured and treatment completed outcomes, was reported in 4,006 (82.8%) patients and 345 (7.1%) patients died during TB treatment. Patients on home-based compared to facility-based DOT were less likely to have treatment success (RR 0.94, 95% CI: 0.92-0.97). We observed no significant difference in loss to follow-up and "not evaluated" treatment outcomes. Treatment failure was only reported in the home-based DOT (24 patients, 0.7%). When restricting the analysis to smear-positive TB patients, patients on home-based DOT were less likely to be cured compared to facility-based DOT (RR, 0.93 95 CI: 0.88-0.98), and were more likely to die but the results were not significant (RR 1.35, 95% CI: 0.88-2.06). We found no statistically significant differences in all other treatment outcomes (Table 4). Of 31 TB patients with unknown DOT preference, 2 patients died during the TB treatment.

Table 4: Differences in TB treatment outcomes among TB patients under home-based DOT compared to facility-based DOT.

Treatment outcome	All n (%)	Home-based DOT n (%)	Facility-based DOT n (%)	RR (95% CI)	p-value
All patients	4,835	3,593 (100)	1,242 (100)		<0.001
Treatment success	4,006 (82.8)	2,932 (81.6)	1,073 (86.4)	0.94 (0.92-0.97)	
Cured	1,738 (35.9)	1,192 (33.2)	546 (44.0)	0.75 (0.70-0.82)	
Treatment completed	2,268 (46.9)	1,741 (48.5)	527 (42.4)	1.14 (1.06-1.23)	
Died	345 (7.1)	295 (8.2)	50 (4.0)	2.04 (1.52-2.73)	
Loss to follow-up	97 (2.0)	68 (1.9)	29 (2.3)	0.81 (0.53-1.25)	
Treatment failed	24 (0.5)	24 (0.7)	0 (0)	ND	
Not evaluated	363 (7.5)	273 (7.6)	90 (7.2)	1.05 (0.83-1.32)	
Smear-positive	2,438 (100)	1,706 (100)	732 (100)		0.024
Treatment success	2060 (84.5)	1431 (83.9)	629 (85.9)	0.98 (0.94-1.01)	
Cured	1,738 (71.3)	1,192 (69.9)	546 (74.6)	0.93 (0.88-0.99)	
Treatment completed	322 (13.2)	239 (14.0)	83 (11.3)	1.23 (0.98-1.56)	
Died	109 (4.5)	83 (4.9)	26 (3.6)	1.35 (0.88-2.06)	
Loss to follow-up	52 (2.1)	34 (2.0)	18 (2.5)	0.83 (0.48-1.46)	
Treatment failed	14 (0.6)	14 (0.8)	0 (0)	ND	
Not evaluated	203 (8.3)	144 (8.4)	59 (8.1)	1.04(0.78-1.40)	

Patients with unknown DOT preference were excluded (n=31, 0.6%; n (%)=absolute number and column percentage

DOT, Directly Observed Treatment; RR, Risk ratio; 95% CI: 95% Confidence Interval; ND, Not Defined; Not evaluated; Transferred out and unknown TB treatment outcome

5.4.3. TB mortality and associated patient factors

TB mortality was strongly associated with home-based compared to facility-based DOT (adjusted OR [aOR] 2.28, 95% CI: 1.64-3.18, p<0.001). Other patient factors associated with mortality included older age (e.g ≥45 years, aOR 2.05, 95% CI: 1.36-3.08, p<0.002), HIV-positive (aOR 1.68, 95% CI: 1.31-2.15, p<0.001), retreatment category (aOR 3.99, 95% CI: 2.33-6.84, p<0.001) and smear-positive TB (aOR 0.49, 95% CI: 0.38-0.64, p<0.001) (Table 5). A complete case analysis (excluding any missing values) produced similar results. Supplementary Table 3 shows the patient characteristics separately for home-based and facility-based DOT for TB patients who were reported as dead or alive.

Table 5: Risk factors for mortality among TB patients.

Characteristic	Crude OR		Adjusted OR	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Place of DOT		<0.001		<0.001
Facility	1 (Ref)		1(Ref)	
Home	2.15 (1.58-2.92)		2.28 (1.64-3.18)	
Sex		0.2		0.8
Male	1 (Ref)		1 (Ref)	
Female	1.14 (0.92-1.43)		0.97 (0.77-1.23)	
Age groups (years)		<0.001		0.002
<25	1 (Ref)		1 (Ref)	
25-34	1.47 (0.97-2.22)		1.37 (0.90-2.08)	
35-44	2.15 (1.44-3.22)		1.69 (1.11-2.57)	
≥45	2.64 (1.78-3.93)		2.05 (1.36-3.08)	
HIV status		<0.001		<0.001
Negative	1 (Ref)		1 (Ref)	
Positive	2.07 (1.65-2.61)		1.68 (1.31-2.15)	
Unknown HIV status	1.11 (0.68-1.82)		0.98 (0.59-1.63)	
Site of disease		0.006		0.8
PTB	1 (Ref)		1 (Ref)	
EPTB	1.46 (1.12-1.89)		0.93 (0.59-1.63)	
AFB smear result at diagnosis		<0.001		<0.001
Smear-negative	1 (Ref)		1 (Ref)	
Smear-positive	0.44 (0.35-0.56)		0.49 (0.38-0.64)	
Unknown	1.82 (0.80-4.17)		1.39 (0.60-3.25)	
Category of TB patient		0.001		<0.001
New	1 (Ref)		1 (Ref)	
Retreatment	2.61 (1.59-4.29)		3.99 (2.33-6.84)	

OR, Odds Ratio; 95% CI=95% Confidence Interval; DOT, Directly Observed Treatment; Ref, Reference Group; HIV, Human Immunodeficiency Virus; TB, Tuberculosis; PTB, Pulmonary tuberculosis; EPTB, Extra-pulmonary tuberculosis:

All variables were included in the adjusted model

Excluded from the analysis: Not evaluated=Transferred-out and TB patients with unknown TB treatment outcomes, n= 363, 7.5%

5.4.4. Successful treatment outcome and associated patient factors

Treatment success was less likely to be associated with home-based compared to facility-based DOT (aOR 0.53, 95% CI: 0.41-0.70, p<0.001). Treatment success was less likely with increasing age, among HIV-positive and patients on retreatment category and smear-negative TB patients (Table 6).

Table 6: Factors associated with treatment success among adult TB patients.

Characteristic	Crude OR		Adjusted OR	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Place of DOT		<0.001		<0.001
Facility-based	1 (Ref)		1 (Ref)	
Home-based	0.56 (0 .43-0.72)		0.53 (0.41-0.70)	
Sex		0.9		0.2
Male	1 (Ref)		1 (Ref)	
Female	1.0 (0.82-1.22)		1.15 (0.94-1.41)	
Age groups (years)		<0.001		0.03
<25	1 (Ref)		1 (Ref)	
25-34	0.79 (0.57-1.09)		0.84 (0.60-1.17)	
35-44	0.60 (0.43-0.83)		0.73 (0.52-1.03)	
≥45 years	0.51 (0.37-0.70)		0.63 (0.45-0.88)	
HIV status		<0.001		<0.001
Negative	1 (Ref)		1 (Ref)	
Positive	0.55 (0.45-0.67)		0.62 (0.50-0.77)	
Unknown HIV status	0.76 (0.51-1.12)		0.81 (0.54-1.20)	
Site of disease		0.046		0.7
PTB	1 (Ref)		1 (Ref)	
EPTB	0.79 (0.62-0.99)		1.09 (0.84-1.41)	
AFB smear results at diagnosis		<0.001		<0.001
Smear-negative	1 (Ref)		1 (Ref)	
Smear-positive	1.74 (1.43-2.12)		1.61 (1.29-2.01)	
Unknown	0.59 (0.27-1.29)		0.74 (0.33-1.64)	
Category of TB patient		0.02		<0.001
New	1 (Ref)		1 (Ref)	
Retreatment	0.45 (0.28-0.72)		0.34 (0.21-0.56)	

OR, Odds Ratio; 95% CI=95% Confidence Interval; DOT, Directly Observed Treatment; Ref, Reference Group; HIV, Human Immunodeficiency Virus; TB, Tuberculosis; PTB, Pulmonary tuberculosis; EPTB, Extra-pulmonary tuberculosis:

All variables were included in the adjusted model

Excluded: Not evaluated=Transferred-out and TB patients with unknown TB treatment outcomes, n= 363, 7.5%

5.4.5. Patient factors associated with preference of home-based DOT

Patients opting for home-based DOT were more likely to be women (aOR 1.55, 95% CI: 1.34-1.80, p<0.001), older (aOR 1.01, 95% CI: 1.00-1.02, p=0.001; for each year increase in age), more likely to have EPTB (aOR 1.45, 95% CI: 1.16-1.81, p<0.001), but less likely to have smear-positive TB (aOR 0.80, 95% CI: 0.68-0.93, p=0.002) and less likely to be on a retreatment category (aOR 0.12, 95% CI: 0.08-0.19, p<0.001). HIV-positive TB patients tended to prefer home-based compared to facility-based DOT (aOR 1.16, 95% CI: 0.99-1.36, p=0.052) (Table 7).

Table 7: Factors associated with preference to home-based DOT.

Characteristic	Crude OR		Adjusted OR	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Sex		<0.001		<0.001
Male	1 (Ref)		1 (Ref)	
Female	1.63 (1.41-1.88)		1.55 (1.34-1.80)	
Age groups (years)		<0.001		<0.001
<25	1 (Ref)		1 (Ref)	
25-34	0.78 (0.63-0.95)		0.73 (0.59-0.90)	
35-44	0.83 (0.67-1.02)		0.77 (0.61-0.96)	
≥45 years	1.24 (0.99-1.54)		1.17 (0.93-1.48)	
HIV status		0.005		0.05
Negative	1 (Ref)		1 (Ref)	
Positive	1.24 (1.08-1.43)		1.16 (0.99-1.36)	
Unknown HIV status	0.92 (0.71-1.19)		0.86 (0.66-1.13)	
Site of disease		<0.001		0.001
PTB	1 (Ref)		1 (Ref)	
EPTB	1.81 (1.49-2.20)		1.43 (1.14-1.78)	
AFB smear results at diagnosis		<0.001		0.001
Smear negative	1 (Ref)		1 (Ref)	
Smear positive	0.64 (0.56-0.74)		0.78 (0.69-0.92)	
Unknown smear result	3.41 (1.04-11.11)		3.38 (1.02-11.23)	
Category of TB patient		<0.001		<0.001
New TB patient	1 (Ref)		1 (Ref)	
Retreatment	0.13 (0.08-0.19)		0.12 (0.08-0.19)	

OR, Odds Ratio; 95% CI=95% Confidence Interval; DOT, Directly Observed Treatment; Ref, Reference Group; HIV, Human Immunodeficiency Virus; TB, Tuberculosis; PTB, Pulmonary tuberculosis; EPTB, Extra-pulmonary tuberculosis.

All variables were included in the adjusted model

n=4,472; Excluded: Not evaluated=Transferred-out and TB patients with unknown TB treatment outcomes, n= 363, 7.5%

5.5. Discussion

We showed that TB patients who opted for home-based DOT had more frequently risk factors for mortality and a higher mortality compared to the facility-based DOT group. The risk factors for mortality were older age (>35 years), HIV infection, smear-negative TB, and being on a TB retreatment regimen.

Our main finding demonstrated that TB mortality was strikingly higher in the home-based compared to the facility-based DOT group under programmatic conditions. This is in contrast to three previous studies from Tanzania (Egwaga et al., 2009; Lwilla et al., 2003; van den Boogaard et al., 2009) which showed that mortality rates were lower or equal in the home-based compared to the facility-based DOT. A cluster randomized trial conducted in a rural district in Tanzania done in 2003, reported mortality to be lower in the home-based compared to facility-based DOT patient group (Lwilla et al., 2003). However, the observed results may be due to the close supervision by community-based DOT observers and health care workers under trial conditions (Lwilla et al., 2003). In 2009, shortly after the introduction of home-based DOT in Tanzania, an observational study conducted in several urban and rural settings reported a lower mortality in the PCT cohort (home-based DOT) compared to a historic cohort (facility-based DOT) (Egwaga et al., 2009). As the authors argue, however, the difference could be explained by the more efficacious and shorter six-month regimen (rifampicin throughout regimen, a more potent anti-TB) during the PCT implementation compared to the eight months regimen in the historic cohort. Moreover, the availability of the treatment supporters in the PCT cohort was verified by the study team. Another observational study in the northern Tanzania reported no difference in TB mortality in home-based compared to facility-based DOT (van den Boogaard et al., 2009). The higher mortality in facility-based DOT could be attributed to TB patients who were admitted at the national TB hospital, who may have been severely sick patients referred from other districts.

The higher mortality in the home-based DOT group could be explained by the fact that TB patients under home-based DOT had more frequently risk factors of death such as older age (>35 years), HIV infection and smear-negative TB. Similarly, a study from the Northern part of Tanzania showed that HIV-positive and smear-negative TB patients preferred home-based DOT (van den Boogaard et al., 2009). In line with other studies from Tanzania (van den Boogaard et al., 2009; Wandwalo et al., 2006), we found that the majority (more than 60%) of TB patients in Tanzania appear to prefer home-based compared to facility-based DOT. Home-based DOT could have been the likely choice because of convenience (e.g., older people who are less mobile or severely sick TB patients) and because patient related treatment costs are considerably lower (Wandwalo et al., 2006, 2005). Furthermore, adequate treatment supervision and adherence under home-based DOT may not have been guaranteed and implemented throughout the treatment period as stipulated in the guidelines (Egwaga et al., 2008; van den Boogaard et al., 2009; Wandwalo et al., 2006), which could have caused the higher mortality in the home-based DOT group. The overall mortality during TB treatment in our study (7%) is comparable to previous studies in Tanzania (9%) (van den Boogaard et al., 2009), and also comparable to studies elsewhere in sub-Saharan Africa (5 %) (Pepper et al., 2015). However, mortality was lower than in the neighboring country Malawi (22%) (Harries A. et al., 2001), and lower than the global average (16%) [1].

We found that patient characteristics such as older age, HIV infection, and smear-negative TB were risk factors for mortality. This is in line with previous reports from several countries in sub-Saharan Africa (Connolly et al., 1998; Kabali et al., 2013; Kang'ombe et al., 2004; Lawn and Churchyard, 2009; van den Broek et al., 1998) and a systematic review (Waitt and Squire, 2011). Smear-negative TB is associated with a higher mortality possibly because smear-negative TB is more common in HIV-positive patients with severe immunosuppression (Sharma et al., 2005). Therefore, HIV-positive patients with severe immunosuppression may have developed the Immune Reconstitution Inflammatory Syndrome (IRIS), which is a consequence of the immune recovery after initiation of ART, and can result in death (Cohen and Meintjes, 2010; Lawn et al., 2005; Leone et al., 2010; Naidoo et al., 2012). We further speculate that other life threatening opportunistic infections such as *Pneumocystis jirovecii* pneumonia (PCP) (Field et al., 2014) may have contributed to the higher mortality in patients opting for home-based DOT. Furthermore, older age was also associated with higher mortality, consistent with several previous studies (Harries A. et al., 2001; Kang'ombe et al., 2004; van den Broek et al., 1998; Zachariah et al., 2002). Older people often have additional comorbidities such as diabetes mellitus, hypertension and chronic obstructive pulmonary disease (COPD) which are independently associated with increased risk of mortality (Waitt and Squire, 2011). In addition, fatal hepatotoxicity due to anti-TB is more common among older patients (Hosford et al., 2015). Finally, we also found that TB retreatment was associated with increased mortality. Retreatment TB patients (treatment failures or treatment after loss to follow-up) may have undetected initial or acquired drug resistance while on TB treatment (Field et al., 2014; Vijay et al., 2011), which may contribute to a higher mortality (Field et al., 2014). Although the prevalence of multi-drug resistance is low in Tanzania (3.9% among retreatment category patients, 1.1% among new TB patients) (Chonde et al., 2010), its contribution to the increased mortality cannot be ruled out.

Our study has several limitations. First, this was a retrospective analysis using routinely collected data. However, we analyzed a large dataset of TB patients notified to the National TB Programme over four years. Also, the patients without the outcome of interest were equally distributed between home-based and facility-based DOT, and therefore we expect no bias of results. Therefore, these findings may reflect the "real life" situation under the programmatic conditions. Second, DOT preference by the patient is as recorded in the TB registers and we could not verify if patient remained with that choice throughout the TB treatment. However, the proportion of patients under home-based DOT in our study is very comparable to others previous studies done in Tanzania (Egwaga et al., 2008; van den Boogaard et al., 2009; Wandwalo et al., 2006); indicating that the data used in our study reflects the practice of DOT implementation in programmatic conditions. Third, additional factors such as socio-economic factors, causes of death, use of cotrimoxazole prophylaxis and anti-retroviral therapy (ART) and treatment supporter characteristics were not available from the routine dataset. However, we can reasonably assume that most of the TB patients included in our study were supported by their family members (Egwaga et al., 2009, 2008; Wandwalo et al., 2005), and the use of CPT and ART initiation was given as per Tanzanian HIV and AIDS treatment guidelines (NACP, 2015). Finally, we analyzed only data from an urban setting, and thus our findings may only be generalizable to other urban areas in sub-Saharan Africa.

In conclusion, our results demonstrated that patients opting for home-based DOT under programmatic conditions are more likely to have risk factors for mortality and have an

increased mortality compared to facility-based DOT. Our findings suggest that there is a need for a risk assessment of patients at the time of TB diagnosis and a need for a careful monitoring of the implementation of DOT under programmatic conditions. The counseling and support of TB patients during TB treatment by trained health care workers may need to be improved, particularly in high-risk groups such as older people and those with comorbidities and co-infections.

Future research should focus on developing simple risk assessment tools based on evidence from prospective cohort studies (Bigna et al., 2015; Waitt and Squire, 2011) to identify patient and treatment supporter factors which may potentially influence mortality during TB treatment. In addition, operational research is needed to monitor the quality of treatment supervision and performance of DOT. Due to the limitations of current DOT strategies to assure proper drug intake (Maher, 2003; Van Deun and Rieder, 2012), additional new innovative public health interventions such as medication monitors and mobile text message reminders (Liu et al., 2015) should also be considered in the transition phase to the “post-DOT” era (Metcalfe et al., 2015). Combined with shortened and simplified TB treatment regimens, this may further reduce mortality and improve clinical outcomes to ultimately meet the “End TB” WHO targets by 2035 (WHO, 2015b).

5.6. Supporting information

Supplementary Table 1. Patient characteristics of TB patients included and excluded in the study.

Characteristics n (%)	All (n=4,866) n (%)	Included (n=4,472) n (%)	Excluded (n=394) n (%)	p-value
Total, n (%)	4,866 (100)	4,472 (91.9)	394 (8.1)	
Sex				0.5
Male	2,960 (60.8)	2,714 (60.7)	246 (62.4)	
Female	1,906 (39.2)	1,758 (39.3)	148 (37.6)	
Age in years, median (IQR)	35 (27-44)	35 (27-44)	34 (28-44)	0.9
Age groups, years				0.9
15-19	285 (5.9)	267 (6.0)	18 (4.6)	
20-24	546 (11.2)	495 (11.1)	51 (12.9)	
25-29	727 (14.9)	670 (15.0)	57 (14.5)	
30-34	825 (17.0)	753 (16.8)	72 (18.3)	
35-39	709 (14.6)	652 (14.6)	57 (14.5)	
40-44	573 (11.8)	531 (11.9)	42 (10.7)	
45-49	435 (8.9)	400 (8.9)	35 (8.9)	
50-54	283 (5.8)	260 (5.8)	23 (5.8)	
≥ 55	483 (9.9)	444 (9.9)	39 (9.9)	
HIV status				0.4
Positive	1,936 (39.8)	1,786 (39.9)	150 (38.1)	
Negative	2,597 (53.4)	2,376 (53.1)	221 (56.1)	
Unknown	333 (6.8)	310 (6.9)	23 (5.8)	
Site of disease				0.054
PTB	4,001 (82.2)	3,663 (81.9)	338 (85.8)	
EPTB	865 (17.8)	809 (18.1)	56 (14.2)	
Patient category				0.080
New	4,735 (97.3)	4,357 (97.4)	378 (95.9)	
Retreatment	131 (2.7)	115 (2.6)	16 (4.1)	
AFB smear results at diagnosis				0.052
Smear-positive	2,455 (50.5)	2,235 (50.0)	220 (55.8)	
Smear-negative	2,366 (48.2)	2,197 (49.1)	169 (42.9)	
Unknown smear results	45 (0.9)	40 (0.9)	5 (1.3)	

n (%), absolute number and column percentage; TB, Tuberculosis; PTB, Pulmonary Tuberculosis; EPTB, Extrapulmonary Tuberculosis; IQR, Inter Quartile Range.

We excluded 31 patients with unknown DOT preference, and 363 patients with the outcome “not evaluated” (“unknown” outcome or “transferred out”). Of the 31 patients with unknown DOT preference, only 2 patients died.

Supplementary Table 2. Baseline characteristics of TB patients, stratified by HIV status.

Characteristic	HIV-positive (n=1,936)	HIV-negative (n=2,597)	p-value	HIV status unknown n =2597	All
Sex			<0.001		
Male	970 (50.1)	1,791 (69.0)		199 (59.8)	2,960 (60.8)
Female	966 (49.9)	806 (31.0)		134 (40.2)	1,772 (39.1)
Age in years, median (IQR)	38 (31-45)	32 (25-42)	<0.001		35 (27-44)
Site of disease			0.022		
PTB	1,570 (81.1)	2,174 (83.7)		257 (77.2)	4,001 (82.2)
EPTB	366 (18.9)	423 (16.3)		76 (22.8)	865 (17.8)
Category			0.1		
New	1,877 (97.0)	2,536 (97.7)		322 (96.7)	4,735 (97.3)
Retreatment	59 (3.0)	61 (2.3)		11 (3.3)	131 (2.7)
TB treatment outcomes			<0.001		
Treatment success	1549 (80.0)	2198 (84.6)			3747 (82.6)
Cured	577 (29.8)	1083 (41.7)		87 (26.1)	1,747 (35.0)
Treatment completed	972 (50.2)	1115 (42.9)		196 (58.9)	2,283 (46.9)
Died	195 (10.1)	133 (5.1)		19 (5.7)	347 (7.1)
Loss to follow-up	43 (2.2)	44 (1.7)		11 (3.3)	87 (1.9)
Treatment failed	7 (0.4)	15 (0.6)		2 (0.6)	24 (0.5)
Not evaluated	142 (7.3)	207 (8.0)		18 (5.4)	363 (7.5)
AFB smear results at TB diagnosis			<0.001		
Smear-positive	839 (43.3)	1,468 (56.5)		148 (44.4)	2,307 (50.9)
Smear-negative	1,065 (55.0)	1,119 (43.1)		182 (54.7)	2,184 (48.2)
Unknown smear results	32 (1.7)	10 (0.4)		3 (0.9)	42 (0.9)
DOT preference			0.003		
Home-based	1,485 (76.7)	1,874 (72.2)		234 (70.3)	3,359 (74.1)
Facility-based	442 (22.8)	708 (27.3)		92 (27.6)	1,150 (25.4)
Unknown	9 (0.5)	15 (0.6)		7 (2.1)	24 (0.5)

n (%), absolute number and column percentage; TB, Tuberculosis; PTB, Pulmonary Tuberculosis; EPTB, Extrapulmonary Tuberculosis; IQR, Inter Quartile Range; DOT, Directly Observed Treatment
HIV-positive and HIV-negative patients were compared using the Chi-square test for categorical variables and the nonparametric Wilcrox-ranksum test for continuous variables

Supplementary Table 3. Patient characteristics of TB patients who died and were alive during TB treatment, stratified by the preference of DOT.

	All patients (n=4,472)	Home-based (n=3,320)			Facility-based (n=1,152)		
		Dead	Alive	p-value	Dead	Alive	p-value
Sex				0.2			0.2
Male	2,714 (60.7)	160 (54.2)	1,759 (58.1)		39 (78.0)	756 (68.6)	
Female	1,758 (39.3)	135 (45.8)	1,266 (41.9)		11 (22.0)	346 (31.4)	
Age in years, median (IQR)	35 (27-44)	40 (32-49)	35 (27-45)	<0.001	35 (26-42)	34 (28-41)	0.8
Age groups in years				<0.001			0.09
15-19	267 (6.0)	13 (4.4)	214 (7.1)		2 (2.0)	38 (3.4)	
20-24	495 (11.1)	15 (5.1)	336 (11.1)		3 (6.0)	141 (12.8)	
25-29	670 (15.0)	19 (6.4)	465 (15.4)		12 (24.0)	174 (15.8)	
30-34	753 (16.8)	52 (17.6)	473 (15.6)		6 (12.0)	222 (20.1)	
35-39	652 (14.6)	44 (14.9)	436 (14.4)		12 (24.0)	160 (14.5)	
40-44	531 (11.9)	43 (14.6)	332 (11.0)		6 (12.0)	150 (13.6)	
45-49	400 (8.9)	40 (13.6)	261 (8.6)		1 (2.0)	98 (8.9)	
50-54	260 (5.8)	28 (9.5)	174 (5.8)		5 (10.0)	53 (4.8)	
≥ 55	444 (9.9)	41 (13.9)	334 (11.0)		3 (6.0)	66 (6.0)	
HIV status				<0.001			0.2
Positive	1,786 (39.9)	175 (59.3)	1,197 (39.6)		19 (38.0)	395 (35.8)	
Negative	2,376 (53.1)	108 (36.6)	1,620 (53.6)		24 (48.0)	624 (56.6)	
Unknown	310 (6.9)	12 (4.1)	208 (6.9)		7 (14.0)	83 (7.5)	
Site of disease				0.007			0.6
PTB	3,663 (81.9)	218 (73.9)	2,434 (80.5)		45 (90.0)	966 (87.7)	
EPTB	809 (18.1)	77 (26.1)	591 (19.5)		5 (10.0)	136 (12.3)	
Patient category				<0.001			0.003
New	4,357 (97.4)	284 (96.3)	3,004 (99.3)		41 (82.0)	1,028 (93.3)	
Retreatment	115 (2.6)	11 (3.7)	21 (0.7)		9 (18.0)	74 (6.7)	
AFB smear results at diagnosis				<0.001			0.6
Smear-positive	2,235 (50.0)	83 (28.1)	1,479 (48.9)		26 (52.0)	647 (58.7)	
Smear-negative	2,197 (49.1)	205 (69.5)	1,516 (50.1)		24 (48.0)	452 (41.0)	
Unknown	7 (2.0)	7 (2.4)	30 (1.0)		0 (0.0)	3 (0.3)	

HIV-positive and HIV-negative were compared using pearson chi-square test for categorical variables and nonparametric Mann-Whitney test for continuous variable

*n (%), absolute number and column percentage; TB, Tuberculosis; PTB, Pulmonary Tuberculosis; EPTB, Extrapulmonary Tuberculosis; IQR, Inter Quartile Range; DOT, Directly Observed Therapy

6. Prevalence and Clinical Relevance of Helminth Co-infections among Tuberculosis Patients in Urban Tanzania

Francis Mhimbira[1,2,3*], Jerry Hella[1,2,3], Khadija Said[1,2,3], Lujeko Kamwela[1], Mohamed Sasamalo[1,2,3], Thomas Maroa[1], Magreth Chiryamkubi[4], Grace Mhalu[1,2,3], Christian Schindler[2,3], Klaus Reither[1,2,3], Stefanie Knopp[2,3,5], Jürg Utzinger[2,3], Sébastien Gagneux[2,3], Lukas Fenner [1,2,3,6 *]

1 Ifakara Health Institute, Dar es Salaam, Tanzania, **2** Swiss Tropical and Public Health Institute, Basel, Switzerland, **3** University of Basel, Basel, Switzerland, **4** Ministry of Health, Community Development, Gender, Elderly and Children, Dar es Salaam, Tanzania, **5** Wolfson Wellcome Biomedical Laboratories, Department of Life Sciences, Natural History Museum, London, United Kingdom, **6** Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland

* E-mail: fmhimbira@ihi.or.tz (FM), lukas.fenner@unibas.ch (LF)

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Competing interest
None of the authors have any competing interests to declare.

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6.1. Abstract

Background

Helminth infections can negatively affect the immunologic host control, which may increase the risk of progression from latent *Mycobacterium tuberculosis* infection to tuberculosis (TB) disease and alter the clinical presentation of TB. We assessed the prevalence and determined the clinical relevance of helminth co-infection among TB patients and household contact controls in urban Tanzania.

Methodology

Between November 2013 and October 2015, we enrolled adult (≥18 years) sputum smear-positive TB patients and household contact controls without TB during an ongoing TB cohort study in Dar es Salaam, Tanzania. We used Baermann, FLOTAC, Kato-Katz, point-of-care circulating cathodic antigen, and urine filtration to diagnose helminth infections. Multivariable logistic regression models with and without random effects for households were used to assess for associations between helminth infection and TB.

Principal findings

A total of 597 TB patients and 375 household contact controls were included. The median age was 33 years and 60.2% (585/972) were men. The prevalence of any helminth infection among TB patients was 31.8% (190/597) and 25.9% (97/375) among controls. *Strongyloides stercoralis* was the predominant helminth species (16.6%, 161), followed by hookworm (9.0%, 87) and *Schistosoma mansoni* (5.7%, 55). An infection with any helminth was not associated with TB (adjusted odds ratio (aOR) 1.26, 95% confidence interval (CI): 0.88-1.80, p=0.22), but *S. mansoni* infection was (aOR 2.15, 95% CI: 1.03-4.45, p=0.040). Moreover, *S. mansoni* infection was associated with lower sputum bacterial load (aOR 2.63, 95% CI: 1.38-5.26, p=0.004) and tended to have fewer lung cavitations (aOR 0.41, 95% CI: 0.12-1.16, p=0.088).

Conclusions/Significance:

S. mansoni infection was an independent risk factor for active TB and altered the clinical presentation in TB patients. These findings suggest a role for schistosomiasis in modulating the pathogenesis of human TB. Treatment of helminths should be considered in clinical management of TB and TB control programs.

6.2. Author summary

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis*, and parasitic worm infections are typical diseases of poverty. They often overlap geographically, and can occur in the same individual. Parasitic worm infections contribute to the down-regulation of the essential immune response against TB, and therefore can increase progression from latent *M. tuberculosis* infection to active TB. We conducted a case-control study in Dar es Salaam, the economic capital of Tanzania, where TB and helminths constitute a considerable burden. We found that infection with the blood fluke *Schistosoma mansoni* was associated with active TB, while none of the other parasitic worms showed such an association. Interestingly, TB patients infected with *S. mansoni* had significantly lower sputum bacterial load at diagnosis and tended to have fewer lung cavitations compared with TB patients without any parasitic worm infection. Diagnosis and treatment of parasitic worm infections, particularly schistosomiasis, should be considered during the management of TB patients and in the context of TB control programs. This could help to reduce the TB burden in settings where TB and parasitic worms co-exist.

6.3. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* remains a challenging disease to control. Indeed, over two billion people are estimated to be infected with *M. tuberculosis* worldwide (WHO, 2015a). Moreover one billion people are infected with soil-transmitted helminths, schistosomes, filarial worms, and food-borne trematodes (Knopp et al., 2012; Pullan et al., 2014; Utzinger et al., 2012). In 2014, an estimated 9.6 million new TB patients were notified and 1.5 million TB patients died from the disease (WHO, 2015a). TB is a leading cause of deaths from an infectious disease (WHO, 2015b).

TB and helminthiases overlap geographically, particularly in areas where poverty persists, for example in countries of sub-Saharan Africa (Simon, 2016; WHO, 2015a). Where TB and helminth infections co-occur, they can affect the same individual and thus exacerbate the course of disease (Simon, 2016). Several conditions such as diabetes mellitus, malnutrition, and malignancies are known to increase the risk of progressing from latent *M. tuberculosis* infection to active TB (Lönnroth et al., 2009). Human immunodeficiency Virus (HIV)-induced immunodeficiency is by far the most important risk factor for developing TB (NTLP, MoHSW, 2013; WHO, 2015a), but parasitic co-infections such as with helminths can also contribute to the development of TB (DiNardo et al., 2016; Potian et al., 2011; Rafi et al., 2012). Immune dysregulations caused by helminth infections are known to negatively affect the prognosis of HIV and malaria (Salgame et al., 2013; Simon, 2016). The immune response to helminth infections is characterized by the induction of $CD4^+$ T-helper 2 (Th2) and down-regulation of $CD4^+$ T-helper 1 (Th1) cells (Babu and Nutman, 2016; Mishra et al., 2014; Monin et al., 2015; Salgame et al., 2013). This immunological imbalance has been suggested to increase the risk of progression from latent *M. tuberculosis* infection to active TB and to worsen the clinical outcomes.

We aimed to study the interaction between TB and helminth co-infections by comparing the prevalence of helminth infections, using a suite of diagnostic techniques, between TB patients and household contact controls without TB in an ongoing cohort study in Dar es Salaam, Tanzania, and to assess the effects of helminth infection on the clinical presentation and outcomes of TB disease.

6.4. Methods

6.4.1. Ethics statement

The study protocol was approved by the institutional review board of the Ifakara Health Institute (IHI; reference no. IHI/IRB/No 04-2015) and the Medical Research Coordinating Committee of the National Institute of Medical Research (NIMR; reference no. NIMR/HQ/R.8c/Vol.I/357) in Tanzania, and the ethics committee of north-west and central Switzerland (EKNZ; reference no.: UBE-15/42). Written informed consent was obtained from all study participants. TB patients were treated according to the National TB and Leprosy Programme (NTLP) treatment guideline (NTLP, MoHSW, 2013). Individuals with a *Schistosoma* spp. infection were treated with praziquantel (40 mg/kg). Other helminth infections were treated with albendazole (400 mg) immediately after diagnosis, as recommended by the national treatment guidelines (MoHSW, 2013). HIV-positive patients were clinically managed according to the Tanzania National HIV and acquired immune deficiency syndrome (AIDS) treatment guideline (NACP, 2015).

6.4.2. Study setting

The study was conducted in the densely populated urban setting of Temeke district in Dar es Salaam, which is the economic capital of Tanzania. The population of Temeke is estimated at 1.4 million. In 2014, about one third of all TB patients from Dar es Salaam were notified in Temeke district (4,373; 32%) (NTLP, MoHSW, 2015). The overall HIV prevalence in the general adult population in Dar es Salaam is 5.2% (PMORALG, 2014). The study area includes two TB sub-districts, Wailes I and Wailes II, whose patients are clinically managed at the Temeke district hospital and the two associated TB diagnostic and treatment centers of Tambukareli and Pasada (Mhimbira et al., 2016).

6.4.3. Study design

The study was conducted within the frame of an ongoing prospective cohort study of TB patients and household contact controls in Dar es Salaam (TB-DAR). We assessed the association of TB and helminth infection in a case-control study design of TB patients (sputum smear-positives for acid-fast bacilli [AFB]) and household contact controls (Xpert MTB/RIF negative), who were matched by age (±5 years) and whenever possible by sex. We prospectively followed-up TB patients and assessed the clinical outcomes comparing TB patients with and without helminth infection at 6 and 12 months after recruitment.

6.4.4. Study population and sample size

We consecutively enrolled study participants starting in November 2013 until October 2015 to reach the required sample size. Over this period, we included adult TB patients (≥18 years of age and sputum-smear positive) and household contact controls. Any individual living in the same household as the index TB patients enrolled in the study is referred to as a household contact control. Controls at recruitment were free of symptoms and signs suggestive of TB, healthy on physical examination, and had a negative Xpert MTB/RIF result (Cepheid; California, United States of America).

Assuming a helminth prevalence of 45% in TB patients and 26% in controls based on results from previous publications (Elias et al., 2006) and a power of 80%, the target sample size was 109 study participants (for each group) to detect a prevalence difference of 19% between the

two groups with a significance level of test 0.05, two-tailed and calculated with Stata version 14.0 (Stata Corp; Texas, United States of America).

6.4.5. Study procedures

TB patients and household contact controls were interviewed and underwent physical examination during recruitment at the study site (see under "Data Collection and Definitions"). We collected skinfold measurements from four body sites (biceps, triceps, subscapular, and suprailiac) using the Harpenden skinfold caliper (Baty International (BI), 2007). The percentage body fat was calculated as previously described (Durnin and Womersley, 1974). Household contacts with no symptoms or signs of TB submitted a sputum sample for Gene Xpert MTB/RIF to rule-out TB. We collected blood, stool, and urine samples from TB patients and controls for subsequent laboratory investigations. Chest X-rays for TB patients were done at the Temeke district hospital and were interpreted by an experienced board certified radiologist who was blinded to patients' clinical data. Trained field workers collected geographic coordinates (global positioning system [GPS]) from the patients' homes using Samsung Tab 4 android tablet (Samsung; Suwon, South Korea).

6.4.6. Laboratory procedures

Microbiological Investigations. A patient was considered as having TB when any of the two submitted sputum samples were positive for AFB by staining sputum smears using the Ziehl-Nielsen (ZN) method, and a positive mycobacterial culture. Sputum smear microscopy was done at the Temeke district hospital under continuous quality control by the central tuberculosis reference laboratory (Dar es Salaam, Tanzania). AFB smear-positive results were graded according to World Health Organization/International Union Against Tuberculosis and Lung Disease (WHO/IUATLD) guidelines: "scanty" with 1-9 AFB per 100 oil immersion fields; "1+" with 10-99 AFB per 100 immersion fields; "2+" with 1-10 AFB per 1 immersion field, and "3+" with >10 AFB per immersion field (NTLP, MoHSW, 2013; WHO, 2013). To rule out TB among household controls, an additional sputum sample from TB patients and controls was sent to the TB laboratory at the Bagamoyo Research and Training Center (BRTC), IHI, for GeneXpert MTB/RIF (controls) and for culture on Löwenstein-Jensen media (TB patients and controls).

Helminthological Investigations. For the diagnosis of helminth infections, single stool and urine samples were collected from each participant before the start of TB treatment (TB patients) and at the time of enrolment (controls). All stool and urine samples were transferred to the Helminth Unit at BRTC and examined for helminth infections using standardized, quality-controlled procedures as described elsewhere (Knopp et al., 2014; Salim et al., 2015, 2014). The Kato-Katz (triplicate thick smears per stool sample) and the FLOTAC methods were used to diagnose *Ascaris lumbricoides,* hookworm, *S. mansoni*, and *Trichuris trichiura* infections. The Baermann method was used to identify *Strongyloides stercoralis* infections (WHO, 1994). In addition, a rapid point-of-care circulating cathodic antigen (POC-CCA) urine cassette test was employed for the diagnosis of *S. mansoni* (Colley et al., 2013). The urine filtration method was applied to detect *S. haematobium* infections (Salim et al., 2015). For quality control, 10% of Kato-Katz slides were randomly selected and re-examined by a second reader.

Blood Testing. In line with national HIV testing algorithms, screening was done using the Alere Determine HIV rapid test (Alere™, USA). The Uni-gold HIV (Trinity Biotech; Wicklow, Ireland) rapid test served as a confirmatory test in case of a positive screening test. The $CD4^+$

T-cells counts were determined using a FACSCount machine (Becton Dickinson Biosciences; California, United States of America). A full blood cell count was done with a MS4 Vet hematology analyzer (Diamond Diagnostics; Massachusetts, United States of America). All blood tests were performed at the Temeke district hospital laboratory, which is under supervision and quality control by the regional laboratory technician.

6.4.7. Data collection and definitions

We collected socio-demographic indicators including age, sex, ethnicity, education, and household income. Anthropometric data included weight, height, and skinfold measurements. Clinical data collected pertained to presenting symptoms of TB patients, TB treatment category, and treatment outcomes. Laboratory data included ZN sputum smear results and Gene Xpert MTB/RIF results, helminth species infections, HIV status, full blood cell count, and $CD4^+$ cell count. All study participants were asked about their use of anthelmintic treatment in the last 12 months prior to the enrollment into the study. Study data were captured by electronic case report forms using the open-source data collection software ODK on Android PC tablets (Andreas Steiner et al., 2016). Data management was done using the *e*Management tool "odk_planner", as previously described (Andreas Steiner et al., 2016). Data were uploaded to a password protected secure server with regular back-ups.

In order to grade the clinical severity of TB, we adopted a previously published clinical TB score (Wejse et al., 2008), with the following modification: 12 points TB score parameters instead of 13 points as tachycardia was not systematically measured. The following TB score parameters were used: (i) coughing; (ii) hemoptysis; (iii) chest pain; (iv) dyspnea; (v) night sweating; (vi) anemic conjunctivae; (vii) positive finding at auscultation; (viii) axillary temperature >37.0 °C; (ix) mid upper arm circumference (MUAC) <220 mm; (x) MUAC <200 mm; (xi) body mass index (BMI) <18 kg/m^2; and (xii) BMI <16 kg/m^2. TB score was then categorized into mild (score of 1-5) and severe (score of ≥6). Low BMI was defined as BMI <18 kg/m^2; high sputum bacterial load as AFB sputum smear result ≥2+ (quantitative scoring), which correlates with GeneXpert Ct values (Blakemore et al., 2011). To assess the clinical outcomes among TB patients, we defined poor gain as a change in absolute body weight (<7 and ≥7 kg), BMI (<2.6 and ≥2.6 kg/m^2) and body fat (<0 and ≥0%) from recruitment to month 6 of follow-up.

"Any helminth infection" was defined as infection with any of the following helminth species: *A. lumbricoides*, *Enterobius vermicularis*, hookworm, *Hymenolepis diminuta*, *S. haematobium*, *S. mansoni*, *S. stercoralis* and *T. trichiura*. High occupational risk for schistosomiasis was defined as working in rice fields, sand harvesting, washing cars, and fishing in freshwater. The intensity of helminth infection was defined according to WHO classification (WHO, 2011). The average egg counts from the triplicate Kato-Katz thick smears per stool sample and per individual were multiplied by a factor of 24 to obtain eggs per gram (EPG) of stool (Knopp et al., 2014).

6.4.8. Statistical analysis

We compared the characteristics of TB patients and household contact controls at the time of TB diagnosis or enrolment. The prevalence of helminth infection was calculated from the generalized estimations equation adjusting for clustering at the household level. We used multilevel mixed-effects logistic regression with random intercepts at the level of households

to assess risk factors for helminth infection. To assess risk factors for TB, we compared cases and controls using unconditional logistic regression because not all TB cases could be assigned a control. In addition, we also performed conditional logistic regression among matched pairs to confirm the results. Additional analyses assessed the association of TB and with specific helminth species separately. We also examined whether the association between the presence of a helminth infection and a recent history of deworming drugs depended on HIV infection status by including an interaction term in the logistic regression model. Among TB patients, logistic regression models were used to study associations between helminth infection and clinical presentation at the time of TB diagnosis (such as TB score, high sputum bacterial load, lung infiltration, and cavitation), and to study the association between helminth infection and clinical outcomes after 6 months of TB treatment (change in absolute weight, BMI, and percentage body fat). Associations were expressed as crude odds ratios (ORs) and adjusted ORs (aORs). All analyses were performed in Stata version 14.0 (Stata Corp; Texas, United States of America).

We used the geographic coordinates of the TB patients' homes to analyze the spatial distribution of TB and helminth co-infections. The prevalence of helminths and helminth species was analyzed at the ward level for optimal readability. The average area per ward in the Dar es Salaam region is 15.5 km^2 (PMORALG, 2014). The maps were produced using the software package ArcGIS Desktop version 10.2 (ESRI; California, United States of America) and the shape files from the National Bureau of Statistics of Tanzania ("Tanzania: shapefiles for EAs, villages, districts and regions," n.d.).

6.5. Results

6.5.1. Characteristics of the study participants

A total of 597 TB patients and 375 household contact controls were included. Table 8 summarizes the socio-demographic and clinical characteristics of TB patients and controls. The study participants' flow diagram is shown in Figure 11. Among all study participants, the median age was 33 years (interquartile range [IQR]: 26-41 years) and 60.2% (585/972) were men. HIV prevalence was 20.4% (95% confidence interval (CI): 17.9-23.0%). TB patients were more frequently male compared with controls (68.8% [411/597] *vs.* 46.4% [174/375]), HIV-positive (27.3% [163] *vs.* 9.3% [35]), and smokers (18.1% [108] *vs.* 8.8% [33]). TB patients also had a lower median BMI (18.3 kg/m^2, IQR: 16.5-20.4 kg/m^2 *vs.* 23.9 kg/m^2, IQR: 21.6-28.1 kg/m^2) and a lower median hemoglobin level (11.3 g/dl, IQR: 9.9-12.7 g/dl *vs.* 12.8 g/dl, IQR: 11.5-14.1 g/dl). The patient characteristics, stratified by HIV status, are shown in Supplementary Table 4.

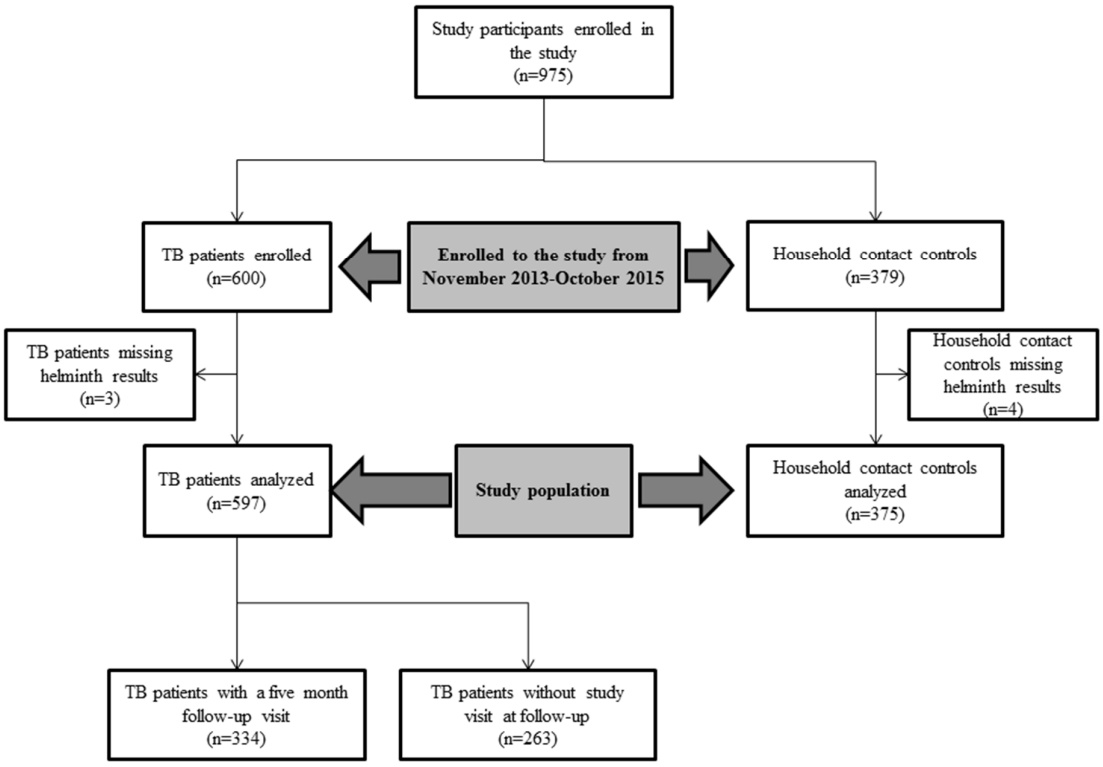


Figure 11. Study participants flow diagram

Table 8. Socio demographic and clinical characteristics of tuberculosis (TB) patients and household contact controls without TB enrolled between November 2013 to October 2015 in Dar es Salaam, Tanzania

Characteristics	Total (n=972)	TB patient (n=597)	Controls (n=375)
Age in years, median (IQR)	33 (26-41)	33 (26-40)	33 (26-42)
Age groups (years)			
18-24	194 (20.0)	107 (17.9)	87 (23.2)
25-34	347 (35.7)	226 (37.9)	121 (32.3)
35-44	266 (27.4)	169 (28.3)	97 (25.9)
≥45	165 (17.0)	95 (15.9)	70 (18.7)
Sex			
Female	387 (39.8)	186 (31.2)	201 (53.6)
Male	585 (60.2)	411 (68.8)	174 (46.4)
HIV status			
Negative	774 (79.6)	434 (72.7)	340 (90.7)
Positive	198 (20.4)	163 (27.3)	35 (9.3)
Education level			
No/Primary	806 (82.9)	500 (83.8)	306 (81.6)
Secondary/University	166 (17.1)	97 (16.2)	69 (18.4)
Occupation			
Unemployed	349 (35.9)	204 (34.2)	145 (38.7)
Employed	623 (64.1)	393 (65.8)	230 (61.3)
Smoking status			
No	831 (85.5)	489 (81.9)	342 (91.2)
Yes	141 (14.5)	108 (18.1)	33 (8.8)
People in the household			
≤3	731 (75.2)	442 (74.0)	289 (77.1)
>3	241 (24.8)	155 (26.0)	86 (22.9)
Household income per month (USD)			
≤100	763 (78.5)	473 (79.2)	290 (77.3)
>100	209 (21.5)	124 (20.8)	85 (22.7)
Body weight at diagnosis, [in kg], (IQR)	54 (48-61)	51 (46-57)	59 (53-67)
BMI (kg/m^2), median (IQR)	20.0 (17.6-23.4)	18.3 (16.6-20.4)	23.9 (21.6-28.1)
BMI categories (kg/m^2)			
Underweight <18.5	337 (34.7)	318 (53.3)	19 (5.1)
Normal, 18.5-24.9	454 (46.7)	256 (42.9)	198 (52.8)
Overweight 25.0-29.9	119 (12.2)	21 (3.5)	98 (26.1)
Obese ≥30	62 (6.4)	2 (0.3)	60 (16.0)
Body fat (%)	10.1 (7.7-14.7)	9.5 (6.8-13.7)	11.5 (8.5-17.0)
MUAC (cm), median (IQR)	24.3 (22.7-26.2)	23.3 (22.0-25.3)	25.3 (23.7-28.0)
Waist hip ratio, median (IQR)	0.89 (0.86-0.94)	0.89 (0.86-0.94)	0.89 (0.86-0.94)
Occupational risk [a]			
No	521 (54.2)	322 (54.2)	199 (54.1)
Yes	441 (45.8)	272 (45.8)	169 (45.9)
Individual deworming (in 12 months)			
Yes	797 (82.0)	484 (81.1)	313 (83.5)
No	175 (18.0)	113 (18.9)	62 (16.5)
Hb level (g/dl), median (IQR)	12 (10.4-13.3)	11.3 (9.9-12.7)	12.8 (11.5-14.1)

[a] Helminth infection occupation risk (working in rice fields, car wash and fishing);
BMI, body mass index; HIV, human immunodefiency virus; Hb, hemoglobin level; IQR, inter-quartile range; MUAC, mid-upper arm circumference; USD, United States Dollars (1 USD=2,190 Tanzanian Shillings in March 2016)

6.5.2. Prevalence and risk factors for helminth infection

Among all participants, the prevalence of any helminth infection was 29.5% (95% CI: 26.7-32.6%). *S. stercoralis* (16.5%, 161) was the predominant helminth species, followed by hookworm (9.0%, 87), *S. mansoni* (5.7%, 55) and *S. haematobium* (2.0%, 19). Overall, TB patients were more frequently co-infected with any helminth species compared with controls (OR 1.34, 95% CI: 1.00-1.78, p=0.048; Table 9). The prevalence of helminth infection was lower in HIV-positive (22.7%, 45) compared with HIV-negative study participants (31.3%, 242; Supplementary Table 4). Similarly, helminth infection was lower among TB patients co-infected with HIV (22.7%, 37) compared with HIV-negative TB patients (35.3%, 153; Supplementary Table 5). We found that most study participants had light-intensity helminth infection. For example, 96.4% (54) of study participants had light-intensity hookworm infection as determined by the Kato-Katz method (Supplementary Table 6). The prevalence and geographic distribution of species-specific helminth infections in the study area is shown in Supplementary Figure 1.

Table 9. Frequency distribution of helminth infections stratified by TB patients and household contact controls.

Helminth Infection	All	TB patients	Controls	Comparing TB patients and controls [a]	
	(n=972)	(n=597)	(n=375)		
	n (%)	n (%)	n (%)	OR (95% CI)	p-value
Any helminth	287 (29.5)	190 (31.8)	97 (25.9)	1.34 (1.00-1.78)	0.048
Helminth species					
Strongyloides stercolaris	161 (16.6)	111 (18.6)	50 (13.3)	1.48 (1.03-2.13)	0.032
Hookworm	87 (9.0)	55 (9.2)	32 (8.5)	1.09 (0.69-1.72)	0.72
Ascaris lumbricoides	6 (0.6)	3 (0.5)	3 (0.8)	0.63 (0.13-3.12)	0.57
Enterobius vermicularis	5 (0.5)	1 (0.2)	4 (1.1)	NA	NA
Trichuris trichiura	9 (0.9)	6 (1.0)	3 (0.8)	1.25 (0.31-5.06)	0.75
Hymenolepis. diminuta	2 (0.2)	1 (0.2)	1 (0.3)	NA	NA
Schistosoma spp[c]	70 (7.2)	49 (8.2)	21 (5.6)	1.51 (0.89-2.56)	0.13
Schistosoma mansoni	55 (5.7)	40 (6.7)	15 (4.0)	1.72 (0.94-3.17)	0.079
Schistosoma haematobium	19 (2.0)	11 (1.8)	8 (2.1)	0.86 (0.34-2.16)	0.75
Helminth infection					0.13
None	685 (70.5)	407 (68.2)	278 (74.1)	1	
Mono-infection	237 (24.4)	158 (26.5)	79 (21.1)	1.37 (1.00-1.86)	
Infection with ≥2 species	50 (5.1)	32 (5.3)	18 (4.8)	1.21 (0.67-2.21)	

[a] Estimates from an unadjusted mixed-effect models with household as a random intercept
Occupational risk for helminth infection (working in rice fields, car wash and fishing; OR, odds ratio; NA, not applicable

Study participants with occupational risk for acquiring schistosomiasis, such as working in rice fields, sand harvesting, washing cars, and fishing had higher odds of being infected with any helminth species (aOR 1.42, 95% CI: 1.04-1.95, p=0.029). HIV-positive patients were less likely to be infected with any helminth species (aOR 0.57, 95% CI: 0.37-0.87, p=0.010; Table 10). Study participants who did not take anthelmintic treatment in the past 12 months did not have significant higher odds of being co-infected with any helminth species (aOR 1.35, 95% CI: 0.92-1.99, p=0.12). There was no statistically significant interaction between the effects of

HIV infection and deworming status on TB incidence (P-value from test for interaction: 0.5). When analyzing the risk factors for helminth infection separately for TB patients and household controls without TB, we found similar results (Supplementary Table 8 and Supplementary Table 9).

Table 10. Risk factors for any helminth infection among TB patients and household controls without TB.

Characteristic	Helminth infection, n (%)		Unadjusted		Adjusted	
	Yes	No				
			OR (95% CI)	p-value	aOR (95% CI)	p-value
Participant				0.054		0.18
Controls	97 (33.8)	278 (40.6)	1.00		1.00	
TB patients	190 (66.2)	407 (59.4)	1.35 (1.00-1.82)		1.29 (0.88-1.87)	
Age group (years)				0.30		0.46
18-24	50 (17.4)	144 (21.0)	1.00		1.00	
25-34	115 (40.1)	232 (33.9)	1.46 (0.96-2.23)		1.38 (0.89-2.17)	
35-44	75 (26.1)	191 (27.9)	1.13 (0.72-1.78)		1.11 (0.68-1.82)	
≥45	47 (16.4)	118 (17.2)	1.16 (0.70-1.92)		1.18 (0.69-2.03)	
Sex				0.003		0.24
Female	93 (32.4)	294 (42.9)	1.00		1.00	
Male	194 (67.6)	391 (57.1)	1.60 (1.17-2.18)		1.23 (0.87-1.75)	
HIV status				0.022		0.010
Negative	242 (84.3)	532 (77.7)	1.00		1.00	
Positive	45 (15.7)	153 (22.3)	0.63 (0.43-0.94)		0.57 (0.37-0.87)	
BMI category (kg/m^2)				0.077		0.47
BMI ≥18	175 (61.0)	460 (67.2)	1.00		1.00	
BMI <18	112 (39.0)	225 (32.8)	1.32 (0.97-1.79)		1.14 (0.79-1.64)	
Education level				0.28		0.50
No/primary	243 (84.7)	563 (82.2)	1.00		1.00	
Secondary/University	44 (15.3)	122 (17.8)	0.80 (0.53-1.20)		0.86 (0.55-1.34)	
Employment status				0.13		0.42
Unemployed	93 (32.4)	256 (37.4)	1.00		1.00	
Employed	194 (67.6)	429 (62.6)	1.28 (0.93-1.76)		1.16 (0.81-1.65)	
Number of people in the household				0.66		0.97
≤3	218 (76.0)	69 (24.0)	1.00		1.00	
>3	513 (74.9)	172 (25.1)	0.93 (0.65-1.32)		0.99 (0.69-1.42)	
Household income per month (USD)				0.47		0.75
≤100	229 (79.8)	534 (78.0)	1.00		1.00	
>100	58 (20.2)	151 (22.0)	0.87 (0.60-1.26)		0.94 (0.63-1.40)	
Individual deworming (in 12 months)				0.043		0.12
Yes	224 (78.0)	573 (83.6)	1.00		1.00	
No	63 (22.0)	112 (16.4)	1.48 (1.01-2.15)		1.35 (0.92-1.99)	
Occupational risk [a]				0.009		0.029
No	136 (47.7)	385 (56.9)	1.00		1.00	
Yes	149 (52.3)	292 (43.1)	1.50 (1.11-2.03)		1.42 (1.04-1.95)	

[a] Helminth infection occupation risk (working in rice fields, car wash and fishing)

BMI, body mass index; HIV, human immunodeficieny virus;

Multilevel mixed-effects logistic regression model with household as a random intercept, adjusted for TB status, age-groups, sex, HIV status, BMI, education level, employment status, number of people living in the same household, individual deworming status, occupational risk and income level.

Note: interaction between the effect of HIV and deworming status on the risk for any helminth infection: p=0.50

6.5.3. Helminth infection as a risk factor for TB

Multiple logistic regression models adjusted for patient characteristics and known risk factors for TB showed that any helminth infection was not statistically significantly associated with TB (aOR 1.26, 95% CI: 0.88-1.80, p=0.22, Table 11 and Supplementary Table 10). However, when analyzing each helminth species separately, we found that *S. mansoni* infection was significantly associated with TB (aOR 2.15, 95% CI: 1.03-4.45, p=0.040), but there was no significant association between TB and *S. stercoralis* or hookworm infection (Supplementary Table 11). Other co-factors that were significantly associated with TB included: male sex, HIV co-infection, smoking, living in a household with ≥3 people, and a low BMI (Table 11). The unadjusted and adjusted ORs for any helminth infection and *S. mansoni* are shown in Supplementary Table 10. Results were more pronounced when using conditional logistic regression model (Supplementary Table 12).

Table 11. Associations of TB disease with helminth infection and other patient characteristics. The full table with unadjusted and adjusted odds ratios is shown in the Supplementary Information (S7 Table).

Characteristics			Any helminth infection (n=972)		*S. mansoni* infection (n=972)	
	TB patients	Controls	Adjusted		Adjusted	
	n (%)	n (%)	aOR (95% CI)	p-value	aOR (95% CI)	p-value
Helminth infection				0.22		0.040
No	407 (68.2)	278 (74.1)	1.00		1.00	
Yes	190 (31.8)	97 (25.9)	1.26 (0.88-1.80)		2.15 (1.03-4.45)	
Age group (years)				0.49		0.25
18-24	107 (17.9)	87 (23.2)	1.00		1.00	
25-34	226 (37.9)	121 (32.3)	1.22 (0.77-1.94)		1.24 (0.78-1.97)	
35-44	169 (28.3)	97 (25.9)	1.00 (0.60-1.67)		1.02 (0.61-1.7)	
≥45	95 (15.9)	70 (18.7)	0.85 (0.48-1.48)		0.88 (0.51-1.54)	
Sex				<0.001		<0.001
Female	186 (31.2)	201 (53.6)	1.00		1.00	
Male	411 (68.8)	174 (46.4)	3.12 (2.13-4.56)		3.16 (2.16-4.63)	
HIV status				<0.001		<0.001
Negative	434 (72.7)	340 (90.7)	1.00		1.00	
Positive	163 (27.3)	35 (9.3)	6.18 (3.83-9.95)		6.23 (3.86-10.05)	
Education level				0.55		0.57
No/primary	500 (83.8)	306 (81.6)	1.00		1.00	
Secondary/University	97 (16.2)	69 (18.4)	1.15 (0.73-1.80)		1.14 (0.72-1.79)	
Employment status				0.63		0.66
Unemployed	204 (34.2)	145 (38.7)	1.00		1.00	
Employed	393 (65.8)	230 (61.3)	0.91 (0.62-1.33)		0.92 (0.63-1.34)	
Smoking status				0.012		0.011
No	489 (81.9)	342 (91.2)	1.00		1.00	
Yes	108 (18.1)	33 (8.8)	1.92 (1.15-3.21)		1.95 (1.16-3.25)	
Number of people in the household				0.018		0.015
≤3 people	442 (74.0)	289 (77.1)	1.00		1.00	
>3 people	155 (26.0)	86 (22.9)	1.58 (1.08-2.30)		1.60 (1.09-2.34)	
Household income per month (USD)				0.85		0.95
≤100	473 (79.2)	290 (77.3)	1.00		1.00	
>100	124 (20.8)	85 (22.7)	1.04 (0.69-1.56)		1.01 (0.68-1.52)	
BMI category (kg/m^2)				<0.001		<0.001
BMI ≥18	279 (46.7)	318 (53.3)	1.00		1.00	
BMI <18	356 (94.9)	19 (5.1)	23.20 (13.91-38.69)		23.52 (14.1-39.24)	
Occupational risk				0.24		0.26
No	322 (54.2)	199 (54.1)	1.00		1.00	
Yes	272 (45.8)	169 (45.9)	0.82 (0.59-1.15)		0.83 (0.59-1.15)	
Individual deworming (in 12 months)				0.20		0.21
Yes	484 (81.1)	313 (83.5)	1.00		1.00	
No	113 (18.9)	62 (16.5)	0.75 (0.48-1.16)		0.76 (0.49-1.17)	

BMI, body mass index; HIV, human immunodeficieny virus; OR, odds ratio; 95% CI, 95% confidence interval

[a] Helminth infection occupation risk (working in rice fields, car wash and fishing);

Logistic regression model for TB disease status as the outcome. Model adjusted for any helminth infection/*S. mansoni*, age, sex, HIV status, BMI, education level, employment status, smoking status, number of people living in the same household, individual deworming status, helminth risk occupation and income level.

6.5.4. Effect of helminth infection on clinical presentation and disease severity in TB patients

TB patients co-infected with any helminth infection were more likely than helminth un-infected TB patients to present with hemoptysis (74 [38.9%] *vs.* 123 [30.2%]), had higher median hemoglobin levels (11.7 g/dl, IQR: 10.1-13.0 g/dl *vs.* 11.3 g/dl, IQR: 9.8-12.5 g/dl) and higher median eosinophil counts (0.2, IQR: 0.1-0.4 cells/µl *vs*. 0.1, IQR: 0.05-0.2 cells/µl; Table 12). TB patients co-infected with *S. mansoni* were more likely to have lower sputum bacterial load than helminth-uninfected TB patients (aOR 2.63; 95% CI: 1.38-5.26, p=0.004). Furthermore, we found that TB patients co-infected with *S. mansoni* tended to have fewer lung cavities, although this association lacked statistical significance (aOR 0.41, 95% CI: 0.12-1.16, p=0.088; Table 13). There were no statistically significant differences in radiological features between TB patients with and without any helminth infection as shown in Supplementary Table 13.

6.5.1. Effect of helminth infection on clinical outcomes in TB patients

Overall, 81.7% (273 TB patients) were cured at the end of TB treatment (at 6 months), 17.4% (58) completed treatment (AFB smear results not available at 6 months, but documented completion of treatment), and 0.9% (3) were treatment failures (positive AFB smear result at 6 months). We found no significant associations between helminth infection (at time of recruitment) and poor gain in absolute weight (aOR 0.89, 95% CI: 0.55-1.45, p=0.63), BMI (aOR 0.74, 95% CI: 0.46-1.21, p=0.23), and body fat percentage (aOR 0.92, 95% CI: 0.55-1.56, p=0.78) after 6 months on TB treatment, as shown in Supplementary Table 14.

Table 12. Patient characteristics of TB patients infected and not infected with helminths at the time of TB diagnosis.

Characteristics	Total	TB and helminth	TB only	
	(n=597)	(n=190)	(n=407)	p-value
Age, median (IQR) (years)	33 (26-40)	31 (26-39)	34 (27-40)	0.22
Age groups (years)				0.13
18-24	107 (17.9)	35 (18.4)	72 (17.7)	
25-34	226 (37.9)	81 (42.6)	145 (35.6)	
35-44	169 (28.3)	42 (22.1)	127 (31.2)	
≥45	95 (15.9)	32 (16.8)	63 (15.5)	
Sex				0.007
Female	186 (31.2)	45 (23.7)	141 (34.6)	
Male	411 (68.8)	145 (76.3)	266 (65.4)	
HIV status				0.003
Negative	434 (72.7)	153 (80.5)	281 (69.0)	
Positive	163 (27.3)	37 (19.5)	126 (31.0)	
CD4$^+$ count, cells/ml[a]	202 (94-273)	185 (90-259)	203 (100-273)	0.74
Education level				0.49
No formal education & Primary	500 (83.8)	162 (85.3)	338 (83.0)	
Secondary/University	97 (16.2)	28 (14.7)	69 (17.0)	
Occupation				0.99
Unemployed	204 (34.2)	65 (34.2)	139 (34.2)	
Employed	393 (65.8)	125 (65.8)	268 (65.8)	
Number of people in the household				0.89
≤3 people	442 (74.0)	140 (73.7)	302 (74.2)	
> 3 people	155 (26.0)	50 (26.3)	105 (25.8)	
Smoking status				<0.004
No	489 (81.9)	143 (75.3)	346 (85.0)	
Yes	108 (18.1)	47 (24.7)	61 (15.0)	
Household income per month (USD)				0.45
≤100	473 (79.2)	154 (81.1)	319 (78.4)	
>100	124 (20.8)	36 (18.9)	88 (21.6)	
Body weight (kg), median (IQR)	51 (46-57)	50.9 (46-56)	51.7 (46-57.5)	0.40
BMI (kg/m^2), median(IQR)	18.3 (16.6-20.4)	18.2 (16.5-20.2)	18.5 (16.6-20.4)	0.22
BMI (kg/m2) groups, n (%)				0.40
Underweight <18.5	318 (53.3)	108 (56.8)	210 (51.6)	
Normal, 18.5-24.9	256 (42.9)	78 (41.1)	178 (43.7)	
Overweight 25.0-29.9	21 (3.5)	4 (2.1)	17 (4.2)	
Obese ≥30	2 (0.3)	0	2 (0.5)	
Body fat (%)	9.5 (6.8-13.7)	9.1 (6.0-12.7)	9.8 (7.4-14.0)	0.008
MUAC (cm), median (IQR)	23.3 (22.0-25.3)	23.7 (22.0-25.0)	23.3 (22.0-25.7)	0.99
Waist hip ratio, median (IQR)	0.89 (0.85-0.94)	0.89 (0.85-0.94)	0.89 (0.86-0.94)	0.75
Occupational risk				0.095
No	322 (54.2)	93 (49.2)	229 (56.5)	
Yes	272 (45.8)	96 (50.8)	176 (43.5)	
Individual deworming (in 12 months)				0.013
Yes	484 (81.1)	143 (75.3)	341 (83.8)	
No	113 (18.9)	47 (24.7)	66 (16.2)	
Symptoms [e]				
Cough	594 (99.5)	189 (99.5)	405 (99.5)	0.96
Fever	551 (92.3)	174 (91.6)	377 (92.6)	0.65
Weight loss	573 (96.0)	181 (95.3)	392 (96.3)	0.54
Night sweats	566 (94.8)	184 (96.8)	382 (93.9)	0.13
Haemoptysis	197 (33.0)	74 (38.9)	123 (30.2)	0.035
TB score, median (IQR)	5 (4-6)	5 (4-6)	5 (4-6)	0.22
TB score (0-5)	372 (62.3)	115 (60.5)	257 (63.1)	
TB score (6-12)	225 (37.7)	75 (39.5)	150 (36.9)	
TB treatment categories				0.40
Retreatment	14 (2.3)	3 (1.6)	11 (2.7)	
New patients	583 (97.7)	187 (98.4)	396 (97.3)	
Blood parameters [e]				
Hemoglobin level	11.3 (9.9-12.7)	11.7 (10.1-13)	11.3 (9.8-12.5)	0.044
Eosinophil, cells per µl [d]	0.15 (0.06-0.32)	0.2 (0.1-0.4)	0.1 (0.05-0.2)	0.003

AFB, acid-fast bacilli; BMI, body mass index; HIV, human immunodefiency virus; IQR, interquartile range; MUAC, mid-upper arm circumference. Helminth infection occupation risk (working in rice fields, car wash and fishing)

[a] TB patient co-infected with HIV and have CD4$^+$ count values (n=80)

[b] Fisher's exact test

[c] TB patients with an available chest X-ray film read by a radiologist (n=335)

[d] TB patients with an available full blood count result (n=322)

[e] "Symptoms", and "blood parameters": categories not mutually exclusive

Table 13. Effect of helminth infection on the clinical severity and clinical presentation in TB patients at the time of TB diagnosis.

Helminth infection	Severe TB score [a]		High sputum bacterial load [b]		Lung infiltration		Lung cavitation	
	aOR (95% CI)	p-value	aOR (95% CI)	p-value	aOR (95% CI)	p-value	aOR (95% CI)	p-value
Any helminth infection		0.55		0.12		0.42		0.82
No	1.00		1.00		1.00		1.00	
Yes	1.12 (0.78-1.61)		0.75 (0.51-1.08)		0.82 (0.50-1.33)		0.95 (0.60-1.50)	
Strongyloides stercolaris [c]		0.44		0.39		0.17		0.76
No	1.00		1.00		1.00		1.00	
Yes	1.19 (0.76-1.86)		0.82 (0.52-1.29)		1.56 (0.83-2.92)		1.09 (0.62-1.91)	
Schistosoma mansoni [d]		0.75		0.004		0.15		0.088
No	1.00		1.00		1.00		1.00	
Yes	0.89 (0.45-1.78)		0.37 (0.19-0.72)		0.51 (0.21-1.27)		0.41 (0.12-1.16)	
Hookworms [e]		0.55		0.40		0.086		0.54
No	1.00		1.00		1.00		1.00	
Yes	1.20 (0.67-2.15)		0.77 (0.42-1.42)		0.51 (0.23-1.10)		0.79 (0.37-1.69)	
Multiple infections		0.82		0.020		0.19		0.40
None	1.00		1.00		1.00		1.00	
Mono	1.13 (0.77-1.67)		0.88 (0.59-1.31)		0.85 (0.51-1.43)		1.06 (0.66-1.72)	
Double or more	1.04 (0.48-2.22)		0.34 (0.16-0.73)		0.67 (0.24-1.84)		0.50 (0.17-1.44)	

Logistic regression model adjusted for age, sex, HIV infection and smoking status.

[a] TB score (Mild [score of 1-5] and severe [score of 6-12])

[b] Sputum bacterial load (according to qualitative AFB smear microscopy grading): mild (scanty and 1+) and severe (≥+2)

[c] 79 TB patients with any helminth infection other than *S. stercoralis* were excluded

[d] 150 TB patients with helminth co-infection other than *S. mansoni* were excluded

[e] 72 TB patients with helminth co-infection other than hookworm were excluded

6.6. Discussion

We present findings on the prevalence and association of TB and helminth co-infection among adult TB patients and household contact controls in a highly-urbanized setting of Dar es Salaam, Tanzania. We found that *S. mansoni* infection was a risk factor for TB disease. This association remained significant after adjustment for other known risk factors for TB, such as HIV infection, smoking, and underweight (Rieder, 1999). None of the other investigated helminth species or the surrogate measure of "any helminth infection" were significantly associated with TB. Importantly, associations between any helminth co-infection and TB were reported in previous epidemiologic studies (Elias et al., 2006; Resende Co et al., 2006; Tristão-Sá et al., 2002), as well as in experimental work using animal or macrophage infection models (Babu and Nutman, 2016; DiNardo et al., 2016; Monin et al., 2015). In line with our findings, a recent study with human peripheral mononuclear cells exposed to *M. tuberculosis* and *S. mansoni* antigens showed that *S. mansoni*-induced $CD4^+$ T cells disrupt the control of *M. tuberculosis* in infected macrophages (DiNardo et al., 2016).

Several studies in humans suggested that helminth infections may increase the risk for progression of latent *M. tuberculosis* infection to active TB (Elias et al., 2006; Monin et al., 2015; Tristão-Sá et al., 2002) as well as for exacerbating the disease (Monin et al., 2015). However, the results of these studies are conflicting, and no differentiation at the helminth species level was made in these analyses. Indeed, the hypothesis of a helminth species-specific impact on the host response is supported by a recent systematic review, which revealed a trend toward an association between a decrease in HIV viral loads and treatment for *S. mansoni*, but not for other helminth species (Sangaré et al., 2011). A case-control study from Ethiopia also found an association between TB and helminth infections, and the association was stronger in patients that were infected with multiple helminth species (Elias et al., 2006). The small number of study participants with *S. mansoni* infection (31 among TB cases, nine among controls) may have masked an association between TB and schistosomiasis in that study (Elias et al., 2006). In contrast, a cohort study from India showed no difference in TB incidence rates in helminth-infected and helminth-free individuals after 2.5 years of follow-up (Chatterjee et al., 2014).

We also found that *S. mansoni*, but not other helminth species, was associated with the clinical presentation among TB patients. Patients co-infected with *S. mansoni* had lower sputum bacterial loads at the time of TB diagnosis than *S. mansoni*-negative TB patients. Similarly, a study in Ethiopia observed lower sputum bacterial loads at TB diagnosis in TB patients co-infected with any helminth species (Abate et al., 2015). Interestingly, our observation in TB patients co-infected with *S. mansoni* resembles the paucibacillary disease in HIV-positive individuals with severe immunosuppression, who frequently have negative or low bacterial *M. tuberculosis* loads in the sputum compared with HIV-negative patients (Abate et al., 2015; Sharma et al., 2005). Hence, the helminth-induced Th1 immunological impairment might have an effect on the sputum bacterial load. Moreover, TB patients with an impaired host immune system rarely present with lung cavitation resulting in fewer *M. tuberculosis* bacilli being expectorated in the sputum (Abate et al., 2015; Sharma et al., 2005). This is in line with our findings that TB patients co-infected with *S. mansoni* tended to present less frequently with lung cavitations compared with *S. mansoni*-negative TB patients. Any helminth co-infection did not appear to have an effect on clinical outcomes during follow-up. We found no evidence for an effect of helminth co-infection on the gain in the percentage of body fat and BMI after 6

months (e.g., at the time of completed TB treatment). This might be explained by the fact that the administration of anthelmintic treatment offered to the study participants after diagnosis might have reversed the Th1 immune response (Monin et al., 2015), and thus attenuated the effect of helminth infections on clinical outcomes. However, the effect of a reversal of the Th1 immune response could be minimal as the anthelmintic drugs target the worms (Martin et al., 2015), which are less immunogenic compared with deposited *S. mansoni* eggs (DiNardo et al., 2016).

We found that TB patients had a higher crude prevalence of helminth infections, as compared with household contact controls. The higher prevalence of helminth infections among TB patients could be the result of the pathogenic role of helminth infection in the progression from *M. tuberculosis* infection to active TB. The higher prevalence of helminth co-infection in TB patients has also been noted in other studies from different settings (Abate et al., 2012; DiNardo et al., 2016). For example, a study conducted in Ethiopia, which reported a higher prevalence of helminth infection among TB patients as compared with household contact controls (Elias et al., 2006). Overall, the prevalence of helminth infection in our study was 32% and lower compared with the 71% observed in the latter study (Elias et al., 2006). It is conceivable that the high proportion of self-reported previous use of anthelmintic drugs in our study (approximately 80%) could have reduced the overall prevalence of helminth infection. Hence, we may have underestimated the effects of helminth infection seen in our study.

We also found that occupation exposing people to regular water contacts (for instance rice field workers, sand harvesters, car washers, and fishermen) were associated with helminth infections. Being exposed to freshwater bodies and being involved in water-related activities have previously been reported to increase the risk of helminth infections (Woodburn et al., 2009). In the current study, HIV-positive individuals were less likely to be co-infected with helminths. A lower prevalence of helminth infections in HIV-positive patients has also been reported in a study conducted in Mwanza in northern Tanzania, which is a highly endemic area for helminthiases (Range et al., 2007). Of note, current clinical practice in Tanzania is to treat any helminth infection in HIV-positive patients at enrolment into HIV care and in case of clinical suspicion of helminth infection during follow-up, as specified in the HIV/AIDS management guideline (NACP, 2015). The use of anthelmintic drugs is safe and might be beneficial in HIV-positive patients by possibly reducing the HIV-RNA viral load and subsequently improving clinical outcomes (Modjarrad and Vermund, 2010). Furthermore, cotrimoxazole preventive therapy (CPT), which is recommended for HIV-positive patients, has also been reported to have limited anthelmintic properties (Abate et al., 2012; Janssen et al., 2015). This might explain the lower prevalence of helminth infection among HIV-positive individuals in our study (NACP, 2015).

Our research has several strengths and limitations that warrant consideration. An important strength of our study is the large sample size and the recruitment of both TB patients and household contact controls with similar socioeconomic profiles and exposure patterns to both TB and helminth infection. Our findings may well apply to other settings with a similar prevalence of TB, HIV, and helminth infections in sub-Saharan Africa. Furthermore, we used recommended TB diagnostics and a suite of standardized, quality-controlled helminth diagnostics, which have comparable diagnostic performance to resource-intensive molecular test assays (Knopp et al., 2014).

Study limitations include the following. First, this is an observational study which cannot establish a causal relationship between helminth infections and TB disease. Second, we could not fully verify whether or not the household contact controls were latently infected with *M. tuberculosis*, which is a prerequisite to develop TB. However, because Dar es Salaam is a high-burden setting for TB with considerable risk of transmission, and because living with a TB patient is a strong risk factor for TB (Rieder, 1999), it is reasonable to assume that the controls have previously been exposed and infected with *M. tuberculosis*. Third, we did not check the helminth infection status for TB patients during and after completion of TB treatment, which could influence the clinical outcomes. However, we do not expect a high helminth re-infection rate after 6 months in our study area (Tukahebwa et al., 2013). Fourth, we did not use molecular diagnostics such as polymerase chain reaction (PCR) which might have identified some more cases, but one of our previous studies revealed that also PCR approaches miss in particular very light intensity infections. Moreover, its performance and sensitivity vary with the helminth species under examination (Knopp et al., 2014). Hence, also a PCR cannot be considered as the diagnostic gold-standard.

In conclusion, co-infection with *S. mansoni*, but not other helminth species, was found to be an independent risk factor for active TB in our study and was associated with the clinical presentation in TB patients. These findings suggest a role for *S. mansoni*, or helminth infection in general, in immunomodulation of human TB. Treatment of helminth infections should be considered in the clinical management of TB patients, and helminthiasis control/elimination through preventive chemotherapy might prove to be useful as an additional component of TB control programs. Further research is needed to establish the underlying mechanisms, and compare helminth-induced immune regulation by different helminth species. Prospective cohort studies that evaluate the effect of preventive anthelmintic chemotherapy on the incidence of *M. tuberculosis* infection and active TB could further help to understand the interaction between these diseases at the population level. Helminthiasis control measures, in combination with traditional TB control strategies, could potentially contribute to the global efforts to reduce TB incidence by 80% until 2030, as stipulated in WHO's ambitious End TB Strategy (WHO, 2015b).

6.7. Supporting information

Supplementary Table 4. Socio-demographic and clinical characteristics of TB patients and household contact controls without TB, stratified by HIV infections status.

Characteristics	Total	HIV status	
	(n=972)	**Positive** (n=198)	**Negative** (n=774)
Type of study participant			
Control	375 (38.6)	35 (17.7)	340 (43.9)
Case	597 (61.4)	163 (82.3)	434 (56.1)
Helminth status			
Negative	685 (70.5)	153 (77.3)	532 (68.7)
Positive	287 (29.5)	45 (22.7)	242 (31.3)
Age, median (IQR), years	33 (26-41)	38 (33-44)	38 (25-39)
Age groups (years)			
18-24	194 (20.0)	6 (3.0)	188 (24.3)
25-34	347 (35.7)	64 (32.3)	283 (36.6)
35-44	266 (27.4)	82 (41.4)	184 (23.8)
≥45	165 (17.0)	46 (23.2)	119 (15.4)
Sex			
Female	387 (39.8)	105 (53.0)	282 (36.4)
Male	585 (60.2)	93 (47.0)	492 (63.6)
Education level			
No formal education & Primary	806 (82.9)	185 (93.4)	621 (80.2)
Secondary/University	166 (17.1)	13 (6.6)	153 (19.8)
Occupation			
Unemployed	349 (35.9)	62 (31.3)	287 (37.1)
Employed	623 (64.1)	136 (68.7)	487 (62.9)
Household income (USD)			
≤100	763 (78.5)	154 (77.8)	609 (78.7)
>100	209 (21.5)	44 (22.2)	165 (21.3)
Weight (kg), median(IQR)	54 (48-61)	53 (47.0-59.7)	54.4 (49.0-61.0)
BMI (kg/m^2), median(IQR)	20.0 (17.6-23.4)	19.3 (17.2-22.1)	20.3 (17.8-23.9)
BMI categories (kg/m^2)			
Underweight, <18.5	337 (34.7)	81 (40.9)	256 (33.1)
Normal, 18.5-24.9	454 (46.7)	96 (48.5)	358 (46.3)
Overweight, 25.0-29.9	119 (12.2)	14 (7.1)	105 (13.6)
Obese, ≥30	62 (6.4)	7 (3.5)	55 (7.1)
Body fat percentage (%), median (IQR)	10.1 (7.7-14.7)	12.2 (8.7-15.8)	9.7 (7.2-14.4)
MUAC (cm), median (IQR)	24.3 (22.6-26.1)	23.6 (22.0-25.7)	24.3 (22.7-26.3)
Waist hip ratio, median (IQR)	0.89 (0.86-0.94)	0.89 (0.86-0.93)	0.89 (0.86-0.94)
Risk occupation			
No	521 (54.2)	102 (51.5)	429 (55.4)
Yes	441 (45.8)	96 (48.5)	345 (44.6)
Individual deworming			
Dewormed within 12months	797 (82.0)	162 (81.8)	635 (82.0)
Never within 12 months	175 (18.0)	36 (18.2)	139 (18.0)
Hemoglobin level (g/dL), median (IQR)	12.0 (10.4-13.3)	10.4 (9.0-11.9)	12.3 (10.9-13.6)
Anemia status			
Normal (Hb ≥11 g/dL)	657 (67.6)	82 (41.4)	575 (74.3)
Anaemia (Hb <11 g/dL)	315 (32.4)	116 (58.6)	199 (25.7)

IQR, inter-quartile range; BMI, body mass index; HIV, human immunodefiency Virus; MUAC, Mid-upper arm circumference; Tshs, Tanzanian Shillings; risk occupation for helminth infection (rice fields, car washing, rice harvesting and fishing)

Supplementary Table 5. Frequency distribution of helminth infections among TB patients and household controls without TB, stratified by HIV status.

Helminth Infection	All (n=972)	TB patients ((n=597)			Controls (n=375)		
		HIV-positive	HIV-negative	p-value	HIV-positive	HIV-negative	p-value[a]
		(n=163)	(n=434)		(n=35)	(n=340)	
Any helminth	287 (29.5)	37 (22.7)	153 (35.3)	0.003	8 (22.9)	89 (26.2)	0.67
Helminth species							
Strongyloides stercolaris	161 (16.6)	19 (11.7)	92 (21.2)	0.008	7 (20.0)	43 (12.6)	0.22
Hookworm	87 (9.0)	7 (4.3)	48 (11.1)	0.011	1 (2.9)	31 (9.1)	0.21
Ascaris lumbricoides	6 (0.6)	1 (0.6)	2 (0.5)	0.62[a]	0 (0)	3 (0.9)	0.75
Enterobius vermicularis	5 (0.5)	0 (0)	1 (0.2)	0.73 [a]	1 (2.9)	3 (0.9)	0.33
Trichuris trichiura	9 (0.9)	4 (2.5)	2 (0.5)	0.050 [a]	0 (0)	3 (0.9)	0.75
Hymenolepis dimunita	2 (0.2)	0 (0)	1 (0.2)	0.73[a]	0 (0)	1 (0.3)	0.91
Schistosoma spp.[b]	70 (7.2)	10 (6.1)	39 (9.0)	0.26	0 (0)	15 (4.4)	0.13
Schistosoma mansoni	55 (5.7)	10 (6.1)	30 (6.9)	0.74	0 (0)	15 (4.4)	0.22
Schistosoma hematobium	19 (2.0)	1 (0.6)	10 (2.3)	0.15[a]	0 (0)	8 (2.4)	0.36
Multiple helminth infection				0.016			1.000
Mono-infection	237 (24.4)	33 (20.2)	125 (28.8)		7 (20)	20 (79)	
Double infection	44 (4.5)	3 (1.8)	24 (5.5)		1 (2.9)	2.9 (17)	
Triple infection	6 (0.6)	1 (0.6)	4 (0.9)		0 (0)	0 (1)	

[a]Fisher's exact; [b] Four patients were co-infected with both *Schistosoma mansoni* and *Schistosoma haematobium*

Supplementary Table 6. Frequency distribution and intensity of helminth infection in TB patients and household contact controls, as determined by the Kato-Katz method (triplicate slides).

Helminth infection [a] n (%)	All (n=89)	TB patients (n=54)	Controls (n=35)	p-value
Hookworm	56 (5.8)	35 (5.9)	21 (5.6)	NA
EPG, median (IQR)	120 (40-480)	104 (32-336)	249 (64-560)	0.16[b]
Infection intensity (epg)				NA
Light (1-1,999)	54 (96.4)	35 (100)	19 (90.5)	
Moderate (2,000-3,999)	1 (1.8)	0	1 (4.8)	
Severe (≥4,000)	1 (1.8)	0	1 (4.8)	
Schistosoma mansoni	25 (2.6)	14 (2.3)	11 (2.9)	NA
EPG, median (IQR)	56 (16-88)	52 (16-88)	56 (16-88)	0.83[b]
Infection intensity (epg)				
Light (1-99)	20 (80.0)	11 (78.6)	9 (81.8)	NA
Moderate (100-399)	4 (16.0)	3 (21.4)	1 (9.1)	
Severe (≥400)	1 (4.0)	0	1 (9.1)	
Trichuris trichiura	8 (0.8)	6 (1.0)	2 (0.5)	NA
EPG, median (IQR)	24 (12-80)	24 (16-120)	24 (8-40)	0.61[b]
Infection intensity (epg)				
Light (1-999)	8 (100)	6 (100)	2 (100)	NA
Ascaris lumbricoides	3 (0.3)	1 (0.2)	2 (0.5)	NA
EPG, median (IQR)	16 (16-80)	80 (80-80)	16 (16-16)	0.16[b]
Infection intensity (epg)				
Light (1-5,000)	3 (100)	1 (100)	2 /100)	

[a] Four patients were co-infected by *Schistosoma mansoni* and *Schistosoma haematobium*;
[b] Wilcoxon signed rank test; Helminth infection risk occupation (rice fields, car wash, rice harvest and fishing); IQR, inter-quartile range; EPG, Eggs per gram; NA, Not Applicable

[x] **Helminth intensity infection: Light**: *A. lumbricoides*, 1-4,999 EPG; *T. trichiura*, 1-999 EPG; hookworms, 1-1,999 EPG; and *S. mansoni*, 1-99 EPG. **Moderate**: *A. lumbricoides*, 5,000-49,999 EPG; *T. trichiura*, 1,000-9,999 EPG; hookworms, 2,000-3,999 EPG; and *S. mansoni*, 100-399 EPG. **Severe:** *A. lumbricoides*, ≥50,000 EPG; *T. trichiura* ≥10,000 EPG; hookworms, ≥4,000 EPG and *S. mansoni*, ≥400 EPG

Supplementary Table 7. Full blood count and hematological parameters in TB patients, stratified by helminth infection status.

Blood parameters	Total	Helminth status		p-value[b]
	(n=566) [a]	**Positive** (n=179)	**Negative** (n=387)	
White blood cells (×10^9/L)	7.4 (5.7-9.6)	7.3 (5.6-9.3)	7.5 (5.7-9.8)	0.48
Red blood cells (×10^9/L)	4.7 (4.1-5.29	4.7 (4.3-5.4)	4.6 (4.0-5.2)	0.11
Haemoglobin level (g/dL)	11.2 (9.7-12-6)	11.5 (10.2-13)	11.1 (9.5-12.5)	0.014
Haematocrit (%)	34.8 (30.5-38.9)	35.9 (31.6-39.6)	34.6 (30.0-38.3)	0.021
MCV (fL)	75.9 (68.9-82.4)	76.0 (70.3-81.6)	75.6 (68.3-83)	0.97
MCH (pg)	24.3 (21.6-26.7)	24.3 (22-27)	24.2 (21.5-26.7)	0.47
MCHC (%)	32.4 (30.6-33-6)	32.5 (30.9-33.7)	32.3 (30.4-33.5)	0.20
Platelets (×10^9/L)	350 (263-460)	366 (247-430)	350 (263-460)	0.29
Differential blood counts (×10^9/L)	(n=332)	(n=112)	(n=220)	
Neuotrophil	4.7 (3.3-6.3)	4.6 (3.1-6.1)	4.8 (3.4-6.4)	0.39
Lymphocyte	1.5 (1.2-2.0)	1.6 (1.2-2.0)	1.5 (1.2-2.1)	0.46
Monoctye	0.77 (0.55-1.0)	0.8 (0.5-1.1)	0.8 /0.6-1.0)	0.86
Eosinophil	0.15 (0.06-0.32)	0.2 (0.1-0.4)	0.1 (0.05-0.2)	0.003
Basophil	0.03 (0.02-0.05)	0.03 (0.02-0.07)	0.03 (0.02-0.05)	0.30

MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume
[a] 31 TB patients were excluded from the analysis because of the missing full blood count results;
[b]Wilcoxon rank-sum test; all measurements are in median (Interquartile Range, [IQR])
[c] 244 TB patients had missing differential count of white blood counts

Supplementary Table 8. Additional analysis: risk factors for any helminth infection among TB patients only.

Characteristics	Helminth Infection		Crude		Adjusted	
n (%)	Yes	No				
	n (%)	(n (%)	OR (95% CI)	p-value	aOR (95% CI)	p-value
Age group (years)				0.13		0.095
18-24	35 (18.4)	72 (17.7)	1.00		1.00	
25-34	81 (42.6)	145 (35.6)	1.15 (0.71-1.87)		1.25 (0.74-2.11)	
35-44	42 (22.1)	127 (31.2)	0.68 (0.40-1.16)		0.70 (0.38-1.26)	
≥45	32 (16.8)	63 (15.5)	1.04 (0.58-1.88)		1.11 (0.58-2.11)	
Sex				0.006		0.058
Female	45 (23.7)	141 (34.6)	1.00		1.00	
Male	145 (76.3)	266 (65.4)	1.71 (1.15-2.53)		1.53 (0.99-2.37)	
HIV status				0.003		0.038
Negative	153 (80.5)	281 (69.0)	1.00		1.00	
Positive	37 (19.5)	126 (31.0)	0.534 (0.36-0.82)		0.62 (0.39-0.97)	
Education level				0.49		0.45
No/primary	162 (85.3)	338 (83.0)	1.00		1.00	
Secondary/University	28 (14.7)	69 (17.0)	0.85 (0.53-1.37)		0.81 (0.48-1.38)	
Employment status				0.99		0.56
Unemployed	65 (34.2)	139 (34.2)	1.00		1.00	
Employed	125 (65.8)	268 (65.8)	1.00 (0.69-1.43)		0.89 (0.59-1.33)	
People in the household				0.89		0.49
≤3 people	140 (73.7)	302 (74.2)	1.00		1.00	
> 3 people	50 (26.3)	105 (25.8)	1.03 (0.69-1.52)		1.16 (0.77-1.74)	
Household income (USD)				0.45		0.96
≤100	154 (81.1)	319 (78.4)	1.00		1.00	
>100	36 (18.9)	88 (21.6)	0.85 (0.55-1.31)		1.01 (0.63-1.62)	
BMI category (kg/m^2)				0.23		0.38
BMI ≥18	82 (43.2)	197 (48.4)	1.00		1.00	
BMI < 18	108 (56.8)	210 (51.6)	1.24 (0.87-1.75)		1.17 (0.82-1.69)	
Occupational risk				0.096		0.14
No	93 (49.2)	229 (56.5)	1.00		1.00	
Yes	96 (50.8)	176 (43.5)	1.34 (0.95-1.89)		1.32 (0.91-1.90)	
Individual deworming (in 12 months)				0.015		0.022
Yes	143 (75.3)	341 (83.8)	1.00		1.00	
No	47 (24.7)	66 (16.2)	1.70 (1.11-2.59)		1.67 (1.08-2.60)	

BMI, body mass index; HIV, human immunodeficieny virus; Helminth infection risk occupation (working in the rice fields, car wash, rice harvest and fishing)
Logistic regression model was used. including the independent variables TB status, age-group, sex, HIV status, BMI, education level, employment status, number of people living in the same household, individual deworming status, occupational risk and income level in quartiles.

Supplementary Table 9. Additional analysis: risk factors for any helminth infection among household controls without TB only

Characteristic	Helminth status		Crude		Adjusted	
	Yes	No				
	n (%)	(n (%)	OR (95% CI)	p-value	aOR (95% CI)	p-value
Age group (years)				0.055		0.091
18-24	15 (15.5)	72 (25.9)	1.00		1.00	
25-34	34 (35.1)	87 (31.3)	1.88 (0.95-3.71)		1.70 (0.82-3.50)	
35-44	33 (34.0)	64 (23.0)	2.47 (1.23-4.97)		2.52 (1.17-5.47)	
≥45	15 (15.5)	55 (19.8)	1.31 (0.59-2.91)		1.29 (0.55-3.02)	
Sex				0.35		0.80
Female	48 (49.5)	153 (55.0)	1.00		1.00	
Male	49 (50.5)	125 (45.0)	1.25 (0.79-1.99)		0.93 (0.55-1.59)	
HIV status				0.67		0.35
Negative	89 (91.8)	251 (90.3)	1.00		1.00	
Positive	8 (8.2)	27 (9.7)	0.84 (0.37-1.91)		0.66 (0.28-1.57)	
Education level				0.57		0.82
No/primary	81 (83.5)	225 (80.9)	1.00		1.00	
Secondary/University	16 (16.5)	53 (19.1)	0.84 (0.45-1.55)		1.08 (0.54-2.18)	
Employment status				0.020		0.099
Unemployed	28 (28.9)	117 (42.1)	1.00		1.00	
Employed	69 (71.1)	161 (57.9)	1.79 (1.09-2.95)		1.63 (0.91-2.92)	
People in the household				0.36		0.63
≤3 people	78 (80.4)	211 (75.9)	1.00		1.00	
> 3 people	19 (19.6)	67 (24.1)	0.77 (0.43-1.36)		0.86 (0.47-1.58)	
Household income (USD)				0.99		0.37
≤100	75 (77.3)	215 (77.3)	1.00		1.00	
>100	22 (22.7)	63 (22.7)	1.00 (0.58-1.74)		0.75 (0.39-1.42)	
BMI category (kg/m^2)				0.62		0.62
BMI ≥18	93 (95.9)	263 (94.6)	1.00		1.00	
BMI < 18	4 (4.1)	15 (5.4)	0.75 (0.24-2.33)		0.75 (0.23-2.40)	
Individual deworming (in 12 months)				0.99		0.87
Yes	81 (83.5)	232 (83.5)	1.00		1.00	
No	16 (16.5)	46 (16.5)	1.00 (0.54-1.86)		0.95 (0.49-1.83)	
Occupational risk				0.028		0.040
No	44 (45.4)	162 (58.3)	1.00		1.00	
Yes	53 (54.6)	116 (41.7)	1.68 (1.06-2.68)		1.69 (1.02-2.78)	

BMI, body mass index; HIV, human immunodeficieny virus; Helminth infection risk occupation (working in the rice fields, car wash, rice harvest and fishing)

Logistic regression model was used, including the independent variables TB status, age-group, sex, HIV status, BMI, education level, employment status, number of people living in the same household, individual deworming status, occupational risk and income level in quartiles.

Supplementary Table 10. Full table with unadjusted and adjusted odds ratios: Associations of TB disease with helminth infection and other patient characteristics comparing TB patients and household contact controls without TB.

Characteristic			Any helminth infection (n=972)				*Schistosoma mansoni* infection (n=972)			
	TB patient	Control								
	n (%)	n (%)	OR (95% CI)	p-value	aOR (95% CI)	p-value	OR (95% CI)	p-value	aOR (95% CI)	p-value
Helminth infection				0.048		0.22		0.07		0.040
No	407 (68.2)	278 (74.1)	1.00		1.00		1.00		1.00	
Yes	190 (31.8)	97 (25.9)	1.34 (1.00-1.79)		1.26 (0.88-1.80)		1.72 (0.94-3.17)		2.15 (1.03-4.45)	
Age group (years)				0.081		0.49		0.081		0.25
18-24	107 (17.9)	87 (23.2)	1.00		1.00		1.00		1.00	
25-34	226 (37.9)	121 (32.3)	1.52 (1.06-2.17)		1.22 (0.77-1.94)		1.52 (1.06-2.17)		1.24 (0.78-1.97)	
35-44	169 (28.3)	97 (25.9)	1.42 (0.97-2.07)		1.00 (0.60-1.67)		1.42 (0.97-2.07)		1.02 (0.61-1.7)	
≥45	95 (15.9)	70 (18.7)	1.10 (0.73-1.68)		0.84 (0.48-1.48)		1.10 (0.73-1.68)		0.88 (0.51-1.54)	
Sex				<0.001		<0.001		<0.001		<0.001
Female	186 (31.2)	201 (53.6)	1.00		1.00		1.00		1.00	
Male	411 (68.8)	174 (46.4)	2.55 (1.96-3.33)		3.11 (2.13-4.56)		2.55 (1.96-3.33)		3.16 (2.16-4.63)	
HIV status				<0.001		<0.001		<0.001		<0.001
Negative	434 (72.7)	340 (90.7)	1.00		1.00		1.00		1.00	
Positive	163 (27.3)	35 (9.3)	3.65 (2.47-5.40)		6.18 (3.83-9.95)		3.65 (2.47-5.4)		6.23 (3.86-10.05)	
Education level				0.39		0.55		0.39		0.57
No/primary	500 (83.8)	306 (81.6)	1.00		1.00		1.00		1.00	
Secondary/University	97 (16.2)	69 (18.4)	0.86 (0.61-1.21)		1.15 (0.73-1.80)		0.86 (0.61-1.21)		1.14 (0.72-1.79)	
Employment status				0.16		0.63		0.16		0.66
Unemployed	204 (34.2)	145 (38.7)	1.00		1.00		1.00		1.00	
Employed	393 (65.8)	230 (61.3)	1.22 (0.93-1.59)		0.91 (0.62-1.33)		1.22 (0.93-1.59)		0.92 (0.63-1.34)	
Smoking status				<0.001		0.012		<0.001		0.011
No	489 (81.9)	342 (91.2)	1.00		1.00		1.00		1.00	
Yes	108 (18.1)	33 (8.8)	2.29 (1.51-4.46)		1.92 (1.15-3.21)		2.29 (1.51-3.46)		1.95 (1.16-3.25)	
People in the household				0.29		0.018		0.29		0.015
≤3 people	442 (74.0)	289 (77.1)	1.00		1.00		1.00		1.00	
>3 people	155 (26.0)	86 (22.9)	1.18 (0.87-1.60)		1.57 (1.08-2.30)		1.18 (0.87-1.6)		1.60 (1.09-2.34)	
Household income (USD)				0.48		0.85		0.49		0.95

≤100	473 (79.2)	290 (77.3)	1.00		1.00		1.00		1.00	
>100	124 (20.8)	85 (22.7)	0.89 (0.65-1.22)		1.04 (0.69-1.56)		0.89 (0.65-1.22)		1.01 (0.68-1.52)	
BMI category (kg/m)				<0.001		<0.001		<0.001		<0.001
BMI ≥18	279 (46.7)	318 (53.3)	1.00		1.00		1.00		1.00	
BMI <18	356 (94.9)	19 (5.1)	21.36 (13.10-34.81)		23.20 (13.91-38.69)		21.36 (13.1-34.81)		23.52 (14.1-39.24)	
Occupational risk				0.880		0.24		0.88		0.26
No	322 (54.2)	199 (54.1)	1.00		1.00		1.00		1.00	
Yes	272 (45.8)	169 (45.9)	1.02 (0.79-1.32)		0.82 (0.59-1.15)		1.02 (0.79-1.32)		0.83 (0.59-1.15)	
Individual deworming (in 12 months)				0.35		0.20		0.21		0.21
Yes	484 (81.1)	313 (83.5)	1.00		1.00		1.00		1.00	
No	113 (18.9)	62 (16.5)	1.18 (0.84-1.66)		0.75 (0.48-1.16)		0.76 (0.49-1.17)		0.76 (0.49-1.17)	

HIV, Human Immunodeficieny Virus; BMI, body mass index; OR, Odds ratio; 95% CI, 95% Confidence Interval

[a] Working in rice fields, car washing, sand harvesting and fishing.

Logistic regression model adjusted for any helminth infection/*S. mansoni*, age, sex, HIV status, BMI, education level, employment status, smoking status, number of people living in the same household, individual deworming status, helminth risk occupation and income level.

Supplementary Table 11. Full table with unadjusted and adjusted odds ratios: Associations of TB disease with *Strongyloides stercolaris* and hookworm infections comparing TB patients and household contact controls without TB.

Characteristic			*Strongyloides stercolaris* (n=972)				*Hookworm* infection (n=972)			
	TB patient	Control								
	n (%)	n (%)	OR (95% CI)	p-value	aOR (95% CI)	p-value	OR (95% CI)	p-value	aOR (95% CI)	p-value
Helminth infection				0.03		0.29		0.717		0.34
No	407 (68.2)	278 (74.1)	1		1.00		1.00		1.00	
Yes	190 (31.8)	97 (25.9)	1.49 (1.03-2.13)		1.27 (0.81-1.99)		1.09 (0.69-1.72)		1.31 (0.75-2.3)	
Age group (years)				0.081		0.25		0.081		0.25
18-24	107 (17.9)	87 (23.2)	1.00		1.00		1.00		1.00	
25-34	226 (37.9)	121 (32.3)	1.52 (1.06-2.17)		1.23 (0.77-1.95)		1.52 (1.06-2.17)		1.24 (0.78-1.97)	
35-44	169 (28.3)	97 (25.9)	1.42 (0.97-2.07)		1.00 (0.6-1.66)		1.42 (0.97-2.07)		1.00 (0.60-1.67)	
≥45	95 (15.9)	70 (18.7)	1.10 (0.73-1.68)		0.84 (0.48-1.47)		1.10 (0.73-1.68)		0.86 (0.49-1.49)	
Sex				<0.001		<0.001		<0.001		<0.001
Female	186 (31.2)	201 (53.6)	1.00		1.00		1.00		1.00	
Male	411 (68.8)	174 (46.4)	2.55 (1.96-3.33)		3.12 (2.14-4.57)		2.55 (1.96-3.33)		3.12 (2.13-4.56)	
HIV status				<0.001		<0.001		<0.001		<0.001
Negative	434 (72.7)	340 (90.7)	1.00		1.00		1.00		1.00	
Positive	163 (27.3)	35 (9.3)	3.65 (2.47-5.40)		6.08 (3.78-9.78)		3.65 (2.47-5.4)		6.16 (3.82-9.91)	
Education level				0.39		0.59		0.39		0.55
No/primary	500 (83.8)	306 (81.6)	1.00		1.00		1.00		1.00	
Secondary/University	97 (16.2)	69 (18.4)	0.86 (0.61-1.21)		1.13 (0.72-1.78)		0.86 (0.61-1.21)		1.15 (0.73-1.8)	
Employment status				0.16		0.59		0.16		0.68
Unemployed	204 (34.2)	145 (38.7)	1.00		1.00		1.00		1.00	
Employed	393 (65.8)	230 (61.3)	1.22 (0.93-1.59)		0.9 (0.61-1.32)		1.22 (0.93-1.59)		0.92 (0.63-1.35)	
Smoking status				<0.001		0.013		<0.001		0.012
No	489 (81.9)	342 (91.2)	1.00		1.00		1.00		1.00	
Yes	108 (18.1)	33 (8.8)	2.29 (1.51-4.46)		1.92 (1.15-3.2)		2.29 (1.51-3.46)		1.93 (1.16-3.22)	
People in the household				0.29		0.021		0.29		0.018
≤3 people	442 (74.0)	289 (77.1)	1.00		1.00		1.00		1.00	
>3 people	155 (26.0)	86 (22.9)	1.18 (0.87-1.60)		1.56 (1.07-2.28)		1.18 (0.87-1.6)		1.58 (1.08-2.3)	
Household income (USD)				0.48		0.83		0.49		0.82

≤100	473 (79.2)	290 (77.3)	1.00		1.00		1.00		1.00			
>100	124 (20.8)	85 (22.7)	0.89 (0.65-1.22)		1.05 (0.70-1.57)		0.89 (0.65-1.22)		1.05 (0.70-1.57)			
BMI category (kg/m)				<0.001		0		<0.001		<0.001		
BMI ≥18	279 (46.7)	318 (53.3)	1.00		1.00		1.00		1.00			
BMI <18	356 (94.9)	19 (5.1)	21.36 (13.10-34.81)		23.2 (13.91-38.7)		21.36 (13.1-34.81)		23.53 (14.10-39.25)			
Occupational risk				0.880		0.28		0.88		0.25		
No	322 (54.2)	199 (54.1)	1.00		1.00		1.00		1.00			
Yes	272 (45.8)	169 (45.9)	1.02 (0.79-1.32)		0.83 (0.60-1.16)		1.02 (0.79-1.32)		0.82 (0.59-1.15)			
Individual deworming (in 12 months)				0.35		0.22		0.21		0.19		
Yes	484 (81.1)	313 (83.5)	1.00		1.00		1.00		1.00			
No	113 (18.9)	62 (16.5)	1.18 (0.84-1.66)		0.76 (0.49-1.18)		0.76 (0.49-1.17)		0.75 (0.48-1.16)			

HIV, Human Immunodeficieny Virus; BMI, body mass index; OR, Odds ratio; 95% CI, 95% Confidence Interval

[a] Working in rice fields, car washing, sand harvesting and fishing.

Logistic regression model adjusted for any helminth infection/*S. mansoni*, age, sex, HIV status, BMI, education level, employment status, smoking status, number of people living in the same household, individual deworming status, helminth risk occupation and income level.

Supplementary Table 12. Additional analysis: Helminth infection and patient characteristics associated with TB among TB patients and household contact controls, using conditional logistic regression.

Characteristic	Any helminth infection (n=722)						*Schistosoma mansoni* infection (n=455)					
	TB patient	Control					TB patient	Control				
	n (%)	n (%)	OR (95% CI)	p-value	aOR (95% CI)	p-value	n (%)	n (%)	OR (95% CI)	p-value	aOR (95% CI)	p-value
Helminth infection				0.398		0.198				0.188		0.008
No	247 (71)	278 (74.3)	1.00		1.00		192 (90.6)	229 (94.2)	1.00		1.00	
Yes	101 (29)	96 (25.7)	1.16 (0.82-1.64)		1.45 (0.82-2.55)		20 (9.4)	14 (5.8)	1.69 (0.77-3.74)		5.29 (1.55-18.1)	
Age group (years)				0.1						0.084		0.0391
18-24	62 (17.8)	86 (23)	1.00		1.00		32 (15.1)	59 (24.3)	1.00	0.834	1.00	
25-34	139 (39.9)	121 (32.4)	1.61 (1.07-2.44)		1.13 (0.58-2.19)		84 (39.6)	79 (32.5)	1.6 (0.96-2.66)		1.76 (0.77-4.03)	
35-44	93 (26.7)	97 (25.9)	1.33 (0.86-2.06)		0.87 (0.42-1.82)		65 (30.7)	57 (23.5)	1.64 (0.95-2.84)		2.04 (0.74-5.61)	
≥45	54 (15.5)	70 (18.7)	1.08 (0.67-1.75)		0.55 (0.25-1.2)		31 (14.6)	48 (19.8)	0.92 (0.5-1.68)		0.56 (0.2-1.51)	
Sex				0		0.001				0		0.007
Female	115 (33)	200 (53.5)	1.00		1.00		74 (34.9)	133 (54.7)	1.00		1.00	
Male	233 (67)	174 (46.5)	2.22 (1.64-3.01)		2.37 (1.41-3.98)		138 (65.1)	110 (45.3)	2.03 (1.4-2.96)		2.44 (1.27-4.67)	
HIV status				0		0				0		0
Negative	250 (71.8)	339 (90.6)	1.00		1.00		144 (67.9)	221 (90.9)	1.00		1.00	
Positive	98 (28.2)	35 (9.4)	4.15 (2.6-6.64)		9.83 (4.64-20.85)		68 (32.1)	22 (9.1)	4.56 (2.55-8.16)		9.36 (3.85-22.73)	
Education level				0.645		0.944				0.834		0.143
No/primary	289 (83)	305 (81.6)	1.00		1.00		173 (81.6)	200 (82.3)	1.00		1.00	
Secondary/University	59 (17)	69 (18.4)	0.91 (0.62-1.35)		0.98 (0.5-1.9)		39 (18.4)	43 (17.7)	1.05 (0.64-1.73)		1.92 (0.8-4.6)	
Employment status				0.383		0.669				0.212		0.223
Unemployed	125 (35.9)	145 (38.8)	1.00		1.00		69 (32.5)	101 (41.6)	1.00		1.00	
Employed	223 (64.1)	229 (61.2)	1.61 (1.07-2.44)		1.13 (0.65-1.95)		143 (67.5)	142 (58.4)	1.27 (0.87-1.86)		1.5 (0.78-2.89)	
Smoking status				0		0.008				0.008		0.176
No	288 (82.8)	341 (91.2)	1.00		1.00		179 (84.4)	223 (91.8)	1.00		1.00	
Yes	60 (17.2)	33 (8.8)	2.28 (1.42-3.67)		2.67 (1.29-5.54)		33 (15.6)	20 (8.2)	2.34 (1.21-4.5)		2.01 (0.73-5.5)	
People in the household				0.091		0.007				0.098		0.008
≤3 people	255 (73.3)	288 (77)	1.00		1.00		154 (72.6)	189 (77.8)	1.00		1.00	
>3 people	93 (26.7)	86 (23)	1.39 (0.95-2.05)		2.46 (1.27-4.74)		58 (27.4)	54 (22.2)	1.51 (0.92-2.48)		2.8 (1.3-6.02)	

Household income (USD)				0.85		0.714				0.44		0.196
≤100	268 (77)	289 (77.3)	1.00		1.00		163 (76.9)	183 (75.3)	1.00		1.00	
>100	80 (23)	85 (22.7)	1.04 (0.72-1.5)		1.12 (0.61-2.04)		49 (23.1)	60 (24.7)	0.84 (0.53-1.32)		0.6 (0.28-1.3)	
BMI category (kg/m)				0		0				0		0
BMI ≥18	167 (48)	355 (94.9)	1.00		1.00		110 (51.9)	228 (93.8)	1.00		1.00	
BMI <18	181 (52)	19 (5.1)	15.5 (8.63-27.85)		21.01 (10.33-42.71)		102 (48.1)	15 (6.2)	11.48 (5.79-22.77)		19.98 (7.89-50.57)	
Occupational risk				0.321		0.015				0.807		0.061
No	204 (58.6)	206 (55.1)	1.00		1.00		121 (57.1)	139 (57.2)	1.00		1.00	
Yes	144 (41.4)	168 (44.9)	0.85 (0.62-1.17)		0.54 (0.33-0.89)		91 (42.9)	104 (42.8)	0.95 (0.63-1.43)		0.53 (0.27-1.03)	
Individual deworming (in 12 months)				0.552		0.18				0.298		0.278
Yes	284 (81.6)	312 (83.4)	1.00		1.00		172 (81.1)	206 (84.8)	1.00		1.00	
No	64 (18.4)	62 (16.6)	1.13 (0.76-1.66)		0.66 (0.36-1.21)		40 (18.9)	37 (15.2)	1.31 (0.79-2.19)		0.63 (0.27-1.45)	

HIV, Human Immunodeficieny Virus; BMI, body mass index; OR, Odds ratio; 95% CI, 95% Confidence Interval

[a] For comparison with *Schistosoma mansoni*, we excluded 232 participants with any helminth infections other than *Schistosoma mansoni*.[b] Working in rice fields, car washing, sand harvesting and fishing Conditional logistic regression model taking into account case-control matching, and adjusted for any helminth infection/*S. mansoni*, age, sex, HIV status, BMI, education level, employment status, smoking status, number of people living in the same household, individual deworming status, helminth risk occupation and household income level.

Excluded TB patients due to unpaired to controls: 250 cases for any helminth infection, and 285 cases for *S. mansoni* infection.

Supplementary Table 13. Radiological findings of chest X-rays in TB patients at the time of TB diagnosis, stratified by helminth infection status.

Radiological findings	Total	TB and helminths	TB only	
	(n=335)	(n=93)	(n=242)	p-value[a]
Infiltrations	276 (82.4)	81 (87.1)	195 (80.6)	0.16
Unilateral	196 (71.0)	55 (67.9)	141 (72.3)	
Bilateral	80 (29.0)	26 (32.1)	54 (27.7)	
Cavitations	146 (43.6)	45 (48.4)	101 (41.7)	0.27
Unilateral	132 (90.4)	38 (84.4)	94 (93.1)	
Bilateral	14 (9.6)	7 (15.6)	7 (6.9)	
Micronodules (< 7mm)	26 (7.8)	9 (9.7)	17 (7.0)	0.42
Unilateral	11 (42.3)	5 (55.6)	6 (35.3)	
Bilateral	15 (57.7)	4 (44.4)	11 (64.7)	
Macronodules (≥7mm)	7 (2.1)	1 (1.1)	6 (2.5)	0.42
Unilateral	5 (71.4)	0 (0)	5 (83.3)	
Bilateral	2 (28.6)	1 (100)	1 (16.7)	
Pleural effusion	52 (15.5)	11 (11.8)	41 (16.9)	0.25
Unilateral	46 (88.5)	9 (81.8)	37 (90.2)	
Bilateral	6 (11.5)	2 (18.2)	4 (9.8)	
Pulmonary edema	18 (5.4)	2 (2.2)	16 (6.6)	0.11
Unilateral	4 (22.2)	1 (50.0)	3 (18.8)	
Bilateral	14 (77.8)	1 (50.0)	13 (81.3)	
Lymph node enlargements (intrathoracic)	38 (11.3)	8 (8.6)	30 (12.4)	0.33
Radiologist severity grading [b]				0.76
Mild/moderate	246 (73.4)	67 (72.0)	179 (74.0)	
Severe	88 (26.3)	26 (28.0)	62 (25.6)	

[a] Pearson chi-squared test; [b] Radiologist grading of the chest-ray severity

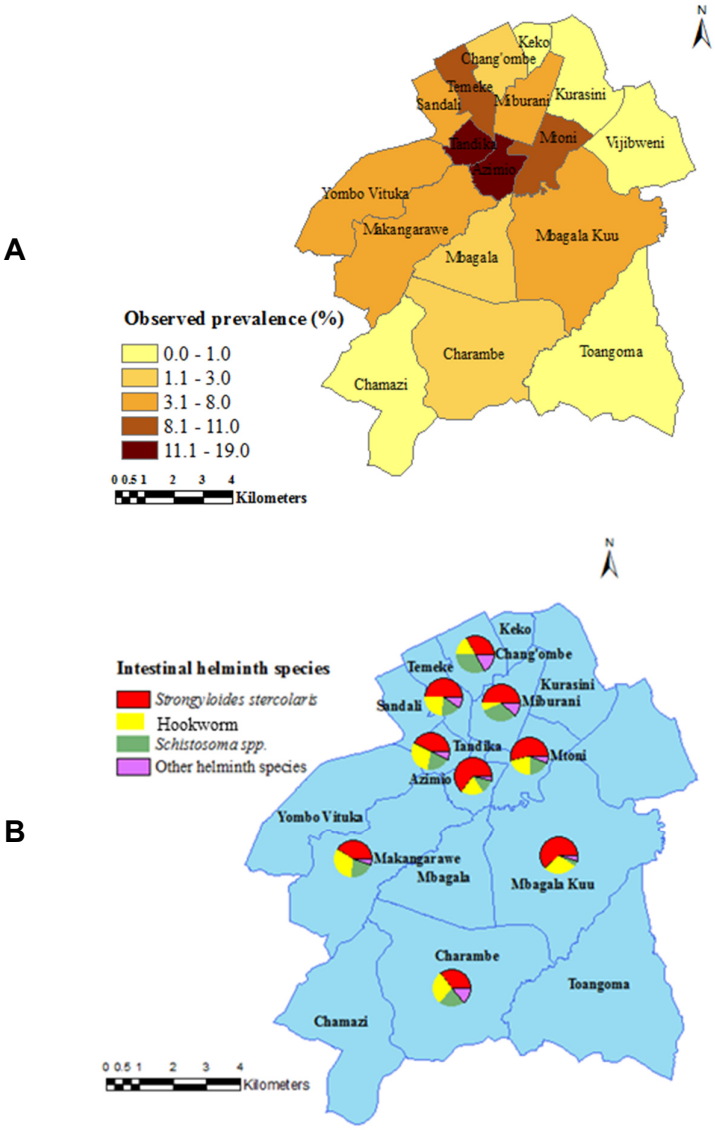
Supplementary Table 14. Association of helminth infection with poor recovery of BMI, poor gain of absolute weight, and percentage body fat in TB patients, between recruitment and after six months of completed TB treatment.

Helminth infection	Poor BMI gain		Poor weight gain		Poor gain in percentage body fat	
	aOR (95% CI)	p-value	aOR (95% CI)	p-value	aOR (95% CI)	p-value
Any helminth		0.23		0.63		0.78
No	1.00		1.00		1.00	
Yes	0.74 (0.46-1.21)		0.89 (0.55-1.45)		0.92 (0.55-1.56)	
Strongyloides stercolaris		0.49		0.11		0.34
No	1.00		1.00		1.00	
Yes	0.81 (0.44-1.47)		0.62 (0.34-1.12)		0.73 (0.39-1.38)	
Schistosoma mansoni		0.35		0.77		0.62
No	1.00		1.00		1.00	
Yes	0.68 (0.29-1.58)		0.88 (0.38-2.03)		0.80 (0.33-1.92)	
Hookworm		0.44		0.79		0.91
No	1.00		1.00		1.00	
Yes	0.72 (0.30-1.68)		0.89 (0.37-2.12)		0.95 (0.39-2.32)	
Multiple infections		0.27		0.44		0.96
None	1.00		1.00		1.00	
Mono	0.67 (0.40-1.13)		0.99 (0.59-1.68)		0.92 (0.52-1.62)	
Double or more	1.17 (0.44-3.11)		0.54 (0.21-1.41)		0.93 (0.35-2.53)	

Poor gain was defined defines as BMI change <2.6 kg/m^2, poor weight change, as weight gain <7 kg; poor body fat gain as percentage body fat <0.0%

Logistic regression model adjusted for: age, sex, HIV status, number of people in the household and income level quartiles.

Patients with any other helminth infection were excluded: 43 TB patients for *S. stercolaris*; 74 TB patients for *S. mansoni*; 75 TB patients for hookworm.



Supplementary Figure 1. Geographical distribution of helminth infections in the study area. (A) The prevalence of helminth infection summarized at the ward level. (B) The helminth species distribution at the study area. Other helminth infections include: *Ascaris lumbricoides*, *Enterobius vermicularis*, *Trichuris trichiura* and *Hymenolepis dimunita.*

7. Prevalence and Clinical Significance of Respiratory Viruses and Bacteria Detected in Tuberculosis Patients Compared to Household Contact Controls in Tanzania: a cohort study.

Authors: Francis Mhimbira [1,2,3 *], Hellen Hiza [1], Emmanuel Mbuba [1], Jerry Hella [1,2,3], Lujeko Kamwela [1], Mohamed Sasamalo [1], Monica Ticlla [2,3,4], Khadija Said [1,2,3], Grace Mhalu [1,2,3], Magreth Chiryamkubi [5], Christian Schindler [3,6], Klaus Reither [3,7], Sebastien Gagneux [2,3], Lukas Fenner [8 *]

[1] Department of Intervention and Clinical Trials, Ifakara Health Institute, Dar es Salaam, Tanzania, [2] Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Basel, Switzerland, [3] University of Basel, Basel, Switzerland, [4] Swiss Institute of Bioinformatics, Lausanne, Switzerland, [5] Department of Curative Services, Ministry of Health, Community Development, Gender, Elderly and Children, Dar es Salaam, Tanzania, [6] Department of Epidemiology and Public Health, [7] Department of Medicine, Swiss Tropical and Public Health Institute, Basel, Switzerland, [8] Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland

Corresponding authors*:
* Corresponding author. F. Mhimbira, Department of Intervention and Clinical Trials, Ifakara Health Institute, PO Box 78373, Dar es Salaam, Tanzania.
E-mail address: fmhimbira@ihi.or.tz (F. Mhimbira).

Dr. Lukas Fenner, Institute of Social and Preventive Medicine, University of Bern, Switzerland (lukas.fenner@ispm.unibe.ch)

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Short Title: Tuberculosis and respiratory pathogens

Competing interest
None of the authors have any competing interests to declare.

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7.1. Abstract

Objectives: To describe the prevalence of respiratory pathogens in tuberculosis (TB) patients and in their household contact controls, and to determine the clinical significance of respiratory pathogens in TB patients.

Methods: We studied 489 smear-positive adult TB patients and 305 household contact controls without TB with nasopharyngeal swab samples within an ongoing prospective cohort study in Dar es Salaam, Tanzania, between 2013 and 2015. We used multiplex real-time PCR to detect 16 respiratory viruses and seven bacterial pathogens from nasopharyngeal swabs.

Results: The median age of the study participants was 33 years; 61% (484/794) were men, and 21% (168/ 794) were HIV-positive. TB patients had a higher prevalence of HIV (28.6%; 140/489) than controls (9.2%; 28/305). Overall prevalence of respiratory viral pathogens was 20.4% (160/794; 95%CI 17.7-23.3%) and of bacterial pathogens 38.2% (303/794; 95%CI 34.9-41.6%). TB patients and controls did not differ in the prevalence of respiratory viruses (Odds Ratio [OR] 1.00, 95%CI 0.71-1.44), but respiratory bacteria were less frequently detected in TB patients (OR 0.70, 95%CI 0.53-0.94). TB patients with both respiratory viruses and respiratory bacteria were likely to have more severe disease (adjusted OR [aOR] 1.6, 95%CI 1.1-2.4; p 0.011). TB patients with respiratory viruses tended to have more frequent lung cavitations (aOR 1.6, 95%CI 0.93-2.7; p 0.089).

Conclusions: Respiratory viruses are common for both TB patients and household controls. TB patients may present with more severe TB disease, particularly when they are co- infected with both bacteria and viruses.

7.2. Introduction

Tuberculosis (TB) caused by the bacterium Mycobacterium tuberculosis affected an estimated 10.4 million new cases and caused 1.7 million deaths in 2016, making TB the leading global cause of death from an infectious disease (WHO, 2017). Influenza pandemics have selectively caused higher mortality among persons with TB compared to the general population (Noymer, 2009; Zürcher et al., 2016). For instance, the 1918 influenza pandemic brought about a sharp decline in TB burden, possibly because of the higher mortality among TB patients coinfected with influenza viruses (Noymer, 2011; Zürcher et al., 2016).

In sub-Saharan Africa, HIV has been the most important risk factor driving the TB epidemic in recent decades (WHO, 2017). The efforts towards understanding other risk factors in TB such as respiratory viruses, helminths (Mhimbira et al., 2017), and bacteria (Wu et al., 2013) are becoming increasingly important (Marais et al., 2013). Evidence from experimental mouse models suggests that respiratory viruses such as influenza viruses may play a pathogenic role in individuals with tuberculous disease by negatively affecting immunity against M. tuberculosis (Redford et al., 2013). The effect of respiratory viruses on TB may mimic the development of bacterial pneumonia immediately after an infection with respiratory viruses (Rohde et al., 2003). Studies of the lung microbiota (the community of microorganisms in the airways), which have focused on respiratory bacterial populations, suggest that there are differences in respiratory bacterial species populations among patients with and without TB, among new and recurrent TB patients, as well as among those in whom TB treatment has failed (Wu et al., 2013).

The differences in airway microorganism populations could indicate that respiratory pathogens can be involved in TB pathogenesis (Wood et al., 2017). However, little is known about the prevalence of respiratory pathogens, whether viral or bacterial, and their role in clinical presentation in TB. We therefore studied the prevalence of respiratory pathogens in TB patients and household contact controls, and assessed the associations between both respiratory viruses and bacterial pathogens and the clinical presentation of TB patients who were prospectively recruited in an area of Dar es Salaam (Tanzania) with a high TB burden.

7.3. Methods

7.3.1. Study setting and study population

This is a prospective cohort study conducted in the densely populated Temeke district of Dar es Salaam, Tanzania; it is a study within a previously described ongoing prospective cohort study of TB patients and household contact controls in Dar es Salaam (TB-DAR) (Mhimbira et al., 2017). Between November 2013 and October 2015, we recruited smear-positive TB patients diagnosed at Temeke district hospital and household contact controls who lived in the same household as the index TB cases (Mhimbira et al., 2017).

Assuming (a) a prevalence of respiratory viruses of 25% in TB patients and of 12.5% in household contact controls (Bigogo et al., 2013), based on the prevalence of respiratory viruses in similar settings, and assuming that respiratory viruses are more frequent in TB patients than in controls, (b) a cluster correlation of 0.2, and (c) a nonparticipation rate of 20%, we estimated that 175 case-control pairs would provide 85% power to observe a statistically significant difference in the prevalence at the 5% level of significance.

7.3.2. Study procedures

At the time of recruitment of study participants, we collected a single sample of nasopharyngeal swabs from TB patients and controls using flexible nasopharyngeal flocked swabs (Copan Diagnostics, CA, USA) ("FLOQSwabs™," n.d.). For TB patients, the nasopharyngeal swabs were taken immediately after diagnosis of TB and prior to initiation of anti-TB treatment. The nasopharyngeal swab samples were then added to 1-mL eNAT tubes for transportation at temperatures between 2° and 8°C to the Ifakara Health Institute (IHI) research laboratory at Bagamoyo where they were stored at -80°C pending analysis.

7.3.3. Laboratory investigations

Detection of respiratory pathogens by multiplex PCR We used the validated multiplex real-time PCRs from Seegene (www.seegene.com/) for detection of a broad panel of respiratory viral and bacterial pathogens in accordance with the manufacturer's instructions, as previously published (Cho et al., 2013). The nasopharyngeal swab samples were also analysed using Anyplex II RV16 simultaneously which detects 16 respiratory viruses, and the Allplex Respiratory Panel 4 assay which detects seven respiratory bacterial pathogens (Supplementary Table 15). Sample processing and analysis to detect respiratory pathogens were all done at the IHI research laboratory in accordance with the manufacturer's instructions and Standard Operating Procedures (SOP).

7.3.4. Other laboratory procedures

TB confirmation was by positive Lowenstein-Jensen (LJ) solid media mycobacterial culture (done at the IHI Research laboratory in Bagamoyo). We ruled out TB in controls with both a negative Gene Xpert MTB/RIF result and no mycobacterial growth in LJ solid media culture. HIV testing for TB patients and controls was done as per Tanzania HIV testing algorithms using an Alere Determine HIV (Alere, USA) and a Uni-Gold HIV (Trinity Biotech; Wicklow, Ireland) confirmatory test rapid tests (NACP, 2015). CD4þ T cells and full blood-cell counts were obtained as previously described (Mhimbira et al., 2017).

7.3.5. Data collection and definitions

Clinical severity of TB was graded as per published clinical TB score (Wejse et al., 2008), but modified to a set of 12 TB score parameters instead of 13, since tachycardia was not systematically measured as previously noted (Mhimbira et al., 2017). Diagnosis delay was defined as a cough duration of ≥3 weeks as previously published from the same cohort study (Said et al., 2017).

Data were captured using electronic case report forms developed from the open source data collection software Open Data Kit (ODK, https://opendatakit.org/) on Android PC tablets, and data were then managed using the eManagement tool 'odk_planner' as published previously (A. Steiner et al., 2016).

7.3.6. Statistical analysis

We compared the baseline characteristics of TB patients and household contact controls using the McNemar test, paired t-test, or Wilcoxon signed rank test, as appropriate. We estimated the prevalence of any respiratory viruses and bacteria using logistic regression models adjusting for clustering at the household level. We used mixed-effects logistic regression models with random household intercepts to assess the risk factors associated with detection of respiratory pathogens in TB patients and controls. The differences in the mean Ct values of respiratory bacteria detected (as a relative measure of the bacterial load) between TB patients and controls were assessed using mixed-effect linear regression models. Logistic regression models adjusting for age, sex, and HIV infection were used to assess the associations between respiratory pathogens and clinical presentations of TB at the time of recruitment among TB patients, with the following outcome variables: severe TB score (score of ≥6) versus mild (score of 1-5), high sputum bacterial load (sputum AFB smear microscopy of ≥2+) versus low bacterial load, and presence versus absence of lung infiltrations and cavitations (chest x-ray findings). Associations were expressed as crude odds ratios (ORs) and adjusted ORs (aORs) with their corresponding 95% confidence intervals (95%CIs). All analyses were performed in Stata version 14.0 (Stata Corp, College Station, Texas, USA).

7.3.7. Ethics approval

The study was approved by the IHI Institutional Review Board (IHI/IRB/No: 04-2015) and the Medical Research Coordinating Committee of the National Institute of Medical Research (NIMR/HQ/R.8c/Vol.I/357) in Tanzania, as well as by the Ethics Committee of the Canton of Basel in Switzerland (EKNZ UBE-15/42). All study participants gave written informed consent. TB patients were managed as per National TB and Leprosy Programme treatment guidelines (NTLP, MoHSW, 2013). Treatment and care for HIV-positive individuals were as per Tanzania National HIV/AIDS treatment guideline (NACP, 2015).

7.4. Results

7.4.1. Characteristics of study participants

Between November 2013 and October 2015, 972 study participants were enrolled in the TB-Dar study. A total of 794 study participants (81.6%; 794/972) had a nasopharyngeal swab taken, of whom 489 were TB patients and 305 were household contact controls (Figure 12). 1). The overall median age was 33 years (interquartile range (IQR) 26.1-41.2 years), and 61% (484/794) were men. The overall HIV prevalence was 21.2% (168/794; 95%CI 18.4-24.1%); 140 TB patients (28.6%; 140/489) and 28 controls (9.2%; 28/305) were HIV-positive (Table 14).

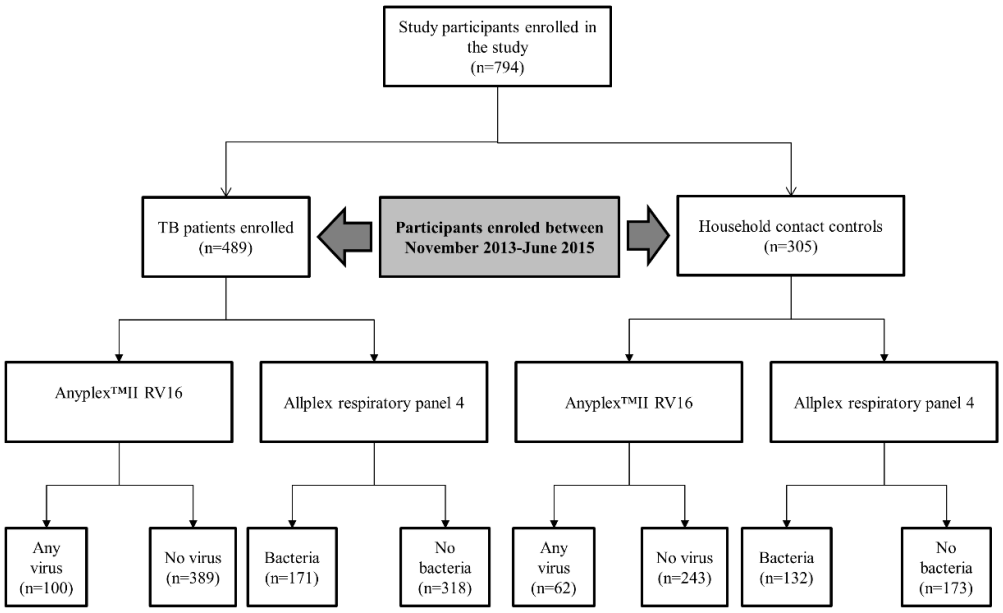

Figure 12. Flow diagram for participants enrolled in the study.

Table 14. Baseline characteristics of 489 tuberculosis (TB) patients and 305 household contact controls without TB in Dar es Salaam, Tanzania

Characteristics n (%)	All (n=794)	TB patients (n=489)	Controls (n=305)	p-value
Age (years), median (IQR)	33.0 (26.1-41.2)	33.0 (27.0-40.0)	32.0 (25.7-42.4)	0.7
Age groups (years)				0.096
<25	153 (19.3)	82 (16.8)	71 (23.3)	
25-34	290 (36.5)	188 (38.4)	102 (33.4)	
35-44	205 (25.8)	132 (27)	73 (23.9)	
≥45	146 (18.4)	87 (17.8)	59 (19.3)	
Male, Sex	484 (61.0)	336 (68.7)	148 (48.5)	<0.001
HIV-positive	168 (21.2)	140 (28.6)	28 (9.2)	<0.001
Education level				0.19
No/primary	285 (35.9)	168 (34.4)	117 (38.4)	
Secondary/University	509 (64.1)	321 (65.6)	188 (61.6)	
Occupation				0.25
Employed	509 (64.1)	321 (65.6)	188 (61.6)	
Current smoker [1]				0.053
Yes	104 (13.1)	73 (14.9)	31 (10.2)	
People in the household				0.22
>3 people	204 (25.7)	133 (27.2)	71 (23.3)	
Weight (kg), median (IQR)	54.0 (48.0-61.0)	51.0 (45.5-57.0)	59.0 (53.0-67.0)	<0.001
BMI (kg/m^2), median (IQR)	20.1 (17.5-23.4)	18.3 (16.5-20.4)	24.0 (21.7-27.9)	<0.001
BMI categories (kg/m^2)				<0.001
Normal/obese, ≥18	517 (65.1)	227 (46.4)	290 (95.1)	
Underweight, <18	277 (34.9)	262 (53.6)	15 (4.9)	
Body fat percentage (%)	9.92 (7.5-14.4)	9.41 (6.8-13.5)	11 (8.5-15.8)	<0.001
Hb level (g/dL), median (IQR)	12.1 (10.4-13.3)	11.4 (9.9-12.7)	12.8 (11.5-14.2)	<0.001
TB categories				
New	477 (97.5)	477 (97.5)	NA	NA
Retreatment	12 (2.5)	12 (2.5)	NA	

BMI, body mass index; IQR, interquartile range; Hb, hemoglobin; HIV, human immunodeficiency virus; NA, not applicable

7.4.2. Prevalence of respiratory viral and bacterial pathogens

The frequency distributions of the respiratory viruses detected from study participants are summarized in (Table 15). The overall prevalence of any respiratory virus among TB patients and controls was 20.4% (161/794; 95%CI 17.7-23.3%), and the odds of detecting any virus was the same in TB patients and controls (OR 1.00, 95%CI 0.71-1.44; p=0.98). The most common respiratory species detected was human rhinovirus A/B/C (HRV), which was found in 9.3% (74/794) of the study participants, followed by influenza A in 3.1% (25/794) and respiratory syncytial virus A (RSVA) in 1.9% (15/794). We detected only minor differences between TB patients and household contact controls in the prevalence (Table 15) and the semiquantitative detection (Supplementary Figure 2) of respiratory viruses. We detected respiratory viruses more frequently during the months of March and April, and October to November (Supplementary Figure 3).

The prevalence of any bacterial pathogen among study participants was 38.2% (303/794; 95%CI 34.9-41.6%, Table 2). Respiratory bacteria were less likely be detected in TB patients than in controls (OR 0.70, 95%CI 0.53-0.94; p 0.02). The most common bacterial species detected were *Haemophilus influenzae*, found in 26.1% of study participants (207/794), followed by *Streptococcus pneumoniae* in 21.5% (171/794). TB patients were less likely than household contact controls to have *H. influenzae* (OR 0.62, 95%CI 0.45-0.86; p=0.004). The mean values of cycle threshold for TB patients were slightly higher (indicating a smaller bacterial load) than those of household contact controls (greater bacterial load), but this did not reach conventional levels of statistical significance (Supplementary Figure 4). There were 12 TB patients on a retreatment drug regimen, and in ten of them (83.3%) both respiratory bacteria and respiratory viruses were detected.

The factors associated with detection of respiratory viruses include smoking and households containing three or more people. Men were the more likely to have respiratory bacteria (Supplementary Table 17)

Table 15. Frequencies of virus detection in the TB patients and household contact controls in Tanzania, and odds ratios of detection in TB patients compared to controls.

Detection of viral species	All	TB patients	Controls	OR (95% CI)	p-value
	(n=794)	(n=489)	(n=305)		
Any respiratory virus	162 (20.4)	100 (20.4)	62 (20.3)	1.00 (0.71-1.44)	0.98
Respiratory viral species					
Human rhinovirus A/B/C	74 (9.3)	42 (8.6)	32 (10.5)	0.80 (0.49-1.30)	0.37
Influenza A	25 (3.1)	15 (3.1)	10 (3.3)	0.93 (0.41-2.11)	0.87
RSV A	15 (1.9)	12 (2.5)	3 (1.0)	2.53 (0.71-9.05)	0.15
Adenovirus	14 (1.8)	9 (1.8)	5 (1.6)	1.13 (0.373.39)	0.83
RSV B	12 (1.5)	9 (1.8)	3 (1.0)	1.89 (0.51-7.03)	0.34
Parainfluenza virus 4	9 (1.1)	4 (0.8)	5 (1.6)	0.49 (0.13-1.86)	0.3
Coronavirus OC43	9 (1.1)	5 (1.0)	4 (1.3)	0.78 (0.21-2.92)	0.71
Coronavirus NL63	4 (0.5)	3 (0.6)	1 (0.3)	1.87 (0.19-18.12)	0.59
Enterovirus	4 (0.5)	4 (0.8)	0 (0)	NA	
Bocavirus 1/2/3/4	3 (0.4)	2 (0.4)	1 (0.3)	1.24 (0.11-13.83)	0.86
Parainfluenza virus 2	3 (0.4)	1 (0.2)	2 (0.7)	0.31 (0.03-3.44)	0.34
Influenza B	2 (0.3)	1 (0.2)	1 (0.3)	0.62 (0.04-10.0)	0.74
Parainfluenza virus 3	2 (0.3)	2 (0.4)	0 (0)	0.90 (0.41-1.97)	0.80
Metapneumovirus	1 (0.1)	1 (0.2)	0 (0)	NA	
Coronavirus 229E	1 (0.1)	0 (0)	1 (0.3)	NA	
Groups of detected viruses					
Influenza A/B	27 (3.4)	16 (3.3)	11 (3.6)	0.90 (0.41-1.97)	0.80
Influenza like (influenza and parainfluenza viruses)	40 (5.0)	23 (4.7)	17 (5.6)	0.84 (0.44-1.60)	0.59
Coronaviruses	14 (1.8)	8 (1.6)	6 (2.0)	0.83 (0.28-2.41)	0.73
Parainfluenza 2/3/4	13 (1.6)	7 (1.4)	6 (2.0)	0.72 (0.24-2.17)	0.56
RSV	25 (3.1)	19 (3.9)	6 (2.0)	2.01 (0.80-5.10)	0.14
Groups according to the number of detected viral species					0.96
One specie	145 (18.3)	89 (18.2)	56 (18.4)	0.99 (0.69-1.44)	
≥2 species	17 (2.1)	11 (2.2)	6 (2.0)	1.15 (0.42-3.13)	
Respiratory bacterial pathogens					
Any bacterial species	303 (38.2)	171 (35.0)	132 (43.3)	0.70 (0.53-0.94)	0.019
Respiratory bacterial species					
Haemophilus influenzae	207 (26.1)	110 (22.5)	97 (31.8)	0.62 (0.45-0.86)	0.004
Streptococcus pneumoniae	171 (21.5)	99 (20.2)	72 (23.6)	0.82 (0.58-1.16)	0.26
Legionella pneumophila	12 (1.5)	9 (1.8)	3 (1.0)	1.89 (0.51-7.03)	0.34
Bordetella parapertussis	4 (0.5)	3 (0.6)	1 (0.3)	1.88 (0.19-18.12)	0.59
Mycoplasma pneumoniae	0 (0)	0 (0)	0 (0)	NA	N/A
Bordetella pertussis	5 (0.6)	4 (0.8)	1 (0.3)	2.51 (0.28-22.54)	0.41
Chlamydophila pneumoniae	0 (0)	0 (0)	0 (0)	NA	NA
Groups according to the number of detected bacterial species					0.062
One specie	209 (26.3)	119 (24.3)	90 (29.5)	0.72 (0.52-1.00)	
≥2 species	94 (11.8)	52 (10.6)	42 (13.8)	0.67 (0.43-1.05)	

OR, odds ratio; 95% CI, 95% confidence interval; NA, not applicable

ORs and p-value calculated from a mixed-effects logistic regression models with random household intercepts

7.4.3. Associations between respiratory pathogens and clinical presentation

TB patients with both viral and bacterial pathogens had significantly more severe TB disease than TB-only patients (aOR 1.64, 95% CI 1.11-2.37; p=0.01; Table 16). Bacterial respiratory pathogens were not significantly associated with the clinical presentation of TB patients at TB diagnosis. Detection of respiratory pathogens was not associated with including diagnostic delay (defined as duration of symptoms of 3 weeks or more) (see Table 16). No association was found between detection of respiratory pathogens and chest x-ray findings among TB patients (Table 17). In addition, the detection of respiratory pathogens was similar for HIV-positive and HIV-negative TB patients (Supplementary Table 16 and Supplementary Table 17).

Table 16. Clinical significance of respiratory pathogens among TB patients at the time of TB diagnosis.

Respiratory pathogens detected	Severe TB score [1]		High sputum bacterial load [2]		Lung cavitation [3]		Lung infiltrations [3]		Diagnostic delay [4]	
	aOR (95% CI)	p-value	aOR (95% CI)	p-value	aOR (95% CI)	p-value	aOR (95% CI)	p-value	aOR (95% CI)	p-value
Respiratory viruses										
Any viral species		0.072		0.69		0.089		0.88		0.46
Yes	1.52 (0.96-2.4)		1.10 (0.69-1.76)		1.58 (0.93-2.68)		1.04 (0.60-1.83)		0.80 (0.45-1.43)	
Respiratory bacteria										
Any bacterial species		0.17		0.89		0.65		0.85		0.77
Yes	1.32 (0.89-1.94)		1.03 (0.69-1.53)		0.90 (0.56-1.44)		0.95 (0.58-1.56)		1.07 (0.67-1.71)	
Combined detection of viral and bacterial species		0.01		0.95		0.7		0.71		0.53
Yes	1.64 (1.11-2.37)		1.00 (0.68-1.46)		1.09 (0.70-1.71)		0.92 (0.57-1.46)		0.87 (0.55-1.37)	

aOR, adjusted odds ratios

Logistic regression model adjusted for age-group, sex, and HIV infection

[1] severe TB score (6 to 12) compared to mild TB score (1 to 5)

[2] High sputum bacterial load (≥2+ according to qualitative AFB smear microscopy grading) compared to low load (scant up to 1+)

[3] As determined by chest X-ray features

[4] Diagnostic delay defined as defined symptoms duration of 3 weeks or more.

Table 17. Chest X-ray findings of TB patients with and without any respiratory pathogen (viruses and bacteria).

Chest X-ray findings	Total	TB and respiratory pathogen(s)	TB only	p-value
	n (%)	n (%)	n (%)	
Any respiratory viruses				
Infiltrates	236 (64)	51 (64.6)	185 (63.8)	0.90
Cavitations	125 (33.9)	33 (41.8)	92 (31.7)	0.09
Pleural effusion	44 (11.9)	7 (8.9)	37 (12.8)	0.34
Lymphnodes	31 (8.4)	6 (7.6)	25 (8.6)	0.77
Micronodules	22 (6)	5 (6.3)	17 (5.9)	0.88
Macronodules	5 (1.4)	1 (1.3)	4 (1.4)	0.94
Any respiratory bacteria				
Infiltrations	236 (64)	78 (63.9)	158 (64)	0.99
Cavitations	125 (33.9)	40 (32.8)	85 (34.4)	0.76
Pleural effusion	44 (11.9)	15 (12.3)	29 (11.7)	0.88
Lymphnodes	31 (8.4)	11 (9)	20 (8.1)	0.77
Micronodules	22 (6)	9 (7.4)	13 (5.3)	0.42
Macronodules	5 (1.4)	1 (0.8)	4 (1.6)	0.53

p-value calculated from chi-square test.

7.5. Discussion

Both respiratory viruses and respiratory bacteria are commonly detected in a high-TB-incidence setting, and the prevalence of respiratory bacteria was lower in TB patients than in household contact controls. Detection of respiratory viruses and respiratory bacteria in TB patients was associated with more severe disease.

The prevalence of any respiratory viruses was the same (20%) for both TB patients and controls without TB. Similar prevalence of respiratory viruses in controls as compared to TB patients could be due to controls being more active than TB patients, hence having increased social contacts. The prevalence shown in our study was lower than that reported in an Indonesian study of influenza viruses that observed respective prevalence of around 46% and 41% for TB patients and controls, respectively (de Paus RA et al., 2013). The difference in the prevalence of influenza in that study compared to ours could be due to the different study region (Asia versus sub-Saharan Africa) and the use of different diagnostic methods (immunological assay versus molecular detection). We detected respiratory viruses from nasopharyngeal swabs using a highly sensitive and specific molecular technique (Niang et al., 2017)[21], whereas the study from Indonesia (de Paus RA et al., 2013) measured influenza virus antibody titres which also detect patients with previous exposure to influenza viruses. The influenza antibody titres were higher in TB patients than in controls, suggesting recent viral infection before the clinical manifestations of TB (de Paus RA et al., 2013).

We did not find any evidence for an association between HIV infection and detection of respiratory pathogens. In line with our results, a household study on respiratory illness surveillance and HIV testing in Kenya (Judd et al., 2015) did not find any association between HIV and influenza viruses. However, household contacts of the HIV-infected influenza index cases were twice as likely to develop a secondary case of influenza-like illness (Judd et al., 2015).

We also found that the presence of both viruses and bacteria could potentially alter the clinical course of TB, and present with a more severe disease as measured by the previously validated clinical TB score (Wejse et al., 2008). Direct evidence of clinical effects of respiratory viruses on TB have only been demonstrated in experimental mouse models that have exhibited higher mycobacterial loads in the lungs and increased lung inflammation (Redford et al., 2013). The immunological pathway responsible for more severe clinical presentation in TB patients may occur either via the type I interferon-receptor dependent pathway (Redford et al., 2013) or via decreased MHC II expression on dendritic cells (Flórido et al., 2013), which may result in poor clearance of *M. tuberculosis* from the lungs.

We found smoking and living in a household with three or more persons to be risk factors for respiratory viruses. Smoking has been shown, at least in animal models, to inhibit the pulmonary T-cell response to influenza viruses, thus increasing susceptibility to infection (Feng et al., 2010), and respiratory viruses were more likely to be detected in children living with a smoker (Nicolai et al., 2016). In addition, overcrowding which we defined as three or more persons in a household - is a common risk factor for most airborne pathogens such as *M. tuberculosis* (Corbett et al., 2009) and respiratory viruses (Judd et al., 2015).

The prevalence of bacterial respiratory pathogens in our study was lower in TB patients than in household contact controls, suggesting interactions between *M. tuberculosis* and the

bacterial populations in the airways. Overall respiratory bacterial load was smaller in TB patients than in controls. This is similar to findings from a microbiota study which reported smaller bacterial loads in TB patients than in controls (Cui et al., 2012). The authors argue that the initial phase of M. tuberculosis invasion in the lungs may prompt an immune response that could also reduce the commensal flora in the lower respiratory tract (Cui et al., 2012). Interestingly, in a mouse model, *M. tuberculosis* infection in the lungs appeared also to have an effect on the gut microbiota, which is part of the collective human microbiota (Winglee et al., 2014). These findings consistently suggest interactions between *M. tuberculosis* and the communities of microorganisms, and a role for these interactions in TB pathogenesis.

We believe that this is the first study to have looked systematically at a wide range of viral and bacterial species in TB patients and controls, and using sensitive molecular techniques and clinical specimens from a well-defined compartment of the airways. A particular strength of the study is that potentially confounding and unmeasured risk factors were minimized by studying patients and controls who lived in the same households. A limitation of the study is its undifferentiated attention to respiratory viruses because of small numbers which precluded assessment of the clinical effects of individual viruses. However, we presume that all respiratory viruses have similar levels of immunomodulation, and thus we could combine all respiratory viruses together.

In conclusion, respiratory pathogens are common in the high-TB setting of Tanzania for both TB patients and household contact controls without TB. However, respiratory bacterial species were more frequently detected in household contact controls than in TB patients. Our findings suggest that TB patients co-infected with both respiratory viruses and respiratory bacteria have severe TB disease. Further research should focus on the pathogenic role of respiratory pathogens in high-TB-incidence settings and their effects on clinical and treatment outcomes.

Transparency declaration
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7.6. Supporting information

Supplementary Table 15. Rv16 kit Panel A/B for detection of respiratory viral pathogens and Allplex respiratory panel 4 for detection of bacterial respiratory pathogens (Seegene, South Korea).

AnyplexII RV16 Detection		**Allplex respiratory**
Panel A	Panel B	Panel 4
Adenovirus	Respiratory syncytial virus A (RSV A)	*Mycoplasma pneumoniae*
Influenza A virus (FluA)	Respiratory syncytial virus B (RSV B)	*Chlamydophila pneumoniae*
Influenza B virus (FluB)	Bocavirus 1/2/3/4 (HBoV)	*Legionella pneumophila*
Parainfluenza virus 1 (PIV1)	Metapneumovirus (MPV)	*Haemophilus influenzae*
Parainfluenza virus 2 (PIV2)	Coronavirus 229E (CoV 229E)	*Streptococcus pneumoniae*
Parainfluenza virus 3 (PIV3)	Coronavirus NL63 (CoV NL63)	*Bordetella pertussis*
Parainfluenza virus 4 (PIV4)	Coronavirus OC43 (CoV OC43)	*Bordetella parapertussis*
Rhinovirus A/B/C (HRV)	Enterovirus (HEV)	

Supplementary Table 16. Characteristics of TB patients at recruitment by viral infection status.

Characteristics n (%)	Total (n=489)	TB and viruses (n=100)	TB only (n=389)	p-value
Age, median (IQR) (years)	33 (27-40)	33 (28-39)	33 (26-41)	0.99
Age groups (years)				0.60
<25	82 (16.8)	13 (13.0)	69 (17.7)	
25-34	188 (38.4)	42 (42.0)	146 (37.5)	
35-44	132 (27)	29 (29.0)	103 (26.5)	
≥45	87 (17.8)	16 (16.0)	71 (18.3)	
Sex, Male	336 (68.7)	67 (67.0)	269 (69.2)	0.68
HIV-positive	140 (28.6)	29 (29.0)	111 (28.5)	0.93
CD4 cell counts, cells/µl [1]	205 (91-280)	260 (160-342)	192 (90-263)	0.20
Number of people in the household				0.65
>3 people	133 (27.2)	29 (29.0)	104 (26.7)	
Current smoker				0.33
Yes	73 (14.9)	18 (18)	55 (14.1)	
Body weight (kg), median (IQR)	51 (45.5-57)	51 (46.0-56.8)	51 (45-57)	0.66
BMI (kg/m^2), median(IQR)	18.3 (16.5-20.4)	17.5 (16.2-20.1)	18.4 (16.7-20.4)	0.11
BMI (kg/m^2) groups				0.15
Normal/Obese, ≥18	227 (46.4)	40 (40.0)	187 (48.1)	
Low weight, <18	262 (53.6)	60 (60.0)	202 (51.9)	
Body fat percentage (%)	9.41 (6.79-13.5)	8.71 (5.91-13.5)	9.58 (7.23-13.5)	0.15
Symptoms				
Cough	486 (99.4)	99 (99.0)	387 (99.5)	0.58
Fever	453 (92.6)	91 (91.0)	362 (93.1)	0.48
Weight loss	474 (96.9)	99 (99.0)	375 (96.4)	0.18
Night sweats	467 (95.5)	94 (94.0)	373 (95.9)	0.42
Hemoptysis	133 (27.2)	32 (32.0)	101 (26.0)	0.23
TB score categories [2]				0.061
TB score (0-5)	313 (64)	56 (56.0)	257 (66.1)	
TB score (6-12)	176 (36)	44 (44.0)	132 (33.9)	
TB treatment categories				0.26
Retreatment	12 (2.5)	4 (4.0)	8 (2.1)	
New patients	477 (97.5)	96 (96.0)	381 (97.9)	
Sputum smear microscopy result [3]				0.78
Scanty and 1+ AFB	172 (35.2)	34 (34.0)	138 (35.5)	
≥2+ AFB	317 (64.8)	66 (66.0)	251 (64.5)	
Any prior antibiotic use				0.31
Yes	436 (89.2)	92 (92)	344 (88.4)	

AFB, acid-fast bacilli; BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range

[1] CD4+ count is for HIV-positive patients only

[2] TB score (mild [score of 1-5] and severe [score of 6-12])

[3] Sputum bacterial load (according to qualitative AFB smear microscopy grading): mild (scant and 1+) and severe (≥+2)

p-value calculated from chi-square test.

Supplementary Table 17. Characteristics of TB patients by bacterial infection status

Characteristics	Total (n=489)	TB and bacteria (n=171)	TB only (n=318)	p-value
Age, median (IQR) (years)	33 (27-40)	34 (29-42)	33 (26-40)	0.20
Age groups (years)				0.53
<25	82 (16.8)	23 (13.5)	59 (18.6)	
25-34	188 (38.4)	70 (40.9)	118 (37.1)	
35-44	132 (27)	47 (27.5)	85 (26.7)	
≥45	87 (17.8)	31 (18.1)	56 (17.6)	
Sex, Male	336 (68.7)	123 (71.9)	213 (67)	0.26
HIV-positive	140 (28.6)	50 (29.2)	90 (28.3)	0.83
CD4 counts, cells/mL [1]	205 (91-280)	234 (113-296)	198 (91-260)	0.39
Number of people in the household				0.75
>3 people	133 (27.2)	45 (26.3)	88 (27.7)	
Current smoker				0.70
Yes	73 (14.9)	27 (15.8)	46 (14.5)	
Body weight (kg), median (IQR)	51 (45.5-57)	51 (45-56)	51 (46-57)	0.22
BMI (kg/m^2), median(IQR)	18.3 (16.5-20.4)	18 (16.1-20)	18.4 (16.7-20.5)	0.10
BMI (kg/m2) groups, n (%)				0.23
Normal/Obese, ≥18	227 (46.4)	73 (42.7)	154 (48.4)	
Underweight, <18	262 (53.6)	98 (57.3)	164 (51.6)	
Body fat (%)	9.41 (6.79-13.5)	9.31 (7.13-13)	9.5 (6.49-13.9)	0.63
Symptoms [2]				
Cough	486 (99.4)	171 (100)	315 (99.1)	0.20
Fever	453 (92.6)	157 (91.8)	296 (93.1)	0.61
Weight loss	474 (96.9)	167 (97.7)	307 (96.5)	0.49
Night sweats	467 (95.5)	166 (97.1)	301 (94.7)	0.22
Haemoptysis	133 (27.2)	46 (26.9)	87 (27.4)	0.91
TB score categories [3]				0.20
TB score (0-5)	313 (64)	103 (60.2)	210 (66)	
TB score (6-12)	176 (36)	68 (39.8)	108 (34)	
TB treatment categories				0.020
Retreatment	12 (2.5)	8 (4.7)	4 (1.3)	
New patients	477 (97.5)	163 (95.3)	314 (98.7)	
Sputum smear microscopy result [4]				0.98
Scant and 1+ AFB	172 (35.2)	60 (35.1)	112 (35.2)	
≥2+ AFB	317 (64.8)	111 (64.9)	206 (64.8)	
Prior antibiotic use	436 (89.2)	152 (88.9)	284 (89.3)	0.89

AFB, acid-fast bacilli; BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range

[1] CD4 cell count is for HIV-positive patients only

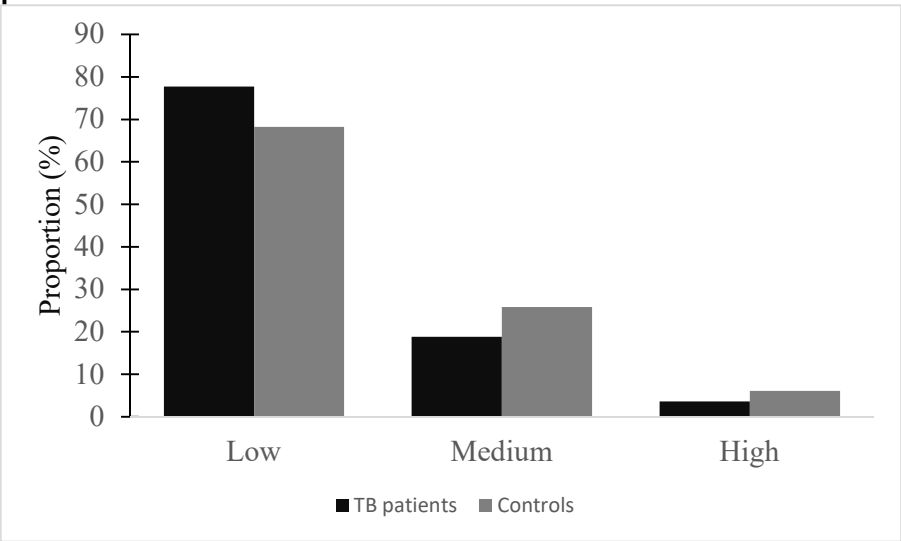
[2] categories mutually exclusive

[3] TB score (mild [score of 1-5] and severe [score of 6-12])

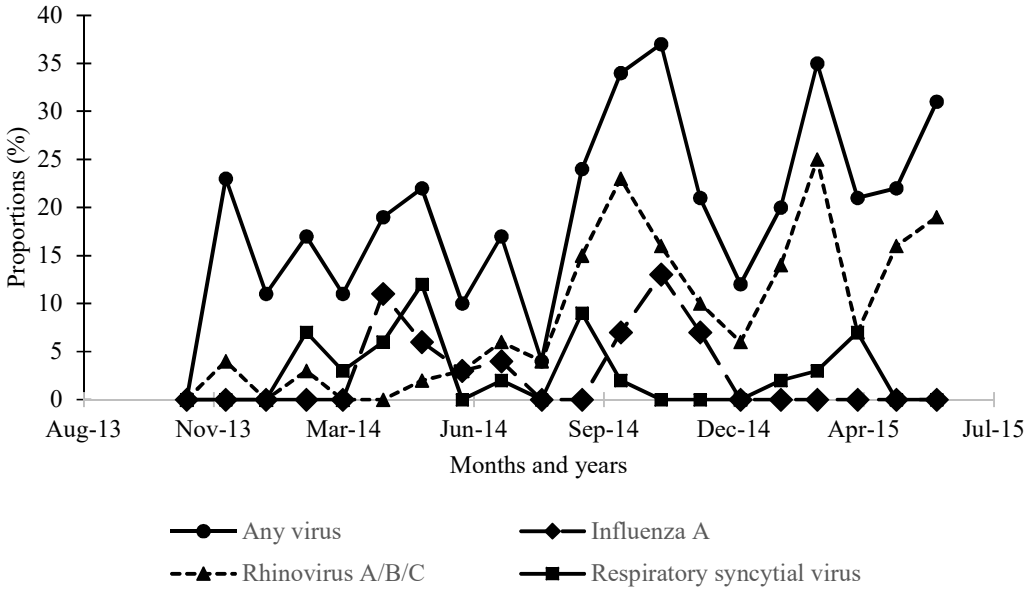
[4] Sputum bacterial load (according to qualitative AFB smear microscopy grading): mild (scant and 1+) and severe (≥+2)

p-value calculated from chi-square test.

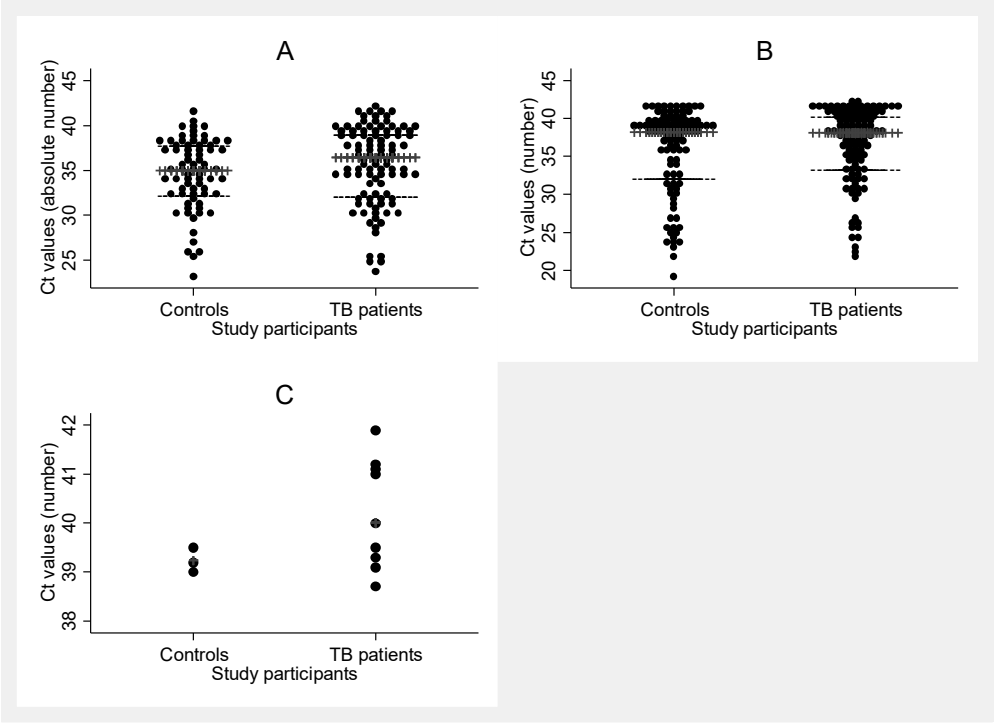
Supplementary Figure 2. Semiquantitative detection of respiratory viruses among TB patients and controls.



Supplementary Figure 3. Seasonal trends of the detection of respiratory viruses during the study period of November 2013 to June 2015.



Supplementary Figure 4. The Dotplot of mean Ct values of (A) *Streptococcus pneumoniae*, (B) *Haemophilus influenza*, and (C) *Legionella pneumophila* in TB patients and controls. Each individual dot represents a study participant.



8. Discussion

8.1. General discussion

This PhD project was done under the framework of TB-DAR study: a prospective cohort study of TB patients and household contact controls. The project is funded by Rudolf Geigy Foundation of Basel Switzerland, and is jointly implemented by IHI and Swiss TPH at Temeke district, Dar es Salaam Region in Tanzania. This PhD thesis has established the burden HIV, helminth infections and respiratory pathogens (viruses and bacteria) among TB patients and household contact controls. In addition, we have demonstrated TB treatment outcomes in high HIV setting, and the clinical significance of helminth infection and respiratory viruses among TB patients. To determine the burden of co-infections, we employed several quality controlled diagnostic tools. For helminth infection diagnosis, we used Kato-Katz, Baermann, urine filtration and CCA to increase overall sensitivity. We used multiplex PCR to simultaneously diagnosed 16 respiratory viruses and seven respiratory bacteria.

A summary discussion of the main findings of this PhD thesis are discussed in the subsequent sections. The findings on TB mortality in high TB and HIV setting under home-based and facility-based DOT are discussed in section 8.1.1 The prevalence and clinical significance of helminth and respiratory pathogens are discussed in sections 8.1.2 and 8.1.3 respectively.

8.1.1. TB mortality in high HIV setting

We found that TB mortality is high among TB patients who opted for home-based DOT compared to facility-based DOT. The uneven burden of mortality in home-based DOT is driven by uneven distribution of TB mortality risk factors. The risk factors we identified included HIV, features of advanced HIV disease (smear-negative PTB) and old age, as also previously described as causes of deaths among TB patients in high TB settings (Waitt and Squire, 2011). TB patients preferred home-based DOT because it is more convenient to them, and not facility based DOT which requires TB patients to make daily visits to the health facility for TB treatment (Wandwalo et al., 2006, 2005). Furthermore, the patients under home-based DOT may not have treatment supporter who could supervise the patient, a concern raised by the health care workers (van den Boogaard et al., 2009). The other assumption relating to this observation is that, mortality risk factors assessment is not done by the health care workers during TB diagnosis. Presuming no risk assessment is done, then very likely no additional clinical care is given to high risk TB patients who have opted home-based DOT.

TB deaths are essentially preventable and therefore can be avoided. TB treatment under DOT is effective and has good treatment success (Van Deun and Rieder, 2012). The absolute number of TB deaths reported in 2015 are actually higher compared to 2014, making TB a leading cause of deaths from an infectious disease (WHO, 2016). To reduce the TB mortality and contribute to the End TB Strategy, a risk assessment tools could prove beneficial as earlier suggested (Waitt and Squire, 2011). We think such a tool has to be robust and comprehensive enough to include risk factors for TB mortality in both low and high TB setting (Waitt and Squire, 2011). We would need to consider all emerging and existing comorbidities that may negatively affect TB treatment outcome (Marais et al., 2013), when designing such a tool.

8.1.2. TB and helminth co-infection

We found that *S. mansoni* infection was a risk factor for TB disease and alters clinical phenotypes of TB patients. TB patients infected with *S. mansoni* presented with lower smear microscopy grading and tended to have less lung cavities. Overall, TB patients had uneven higher burden of helminth infection compared to household contact controls. These findings in our study suggest that helminths, specifically *S. mansoni*, has a pathogenic role in TB pathogenesis. The findings are supported by the experimental mice models which showed helminth infection immunomodulate negatively the Th1 immune by inducing Th2 immune response (Babu and Nutman, 2016; DiNardo et al., 2016; Monin et al., 2015). Interestingly, certain helminth species like *S. haematobium* (Kjetland et al., 2006), *S. mansoni* (Downs et al., 2012) and *Wuchereria bancrofti* (Kroidl et al., 2016) are associated with increased risk of HIV infection, a major risk factor TB. Thus, from our findings and other studies, helminth infection seems to directly increase risk of developing TB, and indirectly through increasing the risk HIV infection.

To compare our findings on the association between TB and helminth infections, we performed a review of published researches reporting the association between TB and helminth as shown in Table 18. TB patients have higher helminth burden than either house or community controls (Abate et al., 2015; Elias et al., 2006; Tristão-Sá et al., 2002) or smear-negative TB patients (Range et al., 2007). We observed that *S. mansoni* is associated with TB, a finding that may suggest that not all helminth immunomodulation effects are equal in TB as observed with HIV (O'Hara and Elliott, 2016). *S. mansoni* eggs have soluble egg antigen (SAE) which is a potent immunogenic inducing a strong Th2 immune response (DiNardo et al., 2016; McKee and Pearce, 2004; Pearce et al., 2004). The assumption that *S. mansoni* is a stronger Th2 inducer, could be supported by our findings that TB patients infected with *S. mansoni* had lower sputum bacterial load and tended to have few lung cavities, compared to other helminth species identified in our study. The clinical picture of lower sputum bacterial load and less cavitary diseases, resembles the clinical manifestation of TB patients with severe immunosuppression caused HIV (Sharma et al., 2005). However, side by side comparison of helminth species on Th2 immune response could be more informative to assess level of immunosuppression caused by different helminth species.

Two studies reported no difference in TB incidence among individuals with or without helminth infection in the general population (Chatterjee et al., 2014) or among HIV-positive individuals (Brown et al., 2006). The use of anthelmintic treatment offered to the study participants after diagnosis in both studies might have reversed the Th1 immune response (Monin et al., 2015), and hence have no substantial Th2 immune response in the course of follow-up period to cause difference in TB incidence.

Table 18. Previous studies on the association of TB and helminth infection

Author-year	Setting	TB burden	Study design	Study population	TB diagnosis	Helminth detection methods	Common helminth species	Prevalence of helminth infection	Effect size (95%CI)	Significance
Abate, 2015	Ethiopia	High	Case-control study of smear-positive and community controls	TB patients (424) Community controls (306) 15-60 years	AFB Smear microscopy	Direct smear microscopy and Kato-Katz technique	*A. lumbricoides*	TB patients 40% (121/306) versus 28% (85/306) for community controls	Lower rate of sputum AFB smear positivity and the association was stronger with increasing smear grade (aOR for a smear grade 2: 0.43 (95% CI: 0.23–0.79), p = 0.007	Association with lower smear grading is indicative of immunosuppression caused by helminth infection.
Brown, 2006	Uganda	High	Prospective cohort study of HIV-infected patients	376 HIV positive patients 17-66 years	Mycobacterial culture, smear-microscopy, clinical diagnosis	Kato-Katz method or CAA ELISA was positive. Microfilaria were diagnosed using a modified Knott concentration method	*S. mansoni* Hookworm *S. stercoralis* *M. perstans*	Helminth prevalence was 54%	TB incidence with gastrointestinal nematodes (RR, 1.18; 95% CI: 0.66–2.10) or *M. perstans* (RR, 0.42; CI, 0.13–1.34). A weak association between *S. mansoni* infection and TB was found (RR, 1.42; CI, 0.86–2.34); after adjusting for potential explanatory variables and using more stringent diagnostic criteria (confirmed TB cases only, n=13), the association was strengthened (RR, 2.31; 1.00–5.33)	A weak association of increased incidence of TB among HIV-positive individuals. More pronounced association is observed with *S. mansoni.* Indicate species differences in inducing Th2.

AFB, Acid fast bacilli; aOR, adjusted Odds ratio; CCA, circulating anodic antigen; ELISA, Enzyme-linked immunosorbent assay; *M. perstans*, *Mansonella perstans*, RR, rate ratio;

Previous studies on the association of TB and helminth infection

Author-year	Setting	TB burden	Study design	Study population	TB diagnosis	Helminth detection methods	Common helminth species	Prevalence of helminth infection	Effect size (95%CI)	Significance
Chatterjee, 2013	India	High	Prospective cohort study	5096 study participants 6-65 years	ZN, followed by fluorescence microscopy and culture.	Direct microscopy and by formal-gasoline concentration techniques. *W. bancrofti* infection - measurement of CFA using an immunocromatographic test	Hookworm *W. bancrofti*	Hookworm (30%) *W. bancrofti* (9%)	No difference in TB risk at recruitment between study participants with and without helminth (RRi 1.60; 95% CI: 0.69, 3.71, p = 0.27), TB incidence during follow-up (RR 1.24; 95% CI: 0.48, 3.18, P = 0.66)	No association between helminth infection and TB incidence.
Elias, 2006	Ethiopia	High	Case-control study	TB patients (230) and healthy household contacts (510) 10-65 years	Sputum microscopy using the sodium hypochlorite concentration techniques	Direct microscopy and formol-ether concentration	*A. lumbricoides* Hookworm *S. stercoralis* *T. trichiura* *S. mansoni* *E. vermicularis*	Prevalence of intestinal helminths was 71% in patients and 36% in controls	Conditional logistic regression analysis showed a strong association between TB and intestinal helminth infection (aOR 4.2, 95% CI 2.7–5.9, p < 0.001). The odds of being a TB patient increased with the number of helminth species per person: mono-infection (aOR 4.3, 95% CI: 2.8–6.8); two species (aOR 4.7, 95% CI 2.5–8.7) and three or more helminths was (aOR 12.2, 95% CI: 3.9–52.6).	Increase risk TB which increases with increasing number of helminth species infecting a person. Dose dependent relationship of Th2 induction by helminth species.

aOR, adjusted Odds ratio; CFA, circulating filarial antigen; RR, rate ratio; RRi, risk ratio; ZN, Ziehl–Neelsen

Previous studies on the association of TB and helminth infection

Author-year	Setting	TB burden	Study design	Study population	TB diagnosis	Helminth detection methods	Common helminth species	Prevalence of helminth infection	Effect size (95%CI)	Significance
Li, 2016	China	High	Bayesian geostatistical modeling.	252,940 TB patients All age-groups	Smear microscopy and culture	Kato-Katz technique, test tube filter paper culture, adhesive cellophane anal swab	*A. lumbricoides* Hookworm *T. trichiura* *Clonorchis sinensis* Taenia spp	Prevalence of helminth infection 27.6%	Active PTB (75.1% [95% CI = 63.0–81.2%]) was spatially correlated to helminth	Geographical overlap of TB and helminth infection distribution. High helminth burden corresponds to high TB burden. .
Range, 2007	Tanzania	High	Cross-sectional	532 PTB+ patients and 123 PTB- (clinical TB patients) 15-85 years	Smear microscopy and culture (LJ)	Kato-katz and urine filtration	Hookworm *S. mansoni* *A. lumbricoides* *T. trichiura* *S. stercoralis*	Prevalence of helminth infection in PTB+ vs PTB- (*S. mansoni,* 35.5% vs. 29.3%: p=0.22); Hookworm 19.4% vs. 13.0%: p=0.11)	Not applicable	No association helminth infection and AFB smear-positive or smear-negative PTB.
Resende Co, 2006	Brazil	High	Prospective cohort study.	40 TB patients 18-53 years	Smear microscopy and culture	Lutz, Kato-katz and Baerman-Moraes methods	*S. stercoralis*	Helminth co-infection prevalence was in 27·5% (11/40) of patients TB	Severe radiological pulmonary disease for TB patients co-infected with helminth (p = 0·013) presenting as the number of involved lung zones at the end of TB treatment	Helminth effect could be long lasting especially on the radiological findings.

AFB, Acid fast bacilli; aOR, adjusted Odds ratio; CFA, circulating filarial antigen; PTB+, smear-positive PTB; PTB-, smear-negative PTB; RR, rate ratio; RRi, risk ratio; ZN, Ziehl–Neelsen

Previous studies on the association of TB and helminth infection

Author-year	Setting	TB burden	Study design	Study population	TB diagnosis	Helminth detection methods	Common helminth species	Prevalence of helminth infection	Effect size (95%CI)	Significance
Tristão-Sá, 2002	Brazil	High	Matched case-control study	57 TB patients and 86 controls Adults (age unspecified)	Unspecified	Lutz-Hoffman method	*A. lumbricoides* *S. stercoralis* *T. trichiura* *Necator americanus* *Ancylostoma duodenale*	Prevalence of intestinal nematodes in PTB compared to a matched control group: 33/57 (57.8%) vs 18/86 (20.9%).	Detection of at least one worm in TB patients compared to controls (OR=5.19; 95% CI= 2.33-11.69; p <0.001)	Disproportionate higher burden of helminth in TB patients compared to controls.

aOR, adjusted Odds Ratio; OR, Odds Ratio

8.1.3. TB and respiratory pathogens

Respiratory pathogens are common in our setting. We observed no difference in the prevalence of either respiratory viruses or bacteria in our study. We found that respiratory bacteria had no clinically significant effect on TB patients. However, TB patients infected with both respiratory and bacteria pathogens may manifest severe TB disease and cavitary disease. The results are consistent with increased mycobacterial growth may be because of decreased MHC II expression on dendritic cells (Flórido et al., 2013) or through type I interferon pathways (Redford et al., 2013). Though immunological pathways are from influenza infection models, we presume such pathways may be common for all other viruses.

We also reviewed the literature of studies reporting the association between TB and respiratory viruses which are shown in Table 19. Only one study measured IgG levels which signified recent viral infection, and higher levels of IgG were associated with severe disease TB disease as seen from the radiological findings (de Paus RA et al., 2013).

The other two studies reported higher mortality among TB patients (Archer et al., 2009; Walaza et al., 2015). The effect of respiratory viruses in humans from published research so far, point to higher TB mortality for patients who are co-infected with specifically influenza viruses. The influenza pandemics are good example of the selective pressure of influenza virus infection among TB patients and causing higher mortality (Noymer, 2011, 2009; Walaza et al., 2015; Zürcher et al., 2016). However, in this study we did not assess the association between influenza virus infection and TB mortality.

Table 19. Previous studies on the association of TB and respiratory viral pathogens

Author-year	Setting	TB burden	Study design	Study population	TB diagnosis	Respiratory virus diagnosis	Common respiratory pathogens	Prevalence of respiratory viruses	Clinical effect	Significance
Archer, 2009	South Africa	High	Prospective study	12,331 H1N1 influenza virus cases	As reported by the attending clinician.	Real-time PCR protocol distributed by the United States Centers for Disease Control and Prevention (US CDC) for the detection and characterization of pandemic influenza A (H1N1) virus	H1N1 influenza virus cases	Active tuberculosis coinfection was present in seven of 72 (10%) fatal cases	NA	Influenza virus and TB co-infection is common high TB burden setting. No definitive interaction between TB and influenza virus.
de Paus, 2013	Indonesia	High	Retrospective study	HIV-negative TB patients Community controls	Smear microscopy and culture.	IgG and IgM antibodies against two subtypes of influenza A virus	H3N2 and H1N1 viruses	46% of the TB patients and 41% of the controls had antibodies against H1N1, while 82% of the TB patients and 82% of the controls had antibodies against H3N2	Similar seroprevalence of antibodies between TB patients and controls. The geometric means of the titers of antibodies against higher for TB patients than controls. TB patient with advanced disease have higher IgG titers.	No correlation between presence of antibodies and manifestation of TB. TB patients have recent reinfection because of high titers of IgG Severity of radiological features may be associated with recent viral infection.

CDC, Center for Disease Control and Prevention; Ig, immunoglobulin; NA, Not applicable

Previous studies on the association of TB and respiratory viral pathogens

Author-year	Setting	TB burden	Study design	Study population	TB diagnosis	Respiratory virus diagnosis	Common respiratory pathogens	Prevalence of respiratory viruses	Clinical effect	Significance
Walaza, 2015	South Africa	High	Modelling of surveillance data of influenza cases	550,769 deaths All age groups				63,596 (12%) were PTB related deaths.	High mortality in PTB patients compared to HIV-infected non-tuberculosis respiratory deaths (relative risk (RR): 5.2; 95% CI: 4.6–5.9) and HIV-uninfected non-tuberculosis respiratory deaths (RR: 61.0; CI: 41.4–91.0) for age <65 PTB deaths were seasonal and increased each winter, coinciding with the period of influenza virus circulation	Increased mortality in persons with TB co-infected with influenza virus. TB mortality follows the influenza epidemics seasonality, signifying possible association between the two diseases.

RR, risk ratio

Table 20. Previous studies on the association of TB and respiratory bacterial pathogens

Author-year	Setting	TB burden	Study design	Sample size	TB diagnosis	Respiratory pathogens diagnosis	Common respiratory pathogens	Prevalence of respiratory pathogens	Effect size (95%CI)	Significance
Lin, 2011	Taiwan	Low	Cross-section	PTB patients (182) 16-93	TST positive, x-ray, culture	Gram stain and microbiological culture.	*M. pneumonia* *L. pneumonia,* *Klebsiella pneumoniae* *S. pneumonia* *H. influenzae*	54 (29.7%) had dual infections	NA	Dual infections are common and cause delay in TB diagnosis. Increased hospital morbidity and mortality with polymicrobial infections.
Wu, 2013	China	High	Cross-section	73 TB patients and 20 controls 13-79	Smear microscopy	PCR amplification of 16S rRNA for microbiota identification by 454 sequencing	*Streptococcus*, *Gramulicatella* and *Pseudomonas* were more abundant in TB patients. *Prevotella, Leptotrichia, Treponema, Catonella* and *Coprococcus* were less abundant in TB patients than in the healthy controls	In the TB patient group, most of the cases were dominated by Streptococcus (36% in new TB, 50% in recurrent TB and 45% in treatment failure TB), followed by Neisseria (24% in new TB, 26.7% in recurrent TB and 30% in treatment failure TB)	NA	Certain bacteria and the disorder of lung microbiota may be associated with not only onset of TB but also its recurrence and treatment failure. Differences in lung microbiota is indicative of pathogenic role of respiratory bacteria.

SED = Standard error of differences of percentages. OR = Odds ratio. ARR = Adjusted risk ratio. AHR = Adjusted hazard ratio. Not clearly stated (-)

8.2. Novel contribution of the thesis

Table 21. The contributions of the different chapters of this PhD thesis

Chapter	Title	Innovation	Finding/Validation	Implication/Application
Chapter 5	Home-based and facility-based Directly Observed Therapy of tuberculosis treatment under programmatic conditions in urban Tanzania	We have evaluated treatment outcome of TB patients under routine care from a large dataset. We analyzed the difference in TB mortality between home- and facility-based DOT.	More TB patients with risk factors for TB mortality opted for home-based DOT compared to facility-based DOT. TB mortality was higher in home-based DOT compared to facility-based DOT.	A thorough evaluation of the TB treatment under DOT to quantify risk factors and design a mortality risk factor tool.
Chapter 6	Prevalence and Clinical Relevance of Helminth Co-infections among Tuberculosis Patients in Urban Tanzania	A first survey in Tanzania to use a suite diagnostic procedure to diagnose helminth infection among TB patients and household contact controls.	*S. mansoni* infection is associated with TB disease and affect the clinical phenotypes of TB patients such as lower sputum smear grading and less formation of lung cavities.	Diagnosis and management of helminth should be an integral part of routine management of TB patients. The approach will reduce morbidity and potential unfavorable treatment outcomes. To consider bidirectional screening of TB and helminth in patients affected by both diseases.
Chapter 7	The clinical significance of respiratory viruses and bacteria among TB patients in Tanzania	The use of PCR molecular test detects respiratory viruses and bacteria from TB patients.	Respiratory viruses cause severe and cavitary TB diseases.	Vaccination may help to reduce the morbidity of TB and possibly improve TB treatment outcome.

DOT, directly observed therapy; PCR, Polymerase chain reaction

8.3. Challenges and opportunities for control of TB and HIV, helminth and respiratory pathogens control

The most challenging aspects of TB control are; i) 2-3 billion people are infected with *M. tuberculosis*, and can develop TB (WHO, 2016), ii) the burden of risk factors both communicable and non-communicable conditions are still high especially in high TB burden countries (Marais et al., 2013), and iii) reduced resources for TB control which threatens sustaining TB control efforts (WHO, 2016). The other emerging TB control challenge, is from the scientific evidence that helminths which affects over a billion (Knopp et al., 2012; Pullan et al., 2014; Utzinger et al., 2012), could actually fuel both TB and HIV epidemics.

Despite the challenges, lies the opportunities to control TB and associated co-infections. TB control is dependent on at least: i) reducing community exposures by reducing risk of *M. tuberculosis* infection and ii) reducing community vulnerability by controlling risk factors for developing TB such as HIV and helminth (Marais et al., 2013). The following sections will discuss opportunities to control TB for HIV (8.3.1), helminth infections (8.3.2) and respiratory pathogens (8.3.3) .

8.3.1. HIV and TB control

Tanzania has implemented the collaborative TB/HIV services since 2007, which aims at reducing the burden of TB and HIV for patients affected by both diseases. Highly active antiretroviral therapy (HAART) has reduced TB incidence (Badri et al., 2002; Houben et al., 2012) and TB mortality (Maruza et al., 2012). The activities that target reduction of co-infection burden of TB and HIV are stipulated in the TB and HIV management guidelines of Tanzania (NACP, 2015; NTLP, MoHSW, 2013).

The treatment modalities under home-based and facility-based DOT could be improved, and offers an opportunity to reduce TB mortality. Our findings propose the introduction of screening tool for TB mortality risk factors. The tools will include already defined risk factors and additional emerging risk factors. We postulate that such a tool will be able to reduce the TB mortality in both HIV-negative and HIV-positive TB patients. Thus, accelerate the progress to attain the End TB Strategy target of reducing TB mortality by 90% by 2035.

8.3.2. Helminth infection and TB control

Tanzanian Neglected Tropical Diseases Control Programme (NTDCP) is responsible in the control of schistosomiasis, STH, lymphatic filariasis and other NTDs. The National schistosomiasis and Soil-transmitted Helminths Control Programme (NSSCP) was established under the National School Health Program (NSHP) to control schistosomiasis and STH in the schools. NTDCP uses the MDA chemotherapy to control helminth burden in the country. The frequency of MDA are dependent on the burden of helminth (Prichard et al., 2012). To complement MDA in the control of helminth infection, environmental improvement, provision of clean water and safe water, and improving sanitation and hygiene are needed (Dangour et al., 2013; Strunz et al., 2014). These interventions will reduce the community vulnerability to helminth infection and subsequently reduce the potential risk of developing TB in the community.

The programmatic activities to be considered as opportunities to address TB and helminth co-infection burden are listed below:

i) Reciprocal screening programmes for TB and helminth infections
ii) Combined health promotion messages and communication
iii) Training activities for health care workers focusing on the relationship on the control of helminth and their effect on other pathogens such as TB, HIV and malaria (Salgame et al., 2013; Simon, 2016).

8.3.3. Respiratory pathogens and TB control

Our results show that respiratory viruses can influence the clinical phenotypes of TB patients. Vaccination of most common circulating viral strains may prove useful in the reducing the morbidity of TB that is associated co-infection with respiratory viruses. The use of respiratory virus vaccines, in particular influenza virus vaccine, can contributed to reduction of TB mortality as previously suggested in studies that reported higher mortality in TB patients infected with influenza virus (Archer et al., 2009; Walaza et al., 2015; Zürcher et al., 2016)

8.4. Conclusions

The following are the main conclusions of the chapters from the chapters of this PhD thesis:

- TB patients under home-based DOT had more frequently risk factors of death such as older age, HIV infection and sputum smear-negative TB, and had higher mortality compared to patients under facility-based DOT.
- *S. mansoni* is a common helminth infection even in an urban setting like Dar es Salaam where it was originally known to be lower. *S. mansoni* infection was an independent risk factor for active TB and altered the clinical presentation in TB patients. These findings suggest a role for schistosomiasis in modulating the pathogenesis of human TB. Treatment of helminths should be considered in clinical management of TB and TB control programs.
- Both respiratory viral and bacterial pathogens are common in TB patients and controls. Respiratory viruses seem to cause severe disease and with a cavitary diseases in TB patients.

8.5. Recommendations

8.5.1. What can be translated into practice and policies

a. Screening of TB mortality risk factors TB diagnosis which may provide a guide to better clinical care of high risk TB patients.
b. Helminth diagnosis and treatment to be an integral part of routine management of TB patient during TB treatment.
c. The control of neglected tropical diseases should be emphasized and resources be mobilized as complementary efforts to control TB morbidity and mortality especially in settings where TB and helminth burden are high.

8.5.2. Potential research questions moving forward

i. Quantifying the risk factors of TB mortality in high burden setting and development of screening tools and offer better management of TB under programmatic setting.

ii. The interventions studies to assess the effect of MDA of anthelminthic drugs on the incidence of tuberculous infection and TB.
iii. To assess differences in immunomodulation of helminth specie and clinical significance of such immunomodulation.
iv. To understand the pathogenic role of respiratory bacteria on the risk of developing TB from tuberculous infection.

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10. Curriculum Vitae

Dr. Francis Apolinary Mhimbira E-mail: fmhimbira@ihi.or.tz/fmhimbira@gmail.com

Bagomoyo Research and Training Centre Mobile No: - +255 754 291657
Ifakara Health Institute
P. O. Box 74
Bagamoyo, Coast Region
Tanzania.
ORCID ID: orcid.org/0000-0001-8989-6832

Career Summary

I am an epidemiologist and a research scientist with interest in infectious diseases epidemiology particularly tuberculosis (TB). I have skills in research designing, implementation, data analysis and scientific write up. I have participated in guideline development for HIV and TB programs in Tanzania.

Education

PhD in Epidemiology	Swiss Tropical and Public Health Institute (Swiss TPH), Basel, (Sept 2013 –expected completion date, February 2017) Basel, Switzerland
Master of Epidemiology (MEpi)	The University of Melbourne (2010) Melbourne, Australia
Doctor of Medicine (MD)	Muhimbili University College of Health Sciences (MUCHS), University of Dar es Salaam, Tanzania (2000 – 2005) Dar es Salaam, Tanzania
Short Courses:	
Summer School on Modern Methods in Biostatistics and Epidemiology	Organized jointly by Harvard School of Public Health, Boston, USA; University of Milano-Bicocca, Milano, Italy and Karolinska Institute, Stockholm, Sweden in Cison di Valmarino, Treviso-Italy **Courses taken:** 1. Flexible modeling of quantitative predictors 2. Missing data in observational and randomized trails 3. Survival analysis 4. Applied longitudinal analysis 5. Meta-analysis using Stata

Working experience and responsibilities

Principal Investigator and National Coordinating Investigator for 4 sites in Tanzania for NC-006 STAND Trial (Shortening Treatment by Advancing Novel Drugs): Jan 2015 - to date IHI, Tanzania

A multi-country and multi-centre Phase 3 Open-Label Partially Randomized Trial to Evaluate the Efficacy, Safety and Tolerability of the Combination of Moxifloxacin plus PA-824 plus Pyrazinamide after 4 and 6 months of Treatment in Adult Subjects with Drug-Sensitive Smear-Positive Pulmonary Tuberculosis and after 6 months of Treatment in Adult Subjects with Multi-Drug Resistant, Smear-Positive Pulmonary Tuberculosis. The study evaluates the combination of Moxifloxacin tablets, PA-824 tablets, Pyrazinamide tablets against standard TB treatment of Rifampicin 150mg/Isoniazid 75mg/Pyrazinamide 400mg/Ethambutol 275mg) and HR (Rifampicin 150 mg/Isoniazid 75 mg)

Principal Investigator, Tuberculosis case finding in pharmacies (TB-Pharm): November 2014 – February 2016 IHI, Tanzania

Tuberculosis case finding at the pharmacy using trained pharmacists and an electronically monitored referral system to reduce TB transmission in the community by shortening the diagnosis delay.

Co-Principal Investigator, Tuberculosis Cohort in Dar es Salaam: November 2013 - to date IHI, Tanzania

TB-DAR: Tuberculosis Cohort Study in the Dar es Salaam region (TB-DAR): a prospective collection of clinical data and biological specimens to study the epidemiology of tuberculosis, including molecular epidemiology and the evaluation of new diagnostics and biomarkers.

Co-Investigator, NC-002 Phase II Clinical Trial (Moxifloxacin tablets, PA-824 tablets, Pyrazinamide): June 2012–February 2014 IHI, Tanzania

A Phase II Open-Label Partially Randomized Trial to Evaluate the Efficacy, Safety and Tolerability of the combination of moxifloxacin plus PA-824 plus pyrazinamide after 8 weeks of treatment in Adult Patients with Newly Diagnosed Drug-Sensitive or Multi Drug-Resistant, Smear-Positive Pulmonary Tuberculosis.

Co-Investigator: Evaluation of new TB diagnostic tools (TB-CHILD): June 2012 – September 2013 IHI, Tanzania

Evaluation of new and emerging diagnostics for childhood tuberculosis in high burden countries (TB CHILD). A multi-centre study evaluating new diagnostics tools for adults and pediatrics in Tanzania and Uganda.

Co-Investigator, Tuberculosis Epidemiology and Management in Tanzania (TB-Cohort) June 2012 – September 2013 IHI, Tanzania

A prospective cohort study with three years of follow-up evaluating the prevalence and incidence of tuberculosis among presumptive TB patients and related co-infections.

Grants and awards

Jan 2015 - to date Principal Investigator for the Phase 3 Open-Label Partially Randomized Trial to Evaluate the Efficacy, Safety and Tolerability of the Combination of Moxifloxacin plus PA-824 plus Pyrazinamide after 4 and 6 months of Treatment in Adult Subjects with Drug-Sensitive Smear-Positive Pulmonary Tuberculosis and after 6 months of Treatment in Adult Subjects with Multi-Drug Resistant, Smear-Positive Pulmonary Tuberculosis.	Funded by Global Alliance for TB drug Development (TB Alliance) and the expected total budget was US $ 1,189,387.50. The STAND trial was stopped due to safety concerns.
November 2014 – February 2016 Principal Investigator for Tuberculosis case finding at the pharmacy using trained pharmacists and an electronically monitored referral system to reduce TB transmission in the community by shortening the diagnosis delay.	Funded by Grand Challenge Canada with a total budget of US $ 112'000

Publications

Hiza H, Fenner L, Hella J, Kuchaka D, Sasamalo M, Blauenfeldt T, Kibiki G, Kavishe RA, **Mhimbira F**, Ruhwald M. Boosting effect of IL-7 in interferon gamma release assays to diagnose Mycobacterium tuberculosis infection. PLoS One. 2018 Aug 29;13(8):e0202525. doi: 10.1371/journal.pone.0202525. eCollection 2018.

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Leadership Positions

September 2016-To Date: Head of Department - Interventions, Clinical Trials and Biomedical Sciences
Main Role: Drive department research agenda and ensure all researches are in line with IHI strategic objective
May 2015-To Date: Head of TB research group
Main role: Provides coordination and overall management of TB researches at IHI TB research group.

Professional Duties/Activities

Scientific Journals Reviewer	Journal: East African Health Research Journal (EAHRJ) Journal: Public Health Action: Health solutions for the poor Journal: Tanzania Medical Journal **Topics**: TB, TB/HIV and drug-resistance TB epidemiology
Memberships	International Union Against TB and Lung Diseases (The Union) 68 Boulevard Saint-Michel, 75006 Paris, France **Scientific sections**: TB, TB/HIV and Childhood TB. Member of following Technical Working Group within Ministry of Health Ministry of Health, Community Development, Gender, Elderly and Children, Dar es Salaam, Tanzania. 1. TB Operational Research 2. TB Laboratory and New Diagnostics 3. Programmatic Management of Drug Resistance TB

Referees

Dr. Honorati Masanja, PhD
Chief Executive Director
Ifakara Health Institute
Plot 463, Kiko Avenue Mikocheni
P. O. Box 78373, Dar es Salaam, Tanzania
Mobile:+255 784 605046
Email: hmasanja@ihi.or.tz

Dr. Fredros Okumu, PhD
Director of Science
Ifakara Health Institute
Plot 463, Kiko Avenue Mikocheni
P. O. Box 78373, Dar es Salaam, Tanzania
Mobile No: +255 686 997269
E-mail: fredros@ihi.or.tz

Prof. Sebastien GAGNEUX, Ph.D.
Head Department of Medical Parasitology & Infection Biology
Head Tuberculosis Research Unit
Swiss Tropical & Public Health Institute
Associated Institute of the University of Basel.
Socinstrasse 57, 4002 Basel, Switzerland
T +41 61 284 8369, F +41 61 284 8101,
http://www.swisstph.ch
E-mail: sebastien.gagneux@unibas.ch