The Effect of HIV and an Antiretroviral Treatment Programme on Tuberculosis Transmission, Incidence and Prevalence in a South African Township

By

Keren Middelkoop, MBChB

Thesis presented for the degree of
DOCTOR OF PHILOSOPHY
in the Department of Medicine
UNIVERSITY OF CAPE TOWN

February 2011

This thesis is presented in fulfillment of the requirements for the degree of Doctor of Philosophy (PhD) in the Department of Medicine, Faculty of Health Sciences, University of Cape Town. The work on which this thesis is based is original research and has not, in whole or in part, been submitted for another degree at this or any other university.

The contents of this thesis are entirely the work of the candidate (except where acknowledgements indicate otherwise), or in the case of multi-authored published papers, constitutes work for which the candidate was the lead author. The contribution of the candidate to multi-authored papers is further delineated in the preface to the thesis.

Keren Middelkoop
February 2011

Table of Contents

Abstract

Tuberculosis (TB) remains a significant cause of morbidity and mortality in countries with high HIV prevalence, such as South Africa. This doctoral work aimed to describe the epidemiology of TB in a high HIV and TB prevalent community from 1997 to 2008, and to assess the effect of HIV and a highly active antiretroviral therapy (HAART) programme on the TB epidemic in this setting.

The study community was a typical South African peri-urban township, with substantial burdens of both TB and HIV disease. The community was geographically well-defined, and population data were available from repeated community censuses. The community clinic provided TB treatment for all resident TB patients, in accordance with the national TB control programme guidelines, based on the World Health Organization's Directly Observed Therapy, Short-course programme. The HAART programme was implemented in 2003, and scaled-up in 2005. By 2008, 22% of the HIV-infected population was receiving HAART.

Data were drawn from a number of sources, including population surveys, TB notification registers, and HIV and HAART databases. Transmission to children was assessed using school-based cross-sectional tuberculin skin test surveys. The risk factors associated with TB transmission to children on residential plots were analyzed retrospectively, using adult and childhood TB cases extracted from the community TB notification register and the tuberculin survey results. Molecular epidemiology and geographic information systems were used to investigate TB transmission between adults, as well as the interaction between the HIV-associated and HIV-unassociated TB epidemics. The impact of the HIV epidemic, and of the HAART programme, on the burden of TB disease was assessed through analysis of TB incidence trends over time (as determined by notification rates) and a repeated cross-sectional community TB prevalence survey.

This thesis reported a substantial rate of TB transmission in the study community, and that risk factors for transmission to children and adults differed. Children were at increased risk of acquiring TB infection and disease from adult source cases on their residential plot, and the HIV status of adult source cases was not a risk factor for this

transmission. In contrast, adult to adult TB transmission did not occur primarily on residential plots. While the adult HIV-associated and HIV-unassociated TB epidemics were inter-related, HIV-infected TB patients contributed proportionally less to the adult transmission of TB than HIV-uninfected counterparts. Further, HIV infection was not associated with an increased risk of acquiring TB infection among adolescents. As a result, the impact of HAART on TB transmission appeared to be relatively small.

The HIV epidemic was responsible for a significant proportion of the burden of TB disease in the study community. Consequently, it was in TB disease that the greatest benefit of a HAART programme was noted, with a significant reduction in TB notification and TB prevalence rates. While the reduction in both TB notifications and prevalence was reported overall, it was predominantly noted in the HIV-infected population.

In conclusion, in a high TB and HIV disease setting, the HIV epidemic contributed significantly to the burden of TB disease, but was responsible for proportionally less TB transmission compared to HIV-uninfected patients. A high coverage HAART programme was associated with a reduction in the overall burden of community TB disease. However, the impact of HAART on TB transmission was relatively minor.

Acknowledgements

I wish to express my heartfelt appreciation and thanks to the following people:

- My supervisors, for so much more than the many valued hours of supervision.
    - Linda-Gail for years of mentorship and guidance, for believing in me and encouraging me to start this PhD journey. You have provided the vision and leadership for the research in this community, and your passion to see our work impact the lives of the people we serve is a constant inspiration to me. Your insights into these two diseases have been invaluable.
    - Robin for your support of this venture, and for hours of thought-provoking and instructive discussion. For helping me to focus on what is relevant, to push past the obvious, and then to step back again and consider the wood.
- Landon Myer for nudging me on to further studies, and for your ongoing encouragement. Thank you also for your guidance in the statistical analyses and especially for the Saturdays spent unravelling the mysteries of auto-regression analysis with me.
- Leigh Johnson for his assistance with the population and HIV models for the study community.
- Drs Catherine Orrell and Jennifer Zeinecker who oversaw the antiretroviral treatment (ART) programme in the study community, without which this project would not have been possible.
- Carl Morrow for hours spent patiently linking and extracting ART data from the databases, and for giving me a crash course in the ArcMap® programme.
- Simon Robertson for assisting with the mathematical models, and providing social interaction data from the study site.
- The incredible team of consultants and collaborators, who have worked with me, trained and mentored me over the last few years, including Gilla Kaplan, Barry Kreiswirth, Barun Mathema, Andrew Whitelaw and Alison Grant.
- Elaine Sebastian and the research team at the community site, without whom these studies would not have been possible.
- My wonderful family and incredible husband, for your love, patience and support, not only over the past three years, but always.
- My God: forever gracious, forever faithful.

The primary school tuberculin skin test surveys were sponsored by the City Bridge and Desmond Tutu HIV Foundations.

The remaining studies reported in this thesis, as well as my tuition fees, were sponsored by the National Institutes of Health's Comprehensive Integrated Programme of Research on AIDS (CIPRA) grant.

Preface

The following publications resulted from this thesis:

1) **Rates of tuberculosis transmission to children and adolescents in a community with a high prevalence of HIV infection among adults**. Middelkoop K, Bekker LG, Myer L, Dawson R, Wood R. *Clin Infect Dis.* 2008 Aug 1;47(3):349-55
2) **Childhood tuberculosis infection and disease in a South African township: a spatial and temporal transmission analysis.** Middelkoop K, Bekker LG, Morrow C, Zwane E, Wood R. *SAMJ* 2009 Oct; 99(10) 738-743.
3) **Molecular epidemiology of Mycobacterium tuberculosis in a South African community with high HIV prevalence**. Middelkoop K, Bekker LG, Myer L, Mathema B, Shashkina E, Kurepina N, Whitelaw A, Fallows D, Morrow C, Kreiswirth B, Kaplan G, Wood R. *J Infect Dis* 2009;200:1207-11.
4) **Effects of antiretroviral therapy on TB notification rates in a high HIV prevalence South African community.** Middelkoop K, Bekker LG, Myer L, Johnson LF, Kloos M, Morrow C, Wood R. *In press*
5) **Antiretroviral programme associated with reduction in untreated prevalent tuberculosis in a South African township**. Middelkoop K, Bekker LG, Myer L, Whitelaw A, Grant A, Kaplan G, McIntyre J, Wood R. *Am J Respir Crit Care Med* 2010 Oct 15;182(8):1080-5. Epub 2010 Jun 17.

The candidate was the project leader for all the studies presented in this thesis. The candidate took the lead in protocol development (under supervision of a number of local and international consultants, ranging in expertise from epidemiology, statistics, clinical medicine and microbiology), and was responsible for the implementation and management of these studies, including oversight of data acquisition. The candidate conducted all the data analyses presented in both the thesis and the publications, although guidance was provided by an epidemiologist and statistician regarding, in particular, the auto-regression and predictive modelling analyses. The candidate was the lead author on all the publications listed above, and was responsible for writing the first and subsequent drafts of all the papers and incorporating, where appropriate, comments from co-authors. Permission has been received from all the co-authors for the inclusion of these publications in the thesis.

Abbreviations

ACF	Active case-finding
AFB	Acid-fast bacilli
ART	Antiretroviral therapy
ARTI	Annual risk of tuberculosis infection
ASSA	Actuarial Society of South Africa
BCG	Bacillus Calmette-Guérin
CI	Confidence interval
CIPRA	Comprehensive International Programme of Research on AIDS
DNA	Deoxyribonucleic acid
DOTS	Directly observed therapy, short-course
DR	Direct repeat
DTHC	Desmond Tutu HIV Centre
ELISA	Enzyme-linked immunosorbent assay
EPTB	Extra-pulmonary tuberculosis
GIS	Geographic Information System
HAART	Highly active antiretroviral therapy
HIV	Human Immunodeficiency Virus
IQR	Interquartile range
IS	Insertion sequence
LJ	Lowenstein-Jensen
MDG	Millennium Development Goals
MDR	Multi-drug resistant
MGIT	Mycobacterial growth indicator tubes
MIRU-VNTR	Mycobacterial Interspersed Repetitive Unit - Variable Number Tandem Repeat
MOTT bacilli	Mycobacteria other than tubercule bacilli
Mtb	Mycobacterium tuberculosis
NIH	National Institutes of Health
OADC	Oleic acid, albumin, dextrose and catalase
OR	Odds ratio
PCR	Polymerase chain reaction
PEPFAR	President's Emergency Plan for AIDS Relief

PHRI	Public Health Research Institute
PPD	Purified protein derivative
PTB	Pulmonary tuberculosis
RFLP	Restriction Fragment Length Polymorphism
RR	Rate ratio
SNP	Single Nucleotide Polymorphism
sSNP	synonymous Single Nucleotide Polymorphism
std dev	Standard deviation
TB	Tuberculosis
TST	Tuberculin skin test
UMDNJ	University of Medicine and Dentistry of New Jersey
UNAIDS	Joint United Nations Programme on HIV/AIDS
UV	Ultra-violet
WHO	World Health Organization

Definitions

Tuberculosis Infection: Latent infection of an asymptomatic individual with *Mycobacterium tuberculosis.* Individuals with latent infection are not infectious.

Annual Risk of TB Infection (ARTI): The probability that a susceptible individual will become infected with *Mycobacterium tuberculosis* during a one year period[1]. ARTI is an averaged measure of risk of TB infection over the lifetime of the study participants[1;2].

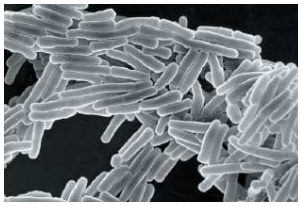
Force of Infection: The proportion of susceptible individuals that have become infected with *Mycobacterium tuberculosis* in a specified period. Force of infection provides a recent estimate of infection incidence, and can be estimated over a range of age groups.

Tuberculosis Disease: Active tuberculosis illness is characterised by actively multiplying *Mycobacterium tuberculosis,* resulting in clinical symptoms and/or the ability to recognize *Mycobacterium tuberculosis* in the patient's sputum or other involved organ.

Incidence of tuberculosis disease: The number of new cases of active tuberculosis disease occurring in a specified period of time[3]. Incident tuberculosis encompasses recognised (notified) and unrecognised tuberculosis disease, as well as TB patients who die before diagnosis.

Prevalence of tuberculosis disease: The proportion of a population with active tuberculosis disease, both treated (diagnosed) and untreated (undiagnosed), at a given point in time.

Highly Active Antiretroviral Therapy (HAART): Combination triple drug therapy used to treat patients infected with HIV. HAART is the current UNAIDS recommendation for the treatment of HIV-infected patients.



Chapter 1

INTRODUCTION

1.1 BACKGROUND

1.1.1 Epidemiology of Tuberculosis and HIV Epidemics

Tuberculosis (TB), cause by the bacterium *Mycobacterium tuberculosis*, is an ancient disease, with evidence of the pathogen dating back to ancient Egypt[4] and pre-Columbian Peru[5]. And yet, far from controlling or eliminating this disease, it remains a major cause of morbidity and mortality worldwide[6-9]. A third of the world's population is estimated to be infected with *Mycobacterium tuberculosis* (*Mtb*)[10], and the World Health Organization (WHO) reported a global TB disease prevalence of 11.1 million cases in 2008[11]. Of the 9.4 million incident TB disease cases in 2008[11], 15% were also HIV-infected. In the same year, TB-associated mortality accounted for approximately 1.3 million deaths among HIV-uninfected TB patients, with an overall mortality rate of 28 deaths/100,000 in HIV-infected and uninfected individuals[11].

HIV, in contrast, is one of the newest diseases known to man but has spread across the world with alarming efficiency. Globally, 33.3 million people are estimated to be living with HIV[12], with 2.7 million new infections occurring in 2008[13]. An estimated two million AIDS-related deaths occurred in the same year[13]. HIV-infected patients have a substantially increased risk of developing TB disease compared to HIV-uninfected individuals[14-16] and as a result the advent of the HIV epidemic is considered one of the primary factors responsible for the dramatic escalations noted in the TB epidemic in the past two decades[9;17-20]. It has been postulated that without the impact of the HIV epidemic, TB epidemics would be "in decline almost everywhere"[21]

Antiretroviral therapy (ART), specifically highly active antiretroviral therapy (HAART), substantially reduces the morbidity and mortality associated with HIV-infection[22-25] and UNAIDS recommends initiating ART in HIV-infected patients with CD4 count <350 cell/µl or WHO stage 3 or 4 disease. There has been a global call for the scale-up of ART availability, and through programmes such as the "3 by 5" initiative[26], the President's

[1] Photograph of *Mycobacterium tuberculosis* by Clifton Barry

Emergency Plan for AIDS Relief (PEPFAR)[27] and the Global Fund[28], there has been substantial progress in patients' access to ART[29] over recent years. By the end of 2009, it was estimated that, in low and middle income countries, 36% of HIV-infected patients who meet the UNAIDS treatment guidelines were receiving HAART[12].

TB and HIV in Sub-Saharan Africa

Africa is hardest hit by both the TB and the HIV epidemics. The African region has the highest per capita TB incidence, prevalence and mortality rates in the world[11], and poverty, a poor health infrastructure and increasing urbanization[30-32] are further challenges for TB control. WHO estimated there to be 3.8 million prevalent TB cases in Africa, with 2.8 million new cases occurring in 2008. The TB-associated mortality in the African region was 48/100,000 in 2008. Of the global incident TB cases, 78% of those co-infected with HIV occurred in Africa[11].

Two thirds of all HIV-infected people live in sub-Saharan Africa. In 2008, 1.9 million new HIV infections and 1.4 million HIV-related deaths were estimated for the region[13]. On a more positive note, sub-Saharan Africa boasts the largest scale-up of HAART access over the past five years, with 44% coverage being achieved by 2008[29]. However, TB remains the most common opportunistic infection[17;33;34] and the most common cause of death in people infected with HIV in Africa, including those receiving antiretroviral therapy[35;36].

TB and HIV in South Africa

With over a quarter of the global burden of TB/HIV[29], South Africa bears the brunt of these two epidemics. The country is undergoing rapid urbanization[37;38], with immigrants to the cities concentrating in poor and crowded peri-urban slums where both HIV prevalence and TB incidence rates are high[39]. By 2008, the HIV epidemic in South African adults aged 15 to 49 years had grown to an estimated prevalence of 16.9%[13] (Figure 1.1).

Over this same period, the incidence rates of TB in South Africa have increased more than 3-fold, with an estimated TB rate of 960/100,000 in 2008 (Figure 1.1). Of these more than 476,000 incident cases, 71% were thought to be co-infected with HIV[7;11]. Case detection of new sputum smear-positive TB cases was estimated to be 57% in

2007 and the treatment success rate was 74%[11]. TB prevalence in South Africa was estimated to be 606/100,000[11], with >110 000 TB-associated deaths in 2007[7].

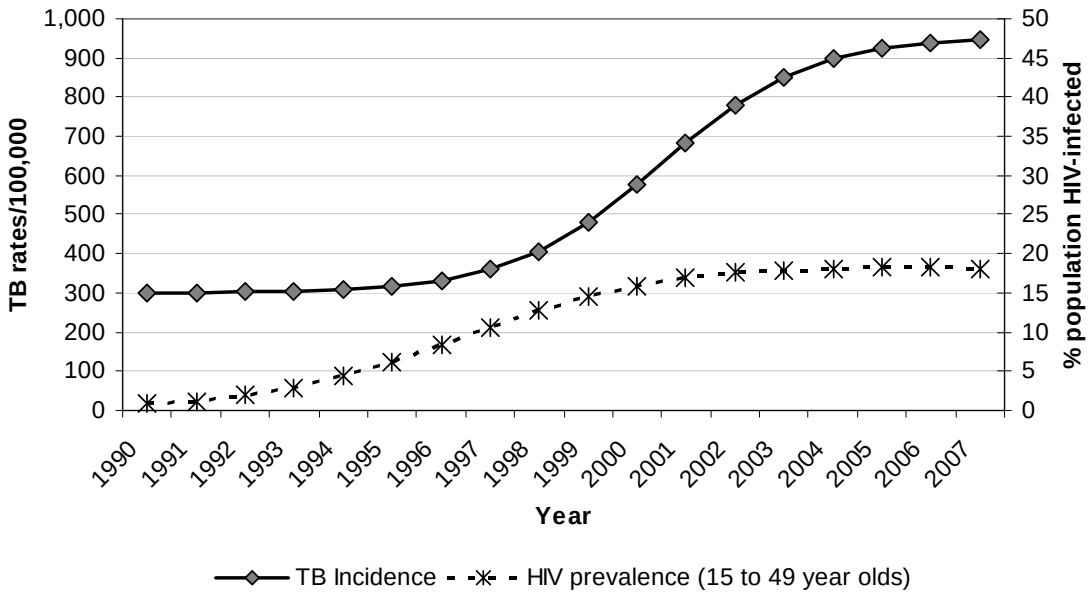


Figure 1.1: TB incidence rates[7] and HIV prevalence[40] in South Africa, from 1990 to 2007

According to the 2008 UNIADS report, 5% of HIV-infected patients in South Africa who meet the UNAIDS HAART treatment guidelines were receiving treatment in 2004, and this increased to 28% in 2007[40]. However, only 39% of all notified TB patients in South Africa had a known HIV test result and only 35% of HIV-infected TB patients were receiving or started on HAART in 2007[40].

Stop TB Partnership Targets

The STOP TB Partnership is a collaboration comprising of the WHO, together with countries, donors and international organisations, whose aim is to eliminate TB as a public health problem. In keeping with the Millennium Development Goals (MDG), the Stop TB Partnership aims to reduce the 1990 per capita TB disease prevalence and TB mortality rates by 50% by the year 2015. By 2050, they aim to have reduced incidence of active TB to <1 case/million population/year[7].

The STOP TB Strategy for achieving these goals includes (1) the pursuit of high-quality Directly Observed Therapy, short-course (DOTS) expansion and enhancement and (2)

addressing HIV-associated TB, multi-drug resistant (MDR) TB, and the needs of poor and vulnerable populations, as well as (3) contributing to primary care health system strengthening, (4) engaging all care providers, (5) empowering people with TB, and communities through partnership and (6) enabling and promoting research[7].

The technical elements of the DOTS strategy include early case detection of symptomatic patients through passive case-finding and utilizing sputum smear microscopy and, where appropriate and available, sputum culture. In addition, standardized short course chemotherapy regimens are recommended, with directly observed treatment during at least the intensive phase (first 2-3 months) of therapy. Key targets in the DOTS programme are the detection of 70% of all smear-positive TB cases and 85% treatment completion of detected cases[7].

Progress towards the Stop TB Partnership's targets for TB prevalence, incidence and mortality has been encouraging on the global scale, with disease incidence rates appearing to have peaked in 2007, disease prevalence rates falling and mortality targets already achieved in four of the nine WHO epidemiological sub-regions. However, it is well documented that the WHO Stop TB strategies are inadequate for controlling TB in African countries with high HIV prevalence[9;39;41;42] (Figure 1.2).

While TB disease incidence appears to have stabilized in the African region, the estimated incidence rates remain over 350/100,000 in 2008[11]. Of all the sub-regions, Africa is furthest from reaching case detection targets of new smear-positive TB cases, with only 39% of cases detected in 2008. It should, however, be noted that treatment success was achieved in 75% of those identified. The estimated 2008 TB prevalence and mortality rates remain substantially higher than those reported in 1990, and the latest WHO report acknowledged that achievement of the prevalence and mortality STOP TB targets "appears impossible in African countries"[11].

In 2005 the WHO Committee for Africa, declared the TB epidemic an Africa regional emergency [43], calling for "urgent and extra-ordinary actions" to assist in TB control. The STOP TB Partnership has proposed adjunctive strategies to address the ongoing difficulties in TB control in this setting, including the "Three I's" strategy for reducing the burden of TB in HIV-infected patients: intensified case finding, isoniazid preventative

therapy, and infection control[44]. However these strategies have not been widely implemented to date. Due to the reduction in TB risk in HIV-infected patients on HAART[45;46], ART has also been proposed as an adjunctive strategy for controlling the TB epidemic in low and middle-income countries with generalised HIV epidemics, such as South Africa[47]. This strategy has been more extensively implemented, as discussed above.

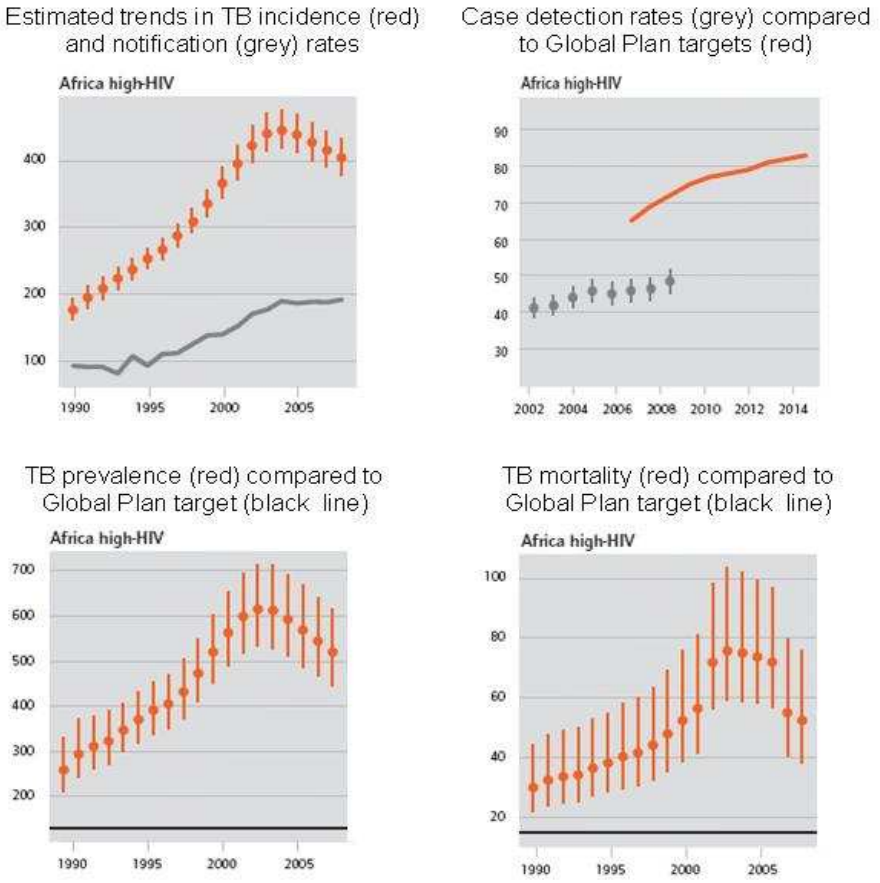


Figure 1.2: Progress towards Global Targets in African countries with high HIV prevalence[11]

1.1.2 Natural History of TB

Tuberculosis infection is caused by the bacterium *Mycobacterium tuberculosis*[48]. The natural history of exposure, infection and disease is illustrated in Figure 1.3.

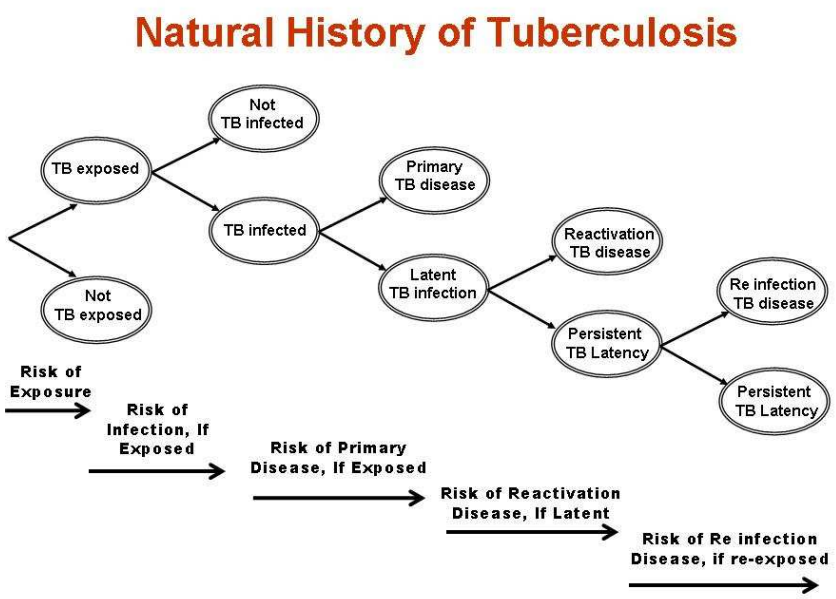


Figure 1.3: The Natural History of Tuberculosis (adapted from C Horsburgh)

TB Exposure and Infection

An individual's risk of exposure to *Mtb* is a function of the prevalence of infectious cases in their community, the duration of time the cases are infectious, and the number and nature of contacts the individual has with these cases[49]. The risk of infection following exposure for an individual who is not infected with TB (susceptible) is determined by a number of factors: the pathogen, the infectious patient, the susceptible individual and the environment[49]. Individuals already infected with *Mtb* can become secondarily infected following repeat exposure[50;51].

Mtb is primarily an airborne pathogen[48;52;53], and as a result tuberculosis is predominantly spread by those patients with respiratory tract disease[49] . TB patients with lung cavitation[20;54] and sputum smear-positive TB are the most infectious cases[54-59], with sputum smear-negative, culture-positive TB patients accounting for approximately 9-18% of transmission[55;57;60-62]. The tubercle bacilli are aerolised in droplet nuclei when the infected person talks or coughs[63]. Droplets of 1-3μm in size may remain suspended in the air indefinitely, and when inhaled are able to reach the alveoli of the lungs[52].

Environmental factors may substantially impact the risk of infection, through destroying or dispersing the tubercle-infected droplets. Air circulation, either as occurs naturally

outdoors or through ventilation, can dilute the concentration of infectious droplets, reducing the risk of inhaling infected droplets. Therefore a simple intervention such as opening windows in a house can significantly reduce an individual's risk of infection[49;60;64]. In addition to the rapid dilution of droplets that occurs outdoors, the ultra-violet (UV) rays of the sun are capable of killing suspended bacilli[53;65]. Therefore contact that occurs outside carries a lower risk of infection, compared to sharing a room with an infected person. Increased time and proximity of contact with an infectious person, such as in overcrowded conditions or intimate contacts, increases a susceptible individual's risk of acquiring TB infection[60;66;67].

Once in the susceptible individuals' alveoli *Mtb* may be eliminated by the host immune response or infection may be established. Of susceptible individuals exposed to *Mtb*, from zero to almost 100% may develop TB infection, depending on the conditions of exposure[55;57;68]. Infection may remain latent for months, years or for the individual's lifetime. In those that acquire infection, there is a 5-10% life-time risk of progressing to disease[55;60;69;70].

TB Disease

For an individual infected with *Mtb* the risk of developing TB disease is, again, a function of a number of factors related to the infected individual, the infecting pathogen and the environment.

TB disease is the result of progression of recent, exogenous TB infection or reactivation of previous, latent infection. The time period since becoming infected is a strong risk factor for developing disease[71-73]: patients with recent infection are 10 times more likely to progress to TB disease compared to those with chronic, latent infection[49]. It is estimated that for a child newly infected with TB at least half the risk for developing disease occurs in the first two to five years following infection[1;57;60;72].

The risk of progressing from TB infection to disease is largely dependent on the ability of the infected individual's immune defenses to contain the TB infection[49]. HIV infection has been identified as the most significant risk factor in progression to TB disease[14;15;49;74] due to increasing immune deficiency[75;76]. Other medical conditions associated with the increased risk of TB disease include diabetes[77;78], silicosis[79;80], cancers such as

lymphoma[81;82] and lung cancer[82], and certain medications such as high dose corticosteroids[83-85].

There is evidence to suggest that the dose of bacilli may be a risk factor for TB disease: studies report that the risk of disease is greater if infection is caused by sputum smear-positive disease compared to sputum smear-negative cases[55-57]. The virulence of the inhaled pathogen is another factor that may influence the risk of disease[86;87]. In addition, there are environmental factors that also increase an individual's risk of TB disease including smoking[88;89], alcohol abuse[73;90-92] and malnutrition[73;93;94].

1.1.3 Interaction between TB and HIV

HIV infection has a major impact on the natural history of tuberculosis, and subsequently on TB epidemics in high prevalence areas. In TB endemic areas, such as South Africa, *Mtb* is the most common opportunistic infectious disease in HIV-infected patients[17;18;34;95] and is often the presenting opportunistic infectious disease that leads to the diagnosis of AIDS[14;18]. TB also occurs more frequently in HIV-infected patients at higher CD4 counts than other opportunistic infectious diseases[34;75;96].

TB Infection and HIV

To date, there is little evidence to suggest that following exposure to TB, HIV-infected patients are at increased risk of developing TB infection. However, studies assessing this risk identify TB infection status using tests reliant on immune response, such as tuberculin skin tests (TST)[14;97]. False negative TST results in HIV-infected patients are common, due to anergic immune responses in advanced HIV disease[98-101]. Therefore TB infection in HIV co-infected patients may be underestimated in these studies. One molecular epidemiological study of a TB outbreak among co-inhabiting HIV-infected individuals in San Francisco suggested that HIV-infected patients may be at increased risk of acquiring HIV infection[102], but evidence for this was not statistically substantiated.

TB Disease and HIV

In contrast, it is well documented that HIV-infected patients have a substantially increased risk of developing TB disease compared to HIV-uninfected individuals[14-16]: a 10% annual risk of TB[14] versus 10% life-time risk[55;69;70].

The immune response to infection by *Mtb* is a complex cell-mediated response, in which the CD4 lymphocyte is the key effector and mediator[103;104]. Interleukin-driven expansion of CD4 lymphoctyes results in the T-cells secreting interferon γ, which in turn activates macrophages. Additional chemokines and cytokines assist with further recruitment and activation of monocytes[105]. The aggregation and differentiation of these activated macrophages and lymphocytes results in the formation of a granuloma[106]: the primary mechanism within which the immune system controls *Mtb* infection[107]. The granuloma contains potentially viable *Mtb* organisms[104], and in this way latent infection is achieved. With the progression of HIV disease, the quantity and the functioning of CD4 T cells is adversely affected[75;108;109], resulting in the compromise of both granuloma formation to new infections, as well as maintenance of established granuloma[104;110].

Consequently, HIV-infected patients have an increased risk of rapid progression to active TB following new infection[14;102;111] and an extraordinarily high rate of re-activation of latent tuberculosis infection compared to HIV-uninfected individuals[14;112]. South African studies among HIV-infected patients have shown that from the time of HIV infection the risk for TB disease doubles[16] and this risk continues to increase as the CD4 cell count decreases[45;75;113].

Impact of HIV on TB epidemics

The HIV-associated TB epidemic has changed the face of the generalized TB epidemic in high burden co-infection areas. As the risk of TB infection declined in developed countries, the incidence of adult TB disease shifted from young adults into the older age groups, occurring predominantly in males >45 and females >65 years of age[114;115]. In the 1980's there was evidence to suggest that TB infection rates were decreasing in developing countries[116], and it was suggested that a similar shift in the distribution of TB disease was starting to emerge in these countries[117]. However, with the escalation of the HIV epidemic among the younger, sexually active population, the incidence of TB disease has concentrated in the 20-40 year old population group[39;118]. HIV-infected patients, particularly those with advanced HIV disease, are recognized to have atypical presentations of TB infection, including an increase in extra-pulmonary[18;118-121] and disseminated TB[18;120;121], and an increased likelihood of smear-negative pulmonary TB[18;118;122-124]. Furthermore, HIV-infected patients are at increased risk of relapse of TB

disease following TB treatment, whether through re-infection or reactivation of latent infection[125-129].

TB disease is the leading cause of death in HIV-infected patients in sub-Saharan Africa[33;95;130;131]. In autopsy studies of HIV-infected patients, TB disease was identified in 40-51% of patients[33;130;131]. TB patients co-infected with HIV have a higher mortality than HIV-uninfected TB patients[131-134] and mortality in HIV-infected patients with TB is two to three times higher than that of HIV-infected patients without TB disease[15;112;135].

Impact of TB on HIV

Tuberculosis, in turn, has been shown to have an effect on the natural history of HIV infection. TB disease accelerates the progression of HIV-associated immune deficiency, as demonstrated by a significant drop in CD4 T cell counts in patients with active TB disease[96;112]. Furthermore, active TB disease enhances viral replication[96;136-140], and studies have indicated that viral load may not return to baseline levels despite an apparently good response to TB treatment[96;140-142]. As a result, HIV-infected TB patients have both an accelerated clinical course[112;143], with more opportunistic infections compared to HIV-infected patients at similar CD4 cell counts who do not have a recent history of TB[135;143], and a significant reduction in survival rates[112;135;144;145].

1.1.4 Impact of HAART on Individual TB Risk

Highly active antiretroviral therapy inhibits viral replication, which in turn is associated with the restoration of the immune system[146-148]. Studies have shown improvement in both the quantity and function of CD4 cells in patients on HAART. However, it must be noted that function of CD4 cells does not appear to be fully restored[146;147], especially when HAART initiation occurs at advanced stages of HIV disease[149;150]. This not withstanding, as a result of immune recovery, HAART has been shown to reduce both morbidity and mortality associated with tuberculosis disease[23;151;152] (Figure 1.4).

While the risk of TB disease incidence is estimated to be 5-10% per annum in untreated HIV-infected cohorts[14;153], HAART treatment cohort studies from sub-Saharan Africa have reported a reduction in incidence of active TB of 61-90%[35;45;46;154-158]. TB incidence rates are high in the first three months of antiretroviral treatment[157;159-161], with rates of up to 22 cases/100 person-years reported in this period[35]. However, with increasing time on HAART, the incidence of TB declines significantly, decreasing to 2.2-7.4/100 person-

years by two to three years on treatment[35;156;157;162], and rates as low as 1.01/100 person years reported at 5 years of HAART[46] (Figure 1.5).

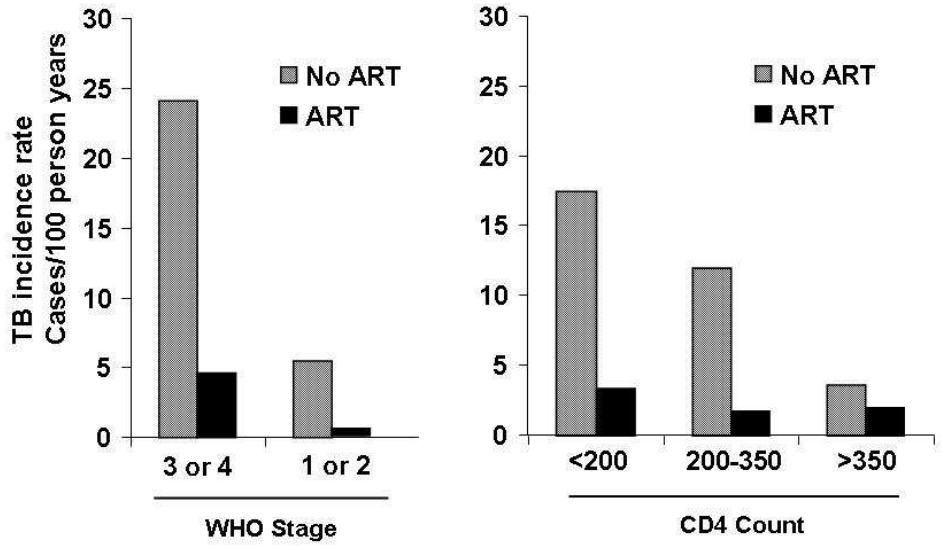


Figure 1.4: Risk of TB disease on and off HAART, stratified by WHO clinical stage and CD4 cell count strata[45]

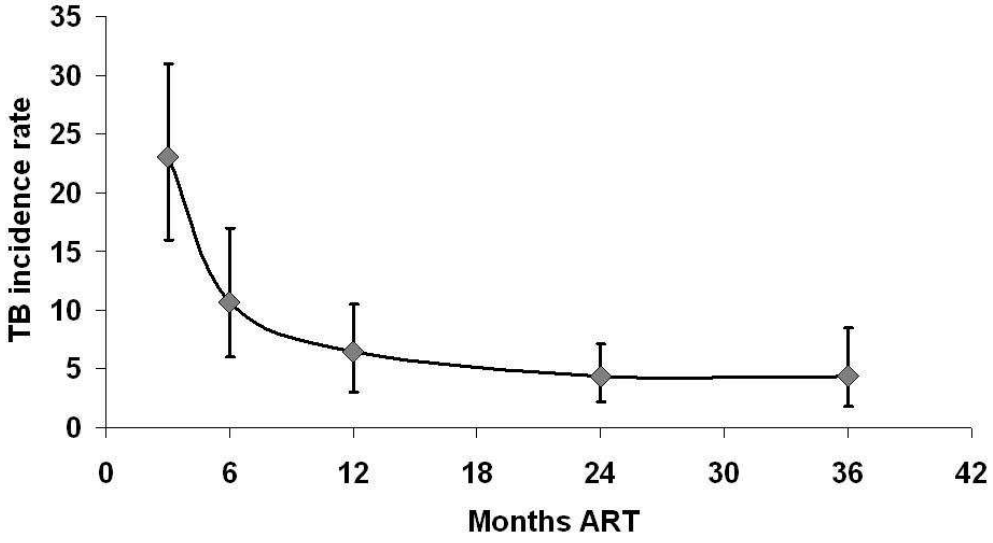


Figure 1.5: Declining TB incidence with increasing duration on HAART[163]

TB is the most common opportunistic infection occurring in the early months of HAART, both in developed and developing countries[155;159;162;164;165]. The high rates of TB in the early months of treatment may be due to risks associated with low baseline CD4 counts[45;46;152;165] and persisting immune deficiency in the early months of HAART[146]. However most patients respond with a rapid decline in viral load and associated increase in CD4 cells[146;147], which may, in turn, result in the unmasking of previously subclinical disease. The rapid recovery and reactivation of the immune system can also result in dysregulation of antigen specific responses, leading to an exaggerated inflammatory response and presentation of TB disease in the first 3 months on HAART[106;161;166-168].

Low CD4 cell count (<100 cells/mm^3), whether at baseline or at five years on treatment, was consistently found to be a key risk factor for developing TB disease on HAART[35;45;46;152;154-156].

Despite these substantial declines in TB risk on HAART, two to three years into HAART treatment patients still have a 2-10 times higher risk of TB disease compared to HIV-uninfected individuals[35;156;165;169]. Studies show that the reduction in TB risk is lowest in those that have a suboptimal immunological response to HAART, as determined by CD4 cell count recovery[35;46;170]. Because TB disease occurs across a wider range of CD4 cell counts than most other opportunistic infections, this persistently elevated risk of TB on HAART may also be due to incomplete functional recovery of the immune system on HAART[35;146;147].

In addition to reducing the risk of TB disease, HAART has been associated with decreased mortality in HIV-infected patients who develop TB[171;172]. This reduction in TB-associated mortality associated with HAART may be as high as 52-60%[157;173].

1.1.5 Population Level Impact of HAART

While the impact of HAART on TB risk for an HIV-infected cohort is well defined, the impact of HAART on TB rates at a population level remains uncertain.

Rationale:

- Impact on HIV-associated TB Rates

As discussed above, the HIV epidemic is driving the TB epidemic in countries with high HIV and TB burdens such as South Africa, where as many as 70% of TB cases are HIV co-infected[7]. As the incidence of active TB disease in HIV-infected patients is substantially reduced on HAART, it can be argued that wide-scale HAART programmes should have a considerable, positive impact on TB epidemics. However, HIV-infected patients on HAART still have a substantially increased risk of TB disease compared to HIV-uninfected individuals, and the combination of prolonged survival and residual increased risk of TB disease will result in an increased number of highly susceptible individuals in the population. In addition, HAART is generally initiated relatively late in the HIV disease process, with the result that as many as 50% of these patients have a prior history of TB in the past two years and have therefore already contributed substantially to the population TB rates[35;174]. Therefore even substantial population coverage with HAART may have a limited impact on TB incidence at a population-level.

- Impact on TB Transmission

In addition to the direct benefits of HAART with regards to the reduction of HIV-associated TB rates, ART programmes may also have indirect benefits on TB epidemics, ie a possible reduction in TB transmission. The impact of HAART programmes on TB transmission will be determined, in part, by the contribution of HIV-associated TB to transmission in communities. As discussed previously, the infectiousness of a TB patient is a function of the bacillary load a patient expectorates and the time they remain infectious in the community.

HIV-co-infected TB patients are considered to be less infectious than HIV-uninfected TB patients due to higher rates of extra-pulmonary TB[18;118-121] and lower rates of smear-positive[18;118;122;123] and cavitatory[122;124] pulmonary TB. These findings are consistent with lower bacillary load in HIV-infected patients compared to HIV-uninfected patients[122]. However, some studies have reported lower rates of transmission from HIV-infected patients[175], even following adjustment for sputum smear status[124;176;177]. It has been suggested that this may be due to a decreased infectious period in the community as a result of faster progression to disease and therefore less time to diagnosis or death of HIV-infected patients compared to HIV-uninfected patients[124;178]. However, prolonged

time pre-diagnosis has also been reported with HIV co-infection due to poor symptom development[179], and other studies have reported a higher risk of transmission from HIV-infected index cases[111;180]. By and large, most studies report no difference in risk of transmission based on HIV status of index case[181-186] including a meta-analysis of 11 studies of household contacts of TB patients[187].
Overall, given the clinical characteristics of HIV-infected TB patients, it has been suggested that HIV-associated TB is responsible for approximately 7% of TB transmission in communities with high TB prevalence[9], in which case a reduction in HIV-associated TB transmission may not have a significant impact on overall TB transmission in such settings.

Modeling in the Impact of HAART on TB Epidemics
Empirical data addressing the impact of HAART on TB epidemics at population level are sparse and most evidence comes from mathematical modeling.

A number of models have predicted limited impact of HAART on population TB epidemics. The model by Currie et al showed that providing HAART at the coverage levels targeted by WHO (3 million patients on HAART by 2005[26]) would have limited impact on TB incidence and deaths, especially compared to a combined strategy of improving both case detection rates and cure rates[188;189]. In addition, this group reported that increasing TB case detection and cure rates would be more cost-effective strategies for TB control than the provision of HAART[188].

A model by Williams et al predicted that in countries with HAART eligibility criteria of CD4 count <200cell/μl, complete ART coverage and 100% compliance on HAART would only reduce TB incidence in HIV-infected patients by 22% over 20 years[190].

Similarly, a modeling paper on the TB epidemic in India reported that a HAART programme would not have a substantial impact on the population TB incidence, but, could lead to a 50% reduction in TB mortality, most notably among HIV infected patients[191].

A more recent modeling paper reported that, under the assumption of 100% of HIV-infected people starting HAART in one year regardless of CD4 count, a HAART

programme would have a dramatic impact on the reduction of TB disease. However this paper did not report the impact of the slower, more practical implementation of a lower coverage HAART programme[192].

In summary, modeling exercises have conflicting results as to the impact of HAART on population-level TB epidemics, although generally HAART is predicted to have little impact on TB incidence in communities. However, it should be noted that the benefit predicted by mathematical models is dependent on the assumptions made and different parameters incorporated, such as the timing of HAART initiation, the coverage achieved by programmes and patient compliance. In addition the benefits predicted for different control strategies will vary widely at different HIV prevalences, and therefore in different settings[193].

1.2 AIMS AND OBJECTIVES

Although control of tuberculosis will undoubtedly require increased investments in health and social infrastructure[194], a better understanding of HIV and TB interactions at the levels of transmission, infection, prevalence and incidence within the prevailing crowded social environment is also required in order to assess the impact of interventions such as HAART on the control of this dual burden at a community level.

The aim of this thesis is to describe the interaction between the HIV-associated and HIV-unassociated TB epidemics in a well-demarcated community with high HIV and TB rates and to assess the impact of a high coverage HAART programme as an intervention strategy on these epidemics. This thesis will describe the TB epidemics at key stages of TB pathogenesis (Figure 1.6) and to describe the impact of HIV and the HAART programme on the epidemics at these stages. The stages in the natural history of TB that will be described include (1) transmission of TB infection and the factors associated with transmission dynamics in children and adults, and (2) the burden of TB disease, using both incidence and prevalence as measures of disease.

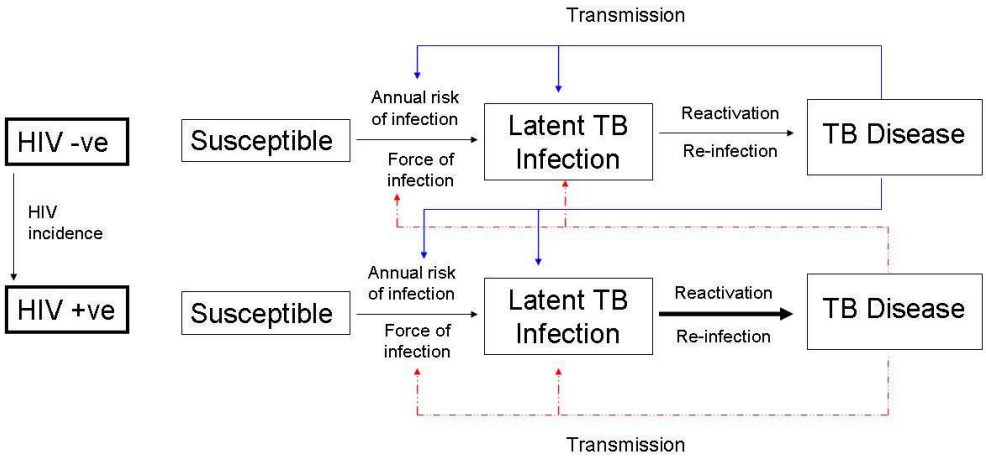


Figure 1.6: A model of the interactions between the TB and HIV epidemics

The specific objectives include:

1) To determine the prevalence of TB infection and the force of TB infection in the study community.
2) To determine the risk of TB transmission to children residing in close contact with adult TB cases, and to determine the importance of HIV-associated TB in this transmission.
3) To describe the molecular epidemiology of TB in the study community, and to utilize molecular epidemiological techniques to investigate the extent of clustering of *Mtb* disease, to assess the impact of the HIV-associated TB epidemic on transmission, and to identify risk factors associated with adult to adult transmission.
4) To describe the changes in TB disease incidence (as determined by TB notification rates) and outcomes in the overall community population and in the HIV-infected and HIV-uninfected subsets of the population. These changes will be assessed over time and following the scale-up of HAART access in the community.
5) To determine the prevalence of microbiologically-confirmed pulmonary TB (PTB) disease in both the HIV-infected and HIV-uninfected subsets of the population and describe changes in the prevalence of PTB disease following the scale-up of HAART access in the community.

1.3 STUDY COMMUNITY

Site M is a peri-urban township typical of many such communities in Southern and South Africa today. Recently urbanized, overcrowded and largely consisting of informal dwellings with low socio-economic status, Site M is a community where both HIV and tuberculosis rates are high.

In the 1980's a few hundred, predominantly Xhosa-speaking individuals, hoping to find work, moved into the area where the study site currently stands. These informal dwellers were repeatedly forcibly removed. However, with the end of Apartheid in the early 1990's, a section of land was serviced for development, and a few thousand people settled in what was to become known as Site M.

The community covers approximately 0.93km^2 and an unusual feature of the township is its clear geographical demarcation and isolation from surrounding areas (Figure 1.7). This feature of the community has facilitated population-based research by enabling an accurate population denominator to be determined. In 1996 a South African national census was performed which included Site M, and more recently Desmond Tutu HIV Centre (DTHC) has carried out community censuses the every second year since 2002. These censuses have enabled the development of a population model for this community and a description of this population model is provided in Appendix A.

Within the perimeter of the township, the community consists of a formal sector with demarcated individually numbers serviced plots and an informal sector of shacks sharing communal services. Approximately 5,239 houses (mostly informal structures) are built in the community, and in the formal sector there are between 1 and 22 dwellings on a plot (mean of 4 houses per plot). Overcrowding is therefore a significant problem in this township.

The community has grown in population size from 5,518 residents in 1996 (based on a South African National census in that year), to 14,592 residents in 2008 (DTHC census). The original residents were predominantly Xhosa-speaking individuals from the Eastern Cape Province. However, over the past few years a growing proportion of foreign nationals for other African countries have also settled in Site M. The community has a

predominantly young population, with 62% of the residents between the ages of 20 and 40 years (2008 DTHC census), as shown in Figure 1.8.

Socio-economic status is poor[2], with unemployment rates of 47-50% consistently reported in community surveys, an average monthly household income of <R2,000 and a high proportion of informal income generation such as hawking, home shops, collecting tins for recycling, etc (unpublished data from 2002, 2005 and 2008).

Figure 1.7: Satellite map of Site M

The community is served by a single primary health care clinic, which is nurse-driven and doctor-supported, with a part-time medical officer assisting with HIV and TB clinics. In 2005, the DTHC established a HAART clinic, with full-time doctors and primary health care nurses running this service. A local referral hospital provides X-ray facilities and specialist referral services, including both outpatient and inpatient services.

[2] Socioeconomic status of the community was assessed using the Cape Metropolitan council per-suburb composite index, which is based on household income, education level, unemployment status, welfare status and overcrowding status (http://www.environment.gov.za/enviro-info/sote/citysoe/cape/povrty). The Site M score correlates well with extremely poor conditions of living.

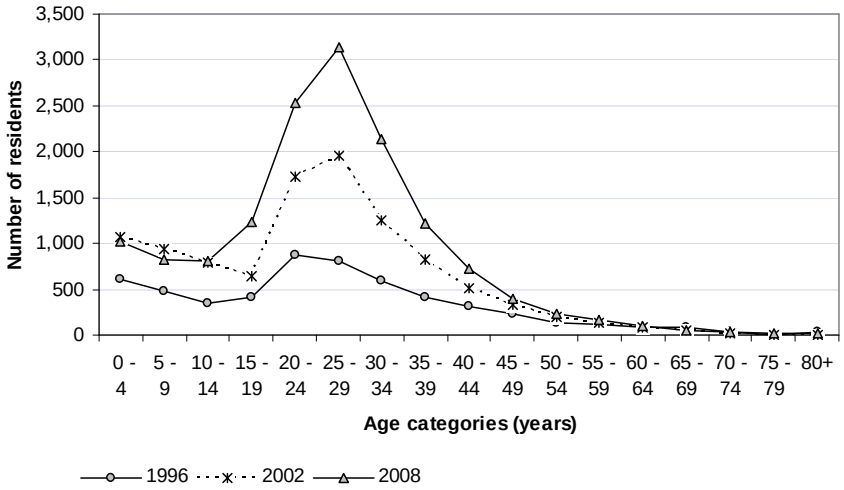


Figure 1.8: Site M population by age categories: 1996, 2002 and 2008

1.3.1 TB Control Programme and Epidemic in Study Community

The community clinic provides TB management and treatment for all TB patients resident in the community. The TB programme follows the National TB Control programme guidelines[195;196], which are based on WHO-recommended DOTS programme.

The TB programme is based on passive case-finding, and is dependent on TB patients presenting to health care services for investigation and treatment. Sputum investigations form the mainstay of the diagnostic approach, together with chest X-rays and, in paediatric cases, tuberculin skin testing. TB patients are treated with standardized, rifampicin-based chemotherapy regimens. The sputum smear-positive TB case detection rates obtained by TB control programme in the study community fall short of the WHO targets of 70% (45% case detection rate in the community)[197], although the programme did average 82% treatment completion rates from 1998 to 2005.

We have previously reported on the escalating TB epidemic in this community: notification rates among adults have increased from 789/100,000 in 1996 to >1,400/100,000 in 2004, with the incidence of smear-positive TB among adults also increasing from 326/100,000 to 1,307/100,000 over the same period[39;179]. HIV-infected individuals have been disproportionally affected.

HIV testing has been routinely offered to all TB patients at the clinic since mid 2001. From 2002 to 2008, on average 89% of adult patients tested for HIV infection and, of those who tested, 68% were HIV-infected (Figure 1.9).

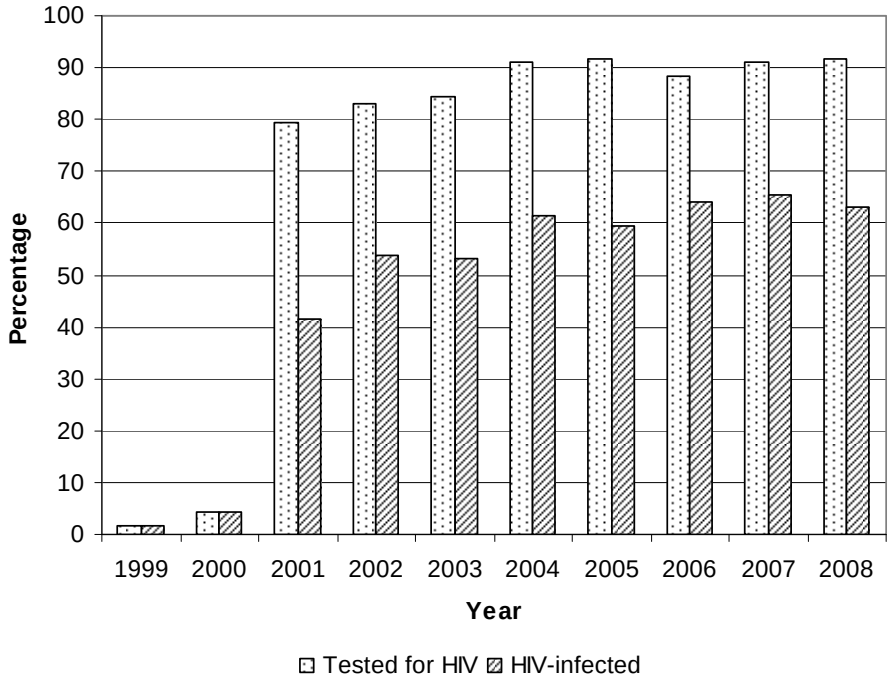


Figure 1.9: HIV testing uptake and HIV-infection prevalence among adult TB patients in study community from 1999 to 2008

While TB management in the study site remained the responsibility of the government clinic, it is worth considering that some research activities may have had an impact on the TB control programme in this community. Of note, is the potential beneficial effect of active case-finding, as performed in two community-based, cross-sectional TB prevalence surveys (in 2005 and 2008), as well as the active TB disease screening in children who were determined to be tuberculin skin test (TST) positive in the school-based TST surveys (Figure 1.10).

1.3.2 HIV Epidemic in Study Community

Based on the Actuarial Society of South Africa (ASSA) 2003 AIDS and Demographic model[198] together with HIV prevalence surveys performed in the community in 2005[179] and 2008[197], an HIV infection model was developed for this community (Appendix A). It is estimated that in 1997 that the overall HIV prevalence in this community was 8%, by 2005 this has increased to 18%, and in 2008, the HIV prevalence was 22%. Figure 1.11

shows the growing HIV epidemic by age strata and year, from 1997 to 2008, based on the community model. For more information on the development of the HIV infection model, see Appendix A.

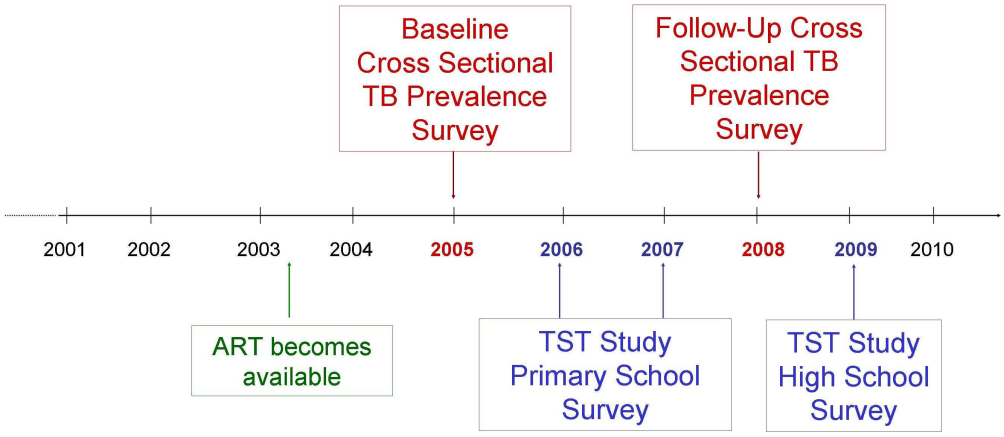


Figure 1.10: Timeline for research studies in Site M

In 2004, the DTHC obtained limited HAART funding for HIV-infected patients in Site M. In 2005 a collaboration between the DTHC, the NIH-funded CIPRA-SA project and provincial health authorities enabled the large scale implementation of a HAART programme in this community. The HAART programme used standard antiretroviral drug regimes, as outlined in the South African HAART treatment guidelines[199]. Prior to February 2005 and after February 2007 (following completion of the CIPRA-SA ART project enrolment), the South African National guidelines for treatment initiation were followed, with patients eligible for HAART at CD4 count <200cells/mm3 or WHO clinical stage 4 (which does not include TB disease)[55]. However, between February 2005 and January 2007, patients were eligible for HAART if they had a CD4+ count <350cells/mm3 or WHO clinical stage 3 or 4 illness (including TB disease[200])[201].

The numbers of patients accessing HAART increased from 11 patients (1% of HIV-infected population) in 2003, to 308 patients (13% of HIV-infected population) in 2005. By 2008, 713 residents were receiving HAART in the study community (22% of HIV-infected population). HAART coverage varied by CD4 strata in the HIV population. An unpublished model for the study site estimated that in 2008, 81% of the adult HIV-infected population with CD4 count <200cells/mm^3 were receiving HAART, and 46% of those with CD4 count <350cells/mm^3 (personal communication L Johnson and K Kranzer). However, it should be noted that, despite a number of years of HAART, a

small proportion of patients do not fully restore their CD4 count[152;202], and this is evidenced by the estimated 18% of patients on ART who have a CD4 count <200cells/mm^3 (unpublished data).

According to the WHO, 12% of HIV-infected patients in South Africa who met UNAIDS treatment guidelines were receiving ART in 2005[203], and this increased to 56% in 2009[12;29]. Given that this coverage reflects the proportion of those "who need antiretroviral therapy", the coverage in the study community of up 21% of the total HIV-infected population, and a possible 81% of those in need, represents a high coverage implementation, achieved rapidly over a 4 year period.

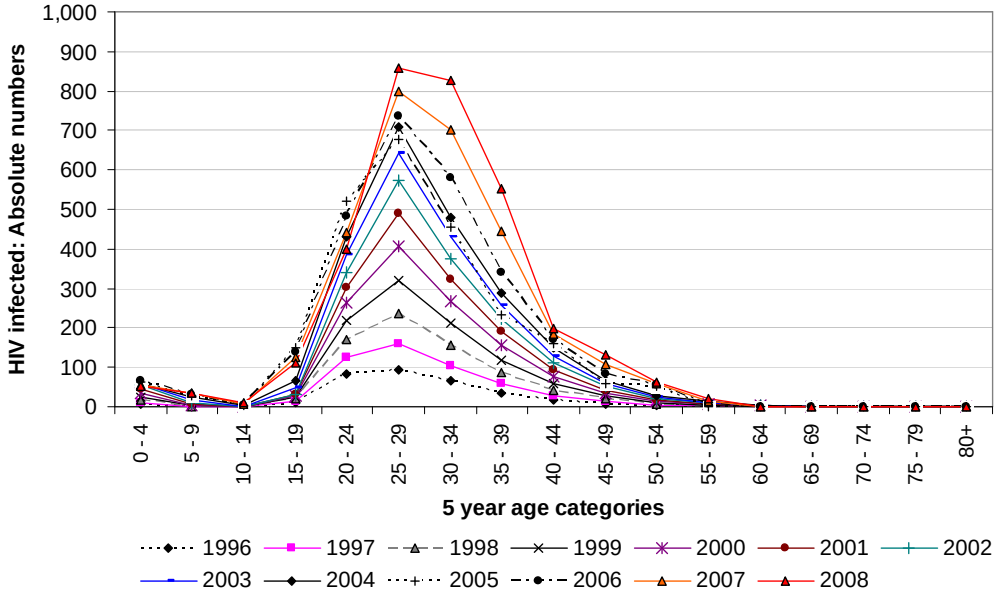


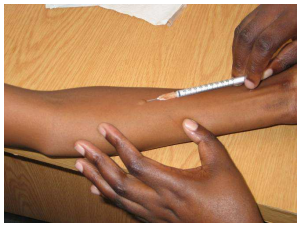
Figure 1.11: HIV infection by year and age

1.3.3 Geographical Information System

Every plot in Site M has been assigned a unique digital location on a Geographical Information System (GIS) database (based on aerial photographs, road plans and surveyor general plans from 1998 to 2007). Residents use this plot number as an address, and therefore each inhabitant of the community can be located on this system by identifying their home address. In addition, clinical and epidemiological data as well as molecular data (such TB genotyping data) can be linked to the geographical location in the community using the ArcMap 9.2 (Esri™) GIS.

1.4 ETHICAL CONSIDERATIONS

All the studies described in this thesis were approved by the University of Cape Town's Human Research Ethics Committee. Genotyping of *Mtb* specimens was performed at the University of Medicine and Dentistry of New Jersey (UMDNJ), and all studies involving genotyping were also approved by the Institutional Review Board of UMDNJ. Written informed consent/assent was obtained from participants in all cross-sectional survey studies performed. Written informed consent/assent was also obtained from TB patients for the collection of epidemiological data and genotyping analysis of *Mtb* specimens.



Tuberculosis Infection: Prevalence and Force of Infection

2.1 RATIONALE

The first step in the natural history of tuberculosis (TB) is the establishment of TB infection in susceptible individuals. Throughout this thesis, TB infection refers to the latent infection of an asymptomatic individual with *Mycobacterium tuberculosis* (*Mtb*), and not to active TB disease. While the conventional risk factors for acquiring TB infection have been well documented, and are outlined in Chapter 1, it is not well understood how HIV-related TB epidemics have impacted transmission of TB infection in communities heavily affected by both diseases.

An increasing proportion of non-infected individuals in a population is required in order to achieve control of a TB epidemic[115] and therefore, in order to develop effective intervention strategies for TB control, it is important to understand the transmission dynamics of the epidemic in HIV and TB prevalent settings. Under the assumption that children with TB infection have been infected in the recent past, assessing the prevalence of TB infection in children may provide insight into the amount of recent TB transmission in a community[204].

This chapter will address the first objective of this thesis, namely to determine the prevalence of TB infection and the force of TB infection in a community with high TB and HIV burdens as well as examining risk factors associated with the transmission of TB infection to children. Data from this chapter have been published in the first paper[205] listed in the preface on page *xi*.

2.2 BACKGROUND

2.2.1 The Tuberculin Skin Test

In 1890 Koch prepared the original tuberculin material as, he thought, a cure for TB[206]. While therapeutic use of tuberculin was rapidly disproved, in 1907 the potential for tuberculin as a diagnostic tool was first realized[207;208]. The tuberculin skin test (TST) is, therefore, one of the oldest diagnostic tests still in clinical use[209], and despite more

recently developed tools such as interferon-gamma release assay, TST remains the most commonly used tool for the diagnosis of latent TB infection.

The test is based on the fact that within 6-12 weeks of infection with *Mtb* a cellular-immune mediated delayed hypersensitivity reaction occurs to components of the mycobacterium[210-212]. The TST is performed by the injection of a standardized dose of PPD (purified protein derivative) intradermally on the volar surface of the individual's forearm. Previously sensitized T-cells and macrophyges migrate to the skin site, where lymphokines are released. The ensuing vasodilation, oedema and recruitment of further inflammatory cells results in induration at the test site[209;213]. This induration (not erythema) is measured to determine the presence of infection. In an infected individual a reaction to the PPD is produced within 48-72 hours.

The tuberculin skin test is routinely utilized in the National TB Control Programme to diagnose TB infection[195;196] and is widely used as an epidemiological tool to measure the prevalence of TB infection in community settings. Advantages of this test include that it is relatively cheap, easy to administer and has an extremely low adverse event rate[209;214;215].

Limitations of TST

However, there are a number of limitations associated with this tool[216]. Firstly, TST cannot differentiate between latent TB infection and active TB disease. Secondly, the sensitivity and specificity of the TST test are variable[210]. TST sensitivity appears to remain relatively constant, at approximately 75-90% (at a 10mm reaction size cut-off for positivity) across countries and regions[216-218]. However, immune response to TST is age-dependent, and tuberculin sensitivity is recognized to wane in TB infected patients over time[213;219-221] . Sensitivity of TST is adversely affected by medical conditions that compromise the immune system, such as malnutrition, cancers (in particular lymphomas) and HIV[100;101;216;221-223]. False-negative reactions may also be due to problems in storing the tuberculin[209], in the technique of TST administration, or errors in reading the test result[1;221;224], although these can be minimized with training[225]. Specificity, in turn, may be influenced by cross-reaction with environmental mycobacteria other than tubercule bacilli (MOTT bacilli)[209;218;226;227], or with Bacillus Calmette-Guérin (BCG) vaccination[228;229]. The degree of cross-reaction with MOTT bacilli varies markedly

in different regions and countries, from virtually absent to substantial[226;227;230], and is less marked in temperate or sub-tropical climates[226]. TST reactions as a result of MOTT bacilli infection tend to be of a smaller diameter[231], and at a 10mm reaction size cut-off for positivity, specificity for TB infection can be as high as >95% in settings with low MOTT bacilli prevalence[209]. Cross-reaction with BCG wanes significantly with time since vaccination[232]. Similar to issues of sensitivity, small reactions may be due to the technique of TST administration, trauma, or errors in reading the test, rather than TB infection[1;221].

While sensitivity and specificity are inherent characteristics of the tuberculin skin test, the positive predictive value of the test (ie the probability that someone with a positive TST result truly has TB infection[233]) is determined by the prevalence of TB infection[233]. Consequently, in high TB prevalence areas, such as South Africa, the positive predictive value of the test is greater than that in low prevalence areas[210].

2.2.2 The Interferon-Gamma Release Assay

A more recently available test for the detection of TB infection is the interferon-gamma (IFN-gamma) release assay. This is a blood-based, ex vivo test that is based on the quantification of IFN-gamma released by effector T cells following stimulation with tuberculin[234;235]. Studies have shown that IFN-gamma release assays have greater specificity for latent TB infection compared to TST, particularly in BCG-vaccinated populations[236]. Other advantages of the IFN-gamma release assays include an objective determination of infection, which is not subject to reader variability, and the absence of activation or boosting of the immune response to TB-related antigens, providing an excellent tool for repeat testing of individuals for the detection of new TB infection. As with TST, these assays are unable to distinguish between TB infection and disease[237]. The literature discussing this assay is only available from the late 1990's and therefore TST was utilized in this study to enable comparison with a large body of both historical and more recent data.

2.2.3 Calculating Incidence of TB Infection

Despite the limitations described above, TST remains a useful tool to determine the prevalence of TB infection, which in turn can provide valuable information on the transmission of *Mtb* in a community.

Incidence of TB infection is a measure of current transmission in a community. While repeated testing of a cohort of uninfected individuals over time is a conventional method for determining incidence of a disease, this methodology is both labour and time intensive, and is further complicated by the boosting of the immune response in immunology-based tests such as TST[2;238]. Therefore alternative approaches for calculating incidence from prevalence data have been developed. I will discuss two methods, in particular, 1) the annual risk of TB infection (ARTI) and 2) the force of infection.

Annual Risk of TB Infection

Annual risk of TB infection has been defined as "the probability that an individual, who has not previously been infected with tubercle bacilli, will be infected during the ensuing period of one year"[1]. ARTI is calculated from TB infection prevalence data, and comprehensive descriptions of the theoretical basis for the relationship between prevalence and ARTI are available in the literature[1;239;240].

ARTI is a cohort's average risk of infection from the birth year of the cohort to the year of the survey, and therefore is a measure of the risk at a calendar time between these two time points[2]. ARTI incorporates all the factors that contribute to the transmission of TB over the specified time period: from the characteristics of the infectious case and susceptible individual, to infecting pathogen, environmental factors, the performance of the local TB control programme[216]. Repeated measurements of ARTI over time will provide information on overall trends in TB infection rates, but because of its "averaging" characteristics ARTI is not sensitive to short term changes[216].

In the pre-chemotherapy era Styblo described a fixed relationship between ARTI and smear-positive disease incidence[241], although this theory has subsequently been criticized[242]. In addition, the availability of TB chemotherapy would have altered any relationship between ARTI and incidence[49;216;242;243]: TB treatment increases survival rates and reduces person-time of infectiousness in a community, thereby reducing infection risk[216] and subsequently decreasing TB prevalence and incidence. However, it should be noted that these factors are all dependent on the performance of the TB control programme[49;216;242] which may vary over time and place. The relationship

between ARTI and TB incidence may again have been altered by the impact of the HIV epidemic on TB. This change is postulated based on the increase in both smear-positive and smear-negative TB cases in areas with high HIV prevalence, and the changes in the duration of infectiousness of HIV-infected TB cases[216;244]. The Styblo ratio of ARTI to smear-positive disease incidence of 1% ARTI: 50 smear-positive cases/100,000[241] is, for the most part, no longer used[7].

Force of Infection

ARTI is an averaged measure of the risk of TB infection over the lifetime of the study participants[1;2]. The obvious limitation of this measure is that ARTI only provides an estimate of current transmission or incidence if calculated in very young participants. In comparison, force of infection provides a recent estimate of infection incidence across a wide range of ages. Force of infection is defined as the proportion of susceptible individuals that became infected in a specified period and can be calculated using changes in age-specific prevalence rates[245-247].

This approach is a relatively new concept that has been utilized to assess incidence in diseases in which incidence is difficult or costly to measure, such as glaucoma[246] and more recently, HIV[248;249]. The mathematical techniques utilized are based on the principle that prevalence is a function of incidence and duration of illness or infectivity[250]. This principle holds true under the assumptions of stability of the population and therefore this approach requires minimal immigration into the community in the relevant age groups, as well as minimal emigration or removals through mortality. In addition this method is most appropriate in diseases that are irreversible. Among children and adolescents, TB infection itself is persistent and not fatal, unless it progresses to TB disease.

2.2.4 Tuberculin Skin Test Surveys

TST surveys have an important role in understanding the impact of changing risk factors, such as an HIV epidemic, or interventions, such as antiretroviral therapy (ART), on TB transmission[251;252]. Yet, there are few current data published on prevalence of TB infection, ARTI or force of infection, particularly in countries with high HIV prevalence[253-255], and this is especially true of the South African context.

In a review of TB infection prevalence and ARTI trends in developing countries, studies ranging from 1949 to the mid 1980's were assessed. In most countries, including many in the African region, ARTI trends were noted to be decreasing[2]. This review did not include South African data, but studies from South Africa have reported low[256] and decreasing ARTIs in the pre-HIV era[257;258].

Since the advent of the HIV epidemic, the few studies that have attempted to assess the impact of the HIV epidemic on TB infection in children have reported mixed findings. One study from Tanzania reported a significant drop in ARTI in 6 to 14 year olds from 1983 to 2003[253] in the context of an increasing HIV epidemic (HIV prevalence of 7-11%[259]). This trend was reported despite increasing TB notification rates, including increasing smear-positive TB notification rates[253]. Similarly, a study of Ugandan children reported a 2.9% drop in ARTI among 10 year old children, from 2.3% in 1970 to 1.2% in 1987[254], at a time of escalating HIV prevalence[260]. In contrast, research from Kenya reported an increased ARTI among young school children from 1986 to 1996 (0.6% to 1.1%), associated with an increasing TB epidemic and high HIV prevalence[255]. Meanwhile the Republic of Djibouti, with a relatively low HIV prevalence of 2.9% in 2002[13], reported a stable ARTI rate of 2.1-3% from 1994 to 2001[261]. While no trends in TB infection rates are available from Malawi, a study performed in 1994 reported an ARTI of 1%[262].

There are few recent, published tuberculin skin test data from southern African countries where HIV prevalence has reached staggering levels, but the few reports available indicate a substantial ARTI, of at least 2.5%, in these countries[205;263;264]. Studies have focused on TB infection in young children in high TB settings[205;253;255;263], and there are few data assessing ongoing TB infection in older children and adolescents in communities with high TB and HIV burdens[265].

To better understand the transmission of TB in communities with substantial TB and HIV prevalence, and the impact of HIV on transmission in this context, we performed a tuberculin skin test survey to determine the prevalence of TB infection and force of infection in school-attending children in the study community.

2.3 STUDY DESIGN

The cross-sectional survey was performed in three stages, among children attending the local government primary and secondary schools in Site M. Children were eligible for participation if they were resident in the community and registered at the local schools. Children from grades 1 to 3 at the primary school were enrolled from October to November 2006 and children in grades 5 to 7 were enrolled from October to November 2007. Children from grades 8 to 12 at the secondary school were enrolled from August to October 2009. All three phases of the school surveys were performed on the primary school premises. Basic demographic information was collected on each participant. All participants were examined for the presence of a BCG scar, and participants received the TST regardless of BCG scar status.

2.3.1 Tuberculin Skin Testing

The World Health Organization (WHO)-recommended standard dose of 2TU of PPD RT23 with Tween 80 (Statens Seruminstitut, Copenhagen) was administered intradermally to the volar surface of the child's left forearm by a trained nurse. The reaction size to the tuberculin was read by a trained assessor at a second visit performed 3 days following the inoculation. The presence or absence of a reaction was noted, and, where present, the size of the induration was measured along perpendicular axes using standard callipers.

The secondary school children also provided an oral transudate specimen for HIV testing, using the Orasure® collection device and Vironostika Uni-Form II HIV-1 and HIV-2 plus 0 ELISA test (bioMérieux SA, Marcy l'Etoile, France). HIV results were anonymous but linked to TST results and all adolescents were encouraged to have voluntary counselling and testing at local facilities. HIV testing was not performed in the primary school surveys.

All children with a TST reaction ≥10mm were recalled for investigation for active tuberculosis and children with signs or symptoms of active disease were referred to the local clinic for further management.

2.3.2 Data Analysis

Data were analyzed using STATA 10.0 (StataCorp, College Station, Texas). Analysis was performed in three parts: firstly on the primary school dataset, secondly on the secondary school dataset, excluding HIV-infected individuals, and thirdly on the secondary school dataset combined with the primary school dataset, excluding the HIV-infected individuals from the secondary school survey.

Primary and Secondary School Surveys

TST results were calculated as the mean of the two diameters of the TST reaction, and a positive reaction was defined at 10mm cut-off, based on the guidelines for infection in clinical settings[195;196;266]. The study participants were divided into age quartiles, and TB infection prevalence and ARTI were calculated overall and for each age group. ARTI was calculated as 1- (1-prevalence)$^{1/(\text{mean age}+0.5)}$. As the age in full years at participants' last birthday was used, 0.5 was added to the mean age for the calculation of ARTI[223]. Bivariate analyses employed Student's t- and Fisher's exact tests, as appropriate; for comparison of TST results between different groups, Wilcoxon sum rank tests were used. Multiple logistic regression models were developed to examine factors associated with positive TST results. Trends in ARTI by age groups were assessed using Cox-Stuart test for trend[267;268].

Sensitivity analysis of TST positivity cut-off point

As the 10mm cut-off for defining TST sensitivity is based on clinical criteria, rather than analytical epidemiologic criteria, we performed a sensitivity analysis on the primary school participant data, in which a TST positive reaction was determined as the mean reaction excluding all non-reactors[223]. This method is recommended in settings in which there is "a distinct separation between reactors and non-reactors"[223], as was shown in our data. Association of TB prevalence with age, gender and BCG status was assessed, and ARTI calculated for this cut-off. Trends in ARTI by age groups were assessed using Cox-Stuart test for trend[267;268].

HIV Infection in Secondary School Participants

Univariate and multivariate logistic regression models were developed to determine the demographic characteristics associated with HIV among the secondary school participants, and to assess for an association between HIV and TB infection. In these

models, a TST reaction size of ≥5mm was used as a positive cut-off for TB infection in HIV-infected adolescents, in keeping with clinical guidelines[195;196].

Repeat TST Participants

In 2009, 159 students who had participated in the 2007 survey also took part in the secondary school survey, resulting in repeat tuberculin testing of these participants. Bivariate analyses used Wilcoxon sum rank tests and Student's t-, as appropriate for comparison of participant groups: participants with repeated TST compared to the participants in the combined surveys; participants who remained TST negative versus those who converted to a positive TST from 2007 to 2009. The McNemar test was used for matched comparison of 2007 and 2009 TST results. A change in TST reaction size was calculated by subtracting the 2007 reading from the 2009 reading for each participant. A multivariate regression model was developed to compare reaction sizes between the groups. A change in reaction size may be the result of random variability in administration or reading of the test[238], or may result from reversion or the "booster" effect of enhanced allergy[238;269]. In keeping with existing literature, we defined a true conversion to TST positivity between the two surveys as a change from a negative result (<10mm) on original TST to a positive result (≥10mm) on the second test, with an absolute reaction size increase of at least 6mm[211;212;270]. In addition, the recent CDC guidelines define a conversion as an increase of TST reaction size of ≥10mm within a two year period, and this definition was also utilised[271].

Combined Primary and Secondary School Surveys

In order to assess the effect of age on TB prevalence and force of infection, we investigated the possibility of combining the primary and secondary school surveys. We compared the prevalence of TST positive results for the two sample populations in the overlapping age range of 14 to 16 years (Figure 2.1). The chi-squared test for comparison was not significant (survey 1 and 2: p=0.66; survey 2 and 3: p=0.46), nor was survey year a significant risk factor in multivariate logistic regression model for TST positivity. Therefore we combined the primary and secondary survey datasets. HIV-infected participants from 2009, as well as the second test in those participants who had repeat tests in 2009 were excluded from the combined database.

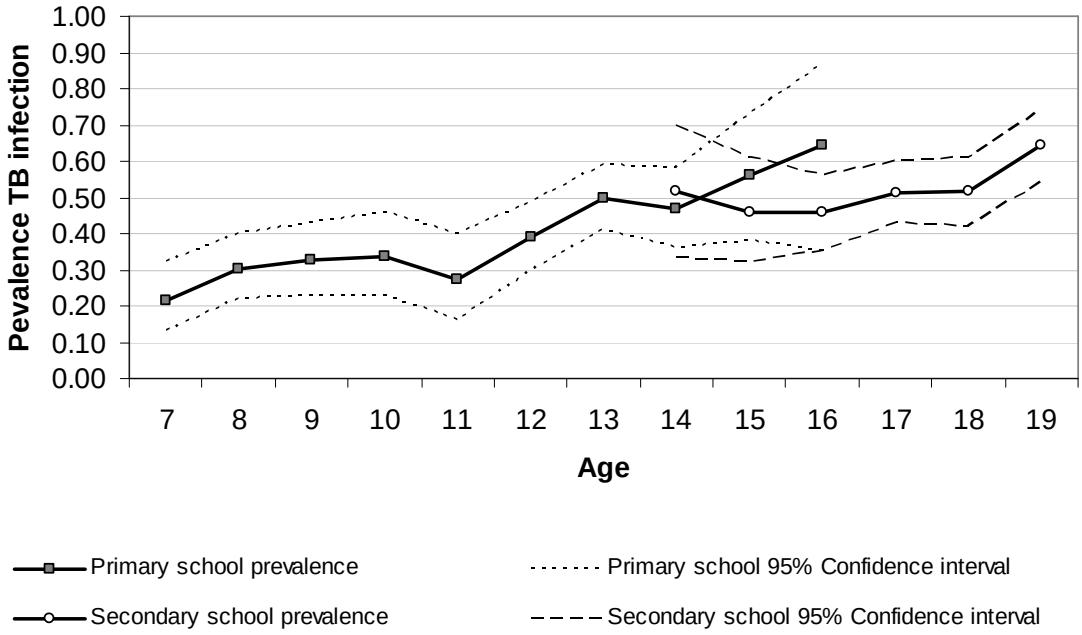


Figure 2.1: TB prevalence in Primary and Secondary School surveys, by age

The participants were divided into age quartiles, and prevalence and ARTI were calculated overall and for each age group. Smoothed prevalence of TB infection by age was calculated from predictive logistic regression models on the combined primary and secondary school dataset, excluding those who tested HIV-infected in the secondary school, and excluding the second test result in participants with a repeat test. Force of infection was defined as the proportion of susceptible individuals that became infected in a specified period, and was calculated as follows: the probability of becoming infected at specific ages for the pool of individuals who remained uninfected = (prevalence$_{Y1+1}$ - prevalence$_{Y1}$) /(1-prevalence $_{Y1}$)]*100. Trends in ARTI and force of infection were assessed using Cox-Stuart test for trend[267;268].

For all analysis, 95% confidence intervals (CI) were based on the Poisson distribution and all statistical tests were 2-sided at alpha=0.05.

2.4 STUDY FINDINGS

2.4.1 Primary School Survey

Of the 1060 children enrolled at the primary school, 1020 were eligible for study participation (96%). Ineligibility was due to living outside of the community (n=18) and

having dropped out of school during the course of the year (n=22). Consent was obtained from the parents of 837 children (82% of those eligible). The parents of 44 children refused consent (4%) and 137 children had incorrect locator information in the school register (13%). We enrolled 832 children (99% of consented children). The outstanding five children were not enrolled due to persistent absenteeism. Tuberculin reaction was assessed in all children who were inoculated, except one who relocated before her reaction was assessed. She has thus been excluded from the analysis (n=831).

Demographic Characteristics

Table 2.1 shows the demographic characteristics of the study sample. Ages ranged from 5 to 17 years, with a mean age of 10.7 years (standard deviation [std dev]=2.7), and 48% of the participants were female. The majority of the children did not have a BCG scar (74%); one child's BCG scar status was unobservable due to burn scars on the upper arm, and he was thus excluded from analysis involving BCG scar status.

Table 2.1: Demographic characteristics of primary school participants

	AGE QUARTILES				**TOTAL**
	5 to 8 yrs n=233 n (%)	**9 to 11 yrs n=222 n (%)**	**12 to 13 yrs n=237 n (%)**	**14 to 17 yrs n=139 n (%)**	**n=831 n (%)**
Mean Age	7.3 years	9.9 years	12.5 years	14.6 years	10.7 years
Gender					
Male	115 (49%)	122 (55%)	110 (46%)	87 (63%)	434 (52%)
Female	118 (51%)	100 (45%)	127 (54%)	52 (37%)	397 (48%)
BCG status					
BCG scar present	30 (13%)	66* (30%)	87 (37%)	30 (22%)	213 (25%)

*One participant's BCG scar status was indeterminable due to burn scars

TST Results

TST reaction sizes ranged from 0 to 27.5mm (median=0mm; interquartile range [IQR]: 0-16.5mm), and 506 (61%) participants had no reaction to the TST. The frequency distribution of reaction sizes for positive TST results is presented in Figure 2.2. There was no significant difference between the mean reactions of participants with BCG scars compared to those without scars (6.2 vs 7.0mm, respectively, p=0.28).

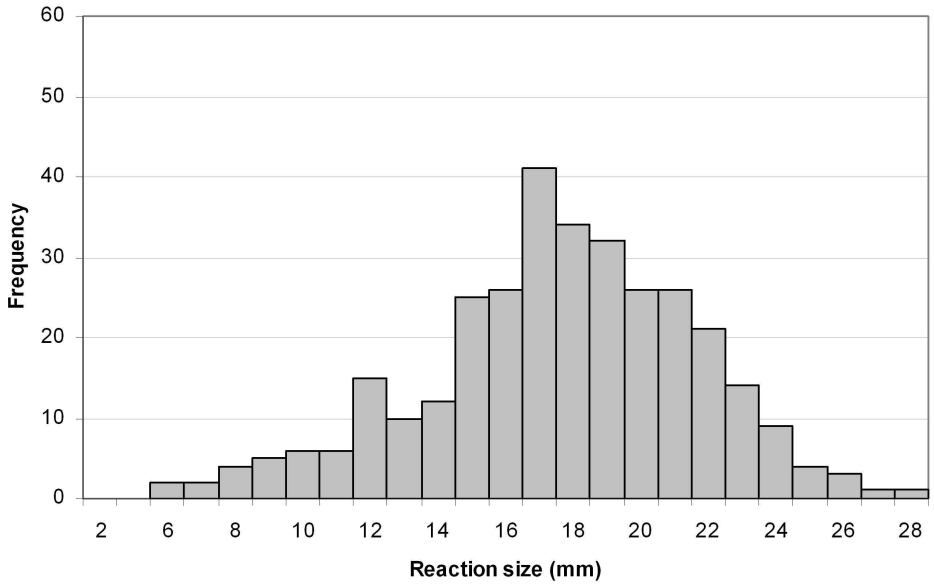


Figure 2.2: Frequency distribution of TST reactions (>0mm) in primary school survey participants

At the 10mm cut-off 311 participants (37%) had a positive TST result. TST positivity (as defined by the 10mm cut-off) was not associated with gender (p=0.97) or BCG status (p=0.39). In a logistic regression model predicting the relative odds of a positive TST result, age was positively associated with a positive TST result at the 10mm cut-off (adjusted odds ratio [OR] for a 1-year increase in age: 1.19, 95% confidence interval [CI]: 1.12 – 1.25; p<0.001).

The total ARTI for this sample was 4.1%. The ARTI did not differ significantly across the age quartiles (p=0.50). Table 2.2 reports the TB prevalence and ARTI by age quartiles.

Table 2.2: TB prevalence and ARTI by age quartile for the 10mm cut-off point

Age Category	Mean Age*	n	TST positive	Prevalence	ARTI%
5-8 yrs	7.8	233	61	26.2	3.8%
9-11yrs	10.4	222	70	31.5	3.6%
12-13yrs	13.0	237	107	45.1	4.5%
14-17yrs	15.1	139	73	52.5	4.8%
TOTAL	**11.2**	**831**	**311**	**37.4**	**4.1%**

* The mean age reported here is the age used for the ARTI calculations, and is based on the mean age for the age category+0.5 years, as described in the methods section

Sensitivity Analysis of TST Positivity Cut-off Point

The cut-off point for TST positivity determined by the mean of non-reactors was 17.4mm. At the 17.4mm cut-off 171 participants (21%) had a positive result. As with the 10mm cut-off, TST positivity at the 17.4 mm cut-off points was not associated with gender (p=0.69) or BCG status (p=0.08). In a logistic regression model predicting the relative odds of a positive TST result, age was positively associated with a positive TST result at both the 10mm cut-off (adjusted OR for a 1-year increase in age: 1.19, 95% CI: 1.12 – 1.25; p<0.001), and at the 17.4mm cut-off (adjusted OR for a 1-year increase in age: 1.19, 95% CI: 1.12 – 1.27).

The total ARTI in the sensitivity analysis was 2.0% at 17.4mm cut-off. Table 2.3 reports the TB prevalence and ARTI by age quartiles for this analysis. The ARTI did not differ significantly across the age quartiles (p=0.50).

Table 2.3: TB prevalence and ARTI by age quartile for the 17.4mm cut-off point

Age Category	Mean Age*	n	TST positive	Prevalence	ARTI%
5-8 yrs	7.8	233	25	10.7	1.4%
9-11yrs	10.4	222	39	17.6	1.8%
12-13yrs	13.0	237	71	30.0	2.7%
14-17yrs	15.1	139	36	25.9	2.0%
TOTAL	**11.2**	**831**	**171**	**20.6**	**2.0%**

* The mean age reported here is the age used for the ARTI calculations, and is based on the mean age for the age category+0.5 years, as described in the methods section

In conclusion, using a cut-off for TST positivity determined as the mean reaction excluding all non-reactors (17.4mm)[223] did not substantively change the study findings when compared to the cut-off determined by clinical guidelines (10mm).

2.4.2 Secondary School Survey

Of the 959 children enrolled in the secondary school, 839 were eligible for study participation (87%). Ineligibility was due to residence outside of the community (n=80) and having dropped out of school (n=40). Consent/assent was obtained for 820 children (98% of those eligible). Refusal by parent or learner accounted for 18 non-consenters

and one child was not consented due to hospitalization for cancer over the study period. We enrolled all 820 consented learners. Four of the children enrolled declined participation in the TST component of the study, and four children declined HIV testing. Of the 816 children who underwent TST, 813 (99.6%) had TST test read within 72-96 hours. No study-related adverse events were noted in the three children assessed outside the window period and these three participants were excluded from the analysis.

Demographic Characteristics

Table 2.4 shows the demographic characteristics of the secondary school study sample. Of the 813 participants who completed TST testing, 159 had received tuberculin skin testing in the 2007 survey in the community. These 159 participants were excluded from the main analysis and their results are presented in a sub-analysis. Of the remaining 654 children in the secondary school survey, all but three of the participants underwent HIV testing. In total 34 of the remaining participants tested HIV-infected, and were excluded from the TB analysis (n=620).

Among the 620 participants, ages ranged from 13 to 22 years, with a mean age of 17.5 years (std dev: 1.9), and 41% of the participants were male. The majority of the children did not have a BCG scar (87%); one child's BCG scar status was not recorded, and this participant was thus excluded from analysis involving BCG scar status.

TST Results

TST reaction sizes ranged from 0 to 30mm (median=11.5mm; IQR: 0-16.5mm), and 222 (36%) participants had no reaction to the TST. The frequency distribution of reaction sizes >0mm are presented in Figure 2.3. There was no significant difference between the median reactions of participants with BCG scars compared to those without scars (11 vs 11.5mm, respectively, p=0.24).

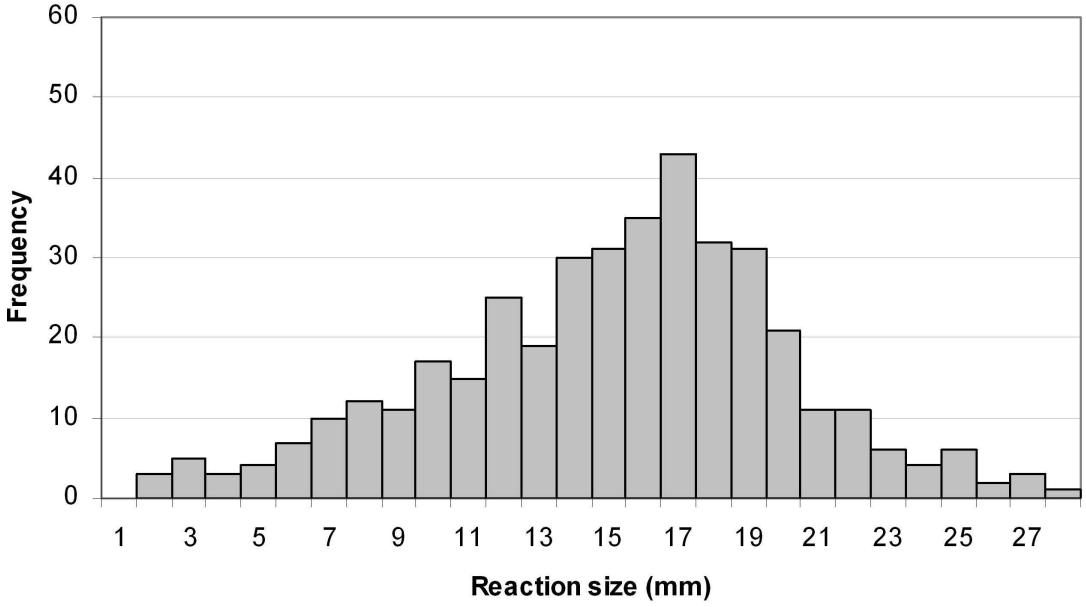


Figure 2.3: Frequency distribution of TST reactions (>0mm) in HIV-uninfected participants in secondary school survey

At the 10mm cut-off, 334 participants (54%) had a positive TST result. There was no significant difference in the defined TB positivity by BCG scar status (p=0.99). In a multivariate logistic regression model predicting the relative odds of a positive TST result, age was positively associated with a positive TST result (adjusted OR for a 1-year increase in age: 1.10, 95% CI: 1.01 – 1.21; p=0.03), as was male gender (adjusted OR for female compared to male: 0.65, 95% CI: 0.47-0.90; p=0.01).

The overall ARTI for this sample was 4.2% (95% CI: 3.8-4.7%). Table 2.5 reports TB prevalence and ARTI overall and by age quartiles. The ARTI did not differ significantly across the age quartiles (p=0.75) or across age by years from 14 to 21 years of age (p=0.63).

Table 2.4: Demographic characteristics of the secondary school sample

	First time TST participants[Φ]			Repeat TST participants[ΦΦ]		
	HIV-uninfected n=617 *n (%)*	**HIV-infected n=34** *n (%)*	**Total n=654** ***n (%)***	**HIV-uninfected n=154** *n (%)*	**HIV-infected n=4** *n (%)*	**Total n=159** ***n (%)***
Age: mean (range)	17.5 (13-22)	18.4 (15-22)	**17.5 (13-22)**	15.4 (13-19)	15.3 (14-17)	**15.4 (13-19)**
13-16 yrs	176 (29)	5 (15)	**183 (28)**	133 (86)	3 (75)	**136 (86)**
17 yrs	148 (24)	4 (12)	**152 (23)**	13 (8)	1 (25)	**15 (9)**
18 yrs	112 (18)	9 (26)	**121 (19)**	6 (4)	0 (0)	**6 (4)**
19-22 yrs	181 (29)	16 (47)	**198 (30)**	2 (1)	0 (0)	**2 (1)**
Gender: Male	255 (41)	9 (34)	**266 (41)**	81 (53)	2 (50)	**84 (53)**
BCG Scar present	80 (13)	6 (18)	**86* (13)**	32 (21)	0 (0)	**32 (20)**
TST positive	331 (54)	11 (32)	**345 (53)**	90 (58)	1 (25)	**91 (57)**

*n = 653: one participant's BCG scar status was not recorded
[Φ] Three participants declined HIV testing
[ΦΦ] One participant declined HIV testing

Table 2.5: Secondary school TB prevalence and ARTI by age quartile for the 10mm cut-off point (excluding HIV-infected and repeat TST participants)

Age Category	Mean Age*	n (%)	TST positive	Prevalence	ARTI% (95% CI)
13-16 yrs	15.8	178 (29)	85	47.8%	4.0% (3.2-5.0%)
17 yrs	17.5	148 (24)	76	51.4%	4.0% (3.2-5.0%)
18 yrs	18.5	112 (18)	58	51.8%	3.9% (2.9-5.0%)
19-22 yrs	20.2	182 (29)	115	63.2%	4.8% (4.0-5.8%)
TOTAL	**18**	**620**	**334**	**53.9%**	**4.2% (3.8-4.7%)**

* The mean age reported here is the age used for the ARTI calculations, and is based on the mean age for the age category+0.5 years, as described in the methods section.

Sub-analysis: HIV and TB infection

Of the 820 participants enrolled in the 2009 secondary school survey, 816 (99.5%) consented to HIV testing. In total 40 (4.9%) participants tested HIV-infected. In a logistic regression model, HIV infection was associated with increasing age (OR: 1.3; 95% CI: 1.1-1.6; p=0.001) and female gender (OR: 2.3; 95% CI: 1.1-4.7; p=0.03).

Among the 809 participants who underwent both TST and HIV testing, 38 were HIV-infected, four of whom had previous TST testing in 2007. The median TST reaction size among HIV-infected participants (n=34; excluding those with a repeat test) was smaller than that of HIV-uninfected participants (0 vs 11.5mm; p=0.08), and this difference was significant when adjusted for age and gender (p=0.04). Using the revised cut-off of ≥5mm, 15 (40%) HIV-infected participants were TST positive. In a multivariate logistic regression model adjusted for age and gender (Table 2.6), HIV infection was not significantly associated with TST positivity (OR = 0.53; 95% CI: 0.27-1.05; p=0.07).

Table 2.6: Risk factors associated with TST positivity in 809 secondary school learners with HIV test results

	TST Negative n=373 n (%)	TST Positive n=436 n (%)	Univariate Analysis OR (95% CI)	Multivariate Analysis OR (95% CI)
Age			1.09 (1.01-1.17)	1.09 (1.01-1.17)
Gender				
Male	132 (35)	215 (49)	1	1
Female	241 (65)	221 (51)	0.56 (0.42 – 0.75)	0.59 (0.44 – 0.78)
HIV status				
HIV-Uninfected	350 (94)	421 (97)	1	1
HIV-Infected	23 (6)	15 (3)	0.54 (0.28 – 1.06)	0.53 (0.27 – 1.05)

Sub-analysis: Repeated TST testing

In 2009, 159 students who had participated in the 2007 survey also took part in the secondary school survey. The median age of these 159 participants was 15 years (IQR: 15-16), which was marginally higher than the median age of the participants in the combined surveys (median 15 vs 14 years; p<0.001). Otherwise, the 159 participants did not differ statistically from the participants in the combined surveys combined in terms of gender (53% male vs 47% respectively; p=0.17), HIV status (2.5% vs 5.5% respectively; p=0.12) or BCG scar status (20% in both groups; p=0.99).

TST results

When assessing change in TST reaction size from 2007 to 2009, 55 (35%) of participants had no change in mean reaction size. Overall, 80 (50%) participants had an increase in reaction size, with a mean increase of 5.8mm. Changes in reaction size are shown in Figure 2.4.

Using a 10mm cut-off for positivity, in 2007, 76 (48%) of these participants had a positive TST result; in 2009, 91 (57%) tested TST positive. Overall, 19 participants who were TST negative in 2007 tested positive in 2009 (23%; p=0.003), and four (5%) reverted to a TST negative result. Table 2.7 shows the comparison of results from 2007 to 2009.

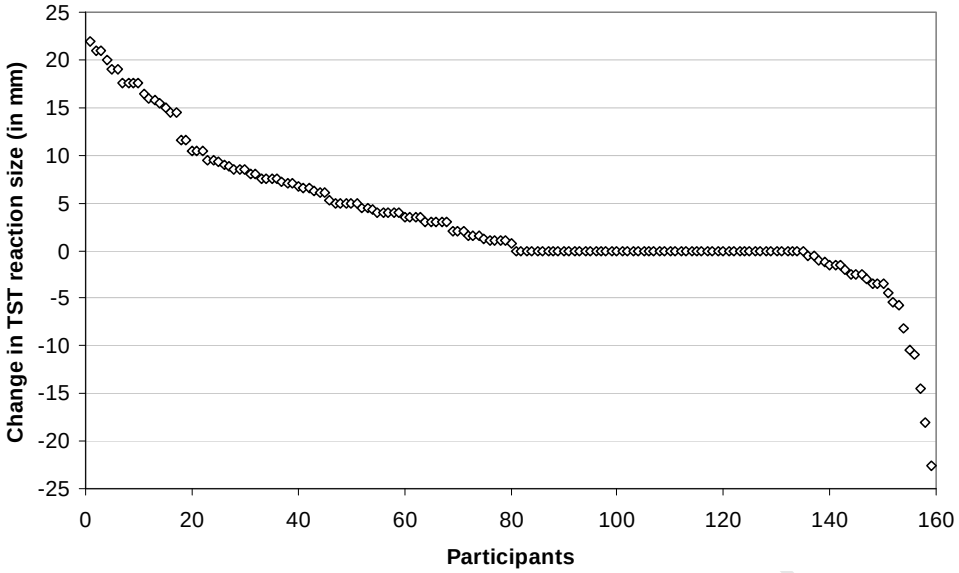


Figure 2.4: Changes in TST reaction size in participants tested in 2007 and 2009

Table 2.7: Matched comparison of TST results in 2007 and 2009

		2007		
		TST Negative	TST Positive	**TOTAL**
2009	TST Negative	64 (40%)	4 (3%)	**68**
	TST Positive	19 (12%)	72 (45%)	**91**
	TOTAL	**83**	**76**	**159**

The 19 participants who converted to a positive TST result in 2009 did not differ statistically from the 64 that remained TST negative in terms of median age (15 years in both groups; p=0.89), gender (63% male vs 45% respectively; p=0.17) or BCG scar status (74% vs 80% respectively, p=0.58). Similarly there was no change between the four participants who reverted to a TST negative result in 2009 and those who remained TST negative in terms of age (p=0.81), gender (p=0.62) or BCG scar status (p=0.61).

Using the definitions outlined above, 18 of the 19 convertors had an increase in reaction size of ≥10mm and were therefore considered true new infections with TB. These 18 participants equate to an incidence of 22% over two years (95% CI: 13-32%), or an annual incidence of infection of 11%.

In 2009, the mean reaction size among participants with repeat tests was larger compared to those participants who tested for the first time (15 vs 11.5mm; p<0.001), and this finding persisted when adjusted for age and gender (p<0.001). Overall TB prevalence did not differ significantly between those that had a repeat TST test and those participants who were testing for the first time (58 vs 54%, p=0.29).

2.4.3 Combined Primary and Secondary School Surveys

TST results from the primary school children were combined with those of the 620 HIV-uninfected secondary school children, excluding the second test in participants who had a repeat test in 2009, providing a sample of 1,451 participants.

Demographic Characteristics

Ages of the combined sample ranged from 5 to 22 years, with a mean age of 13.6 years (std dev: 4.1), and 52% of the participants were female. Overall 80% of the children did not have a BCG scar. Demographic characteristics are reported in Table 2.8

Table 2.8: Demographic characteristics of the participants in the combined surveys

	AGE QUARTILES				TOTAL
	5 to 9 yrs n=325 n (%)	**10 to 14 yrs n=488 n (%)**	**15 to 17 yrs n=344 n (%)**	**18 to 22 yrs n=294 n (%)**	**n=1,451 n (%)**
Mean Age	7.8 years	12.3 years	16.2 years	19.1 years	13.6 years
Gender					
Male	165 (51)	241 (49)	142 (41)	143 (49)	691 (48)
Female	160 (49)	249 (51)	202 (59)	151 (51)	760 (52)
BCG status					
BCG scar present	54* (17)	159* (33)	44 (13)	36 (12)	293 (20)

*One BCG scar status unknown

TST Results

TST reaction sizes ranged from 0 to 30mm (median=0mm; IQR:0-16.5mm). Overall, 728 (50%) of the participants had no reaction to the TST. The frequency distribution of reaction sizes >0mm are presented in Figure 2.5.

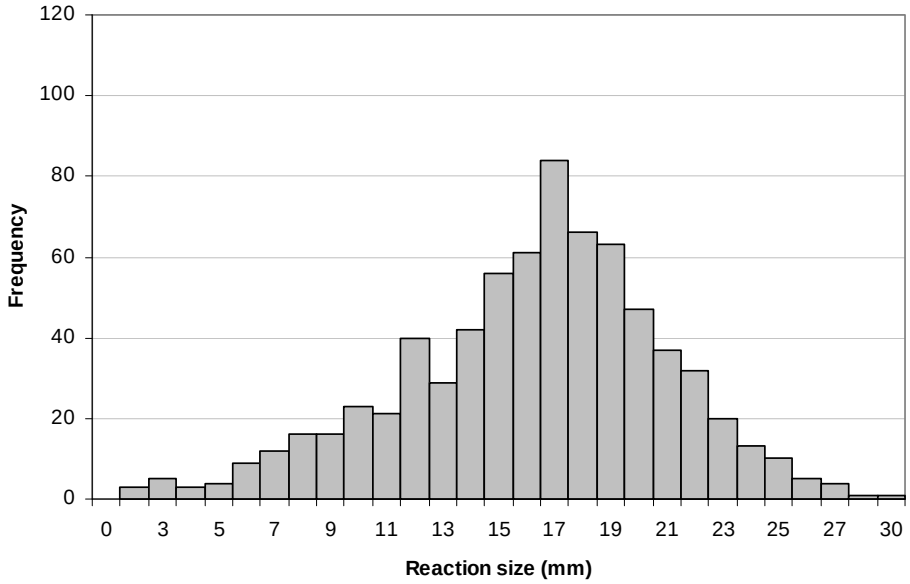


Figure 2.5: Frequency distribution of TST reactions (>0mm) in combined primary and secondary school surveys

At the 10mm cut-off, 645 participants (45%) had a positive TST result (Table 2.9). TB prevalence by age, and by age and gender are presented in Figures 2.6 and 2.7 respectively. In a multivariate logistic regression model, a positive TST result was significantly associated with age (adjusted OR for a 1-year increase in age: 1.17, 95% CI: 1.10 – 1.25; p<0.001) and male gender (adjusted OR for female compared to male: 0.74, 95% CI: 0.60-0.92; p=0.01). TST positivity was not associated with BCG scar status (p=0.40) or year of survey (p=0.23).

Table 2.10 reports the smoothed prevalence of TB infection by age, as predicted by logistic regression model. Also reported in Table 2.10 are the ARTI and force of infection by age for this sample, calculated from the smoothed prevalence. The overall ARTI for this sample was 4.1% (95% CI: 3.8-4.4%). The ARTI did not differ significantly across age by years from 6 to 22 years of age (p=0.15), or from 6 to 14 years of age (p=1.00).

The force of infection, as determined by the probability of becoming infected at a specific age, increased with increasing age, and this trend was significant (p=0.01).

Table 2.9: TB prevalence and ARTI by age quartile and gender for the 10mm cut-off point for the three surveys combined (excluding HIV-infected and second tests in repeat TST participants)

	Mean Age*	n (%)	TST positive	Prevalence	ARTI% (95% CI)
Age Category					
5-9yrs	8.3	325 (22)	91	28.0%	3.9% (3.1-4.7%)
10-14yrs	12.8	488 (34)	207	42.4%	4.0% (3.2-5.0%)
15-17yrs	16.7	344 (24)	174	50.6%	3.9% (2.9-5.0%)
18-22yrs	19.6	294 (20)	173	58.8%	4.8% (4.0-5.8%)
Gender					
Male	13.8	691 (48)	330	47.8%	4.6% (4.1-5.1%)
Female	14.3	760 (52)	315	41.4%	3.7% (3.3-4.1%)
TOTAL	**14.1**	**1,451**	**645**	**44.5%**	**4.1% (3.8-4.4%)**

* The mean age reported here is the age used for the ARTI calculations, and is based on the mean age for the age category+0.5 years, as described in the methods section.

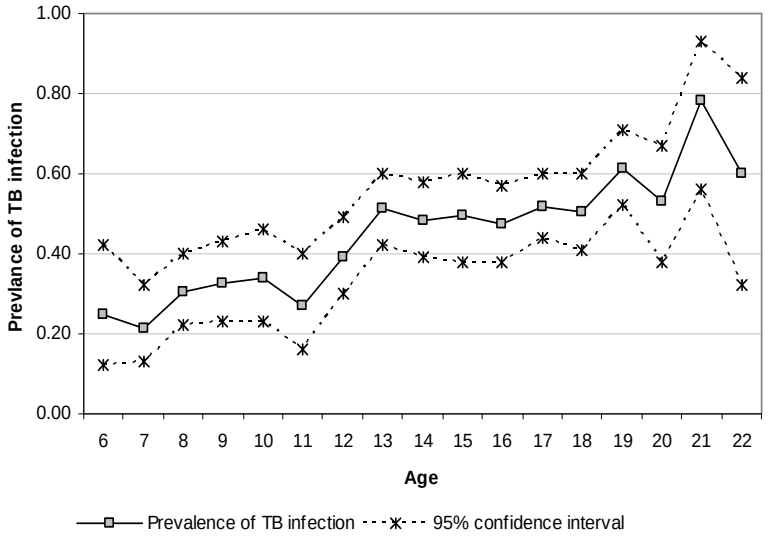


Figure 2.6: Prevalence of TB infection in combined survey by age

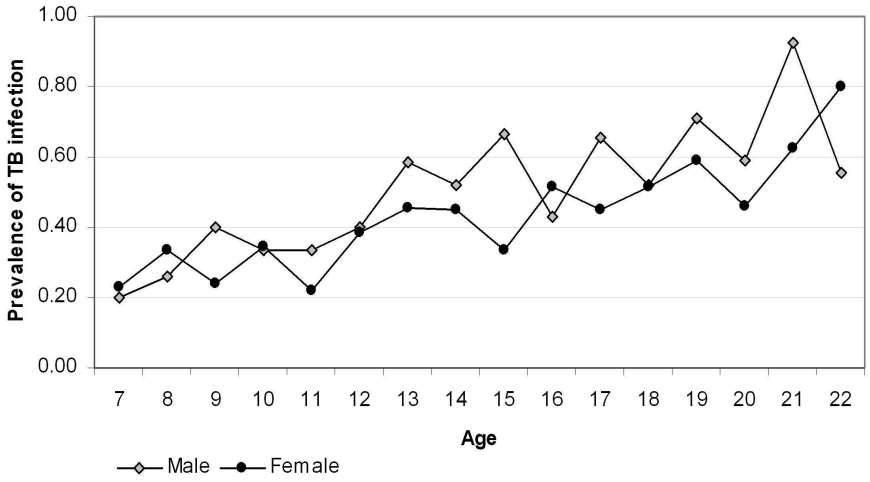


Figure 2.7: Prevalence of TB infection in combined survey by age and gender

Table 2.10: Predicted prevalence, ARTI and force of infection by age in study sample

Age	Number of participants	Predicted Prevalence* (95% CI)	ARTI**	Force of infection $^{\Phi}$
5	1	0.22 (0.17 - 0.27)	4.85	
6	36	0.24 (0.19 - 0.29)	4.47	2.93
7	84	0.26 (0.22 - 0.30)	4.21	3.15
8	112	0.28 (0.24 - 0.32)	4.06	3.39
9	92	0.31 (0.27 - 0.34)	3.96	3.81
10	71	0.33 (0.29 - 0.36)	3.91	3.96
11	59	0.35 (0.32 - 0.39)	3.89	4.28
12	107	0.38 (0.35 - 0.41)	3.91	4.47
13	135	0.41 (0.38 - 0.43)	3.93	5.03
14	116	0.43 (0.41 - 0.46)	3.97	5.11
15	84	0.46 (0.44 - 0.49)	4.04	5.58
16	106	0.49 (0.46 - 0.52)	4.10	5.70
17	154	0.52 (0.48 - 0.55)	4.18	6.05
18	112	0.54 (0.51 - 0.58)	4.27	6.44
19	101	0.57 (0.53 - 0.61)	4.36	6.63
20	46	0.60 (0.55 - 0.64)	4.45	7.11
21	21	0.62 (0.58 - 0.67)	4.55	7.37
22	14	0.65 (0.60 - 0.70)	4.66	7.34

*** Predictive logistic regression model:** logit{P(Age)} = -1.82+0.111 age.
****** The ARTI calculations are based on the mean age for the age category+0.5 years, as described in the methods section.
$^{\Phi}$ Force of infection = annual change in prevalence/(1-prevalence)

2.5 DISCUSSION

A key finding of this study was the staggering ARTI and force of infection rates identified among children and adolescents in this community. These findings are in keeping with the substantial burden of TB disease prevalence[179] and notifications[39] in Site M, and are consistent with the prevalence of latent TB infection of 67 – 88% reported among young HIV-uninfected adult controls from similar communities in Cape Town[265;272;273].

2.5.1 Interpreting the ARTI

The ARTI of 4.1% observed in this study was markedly higher than those documented both in sub-Saharan Africa prior to the HIV epidemic (1.5-2.5% in late 1980's) and in many other sub-Saharan countries which are heavily affected by HIV (0.68-2% reported in other countries). If ARTI provides a measure of the risk of infection from birth to time of study, this survey covers the annual risk from 1987 through to 2009, a period of escalating HIV and TB epidemics in Site M[39].

We found that the ARTI remained constant across the age range of 5-22 years, despite children being exposed to a rapidly increasing TB epidemic. Unfortunately, the difficulty in separating the effect of age and calendar year in ARTI complicates the interpretation of ARTI across age groups. Figure 2.8 illustrates three hypothetical conditions under which an ARTI of 4.1% could be obtained for this community.

The consistency of ARTI across age groups may seem to imply that the risk of TB infection has remained constant over the entire calendar period (Figure 2.8a). Given the relatively constant rates of TB notification among HIV-uninfected patients over this time period (Chapter 5), as well as the possibility that HIV-associated TB cases may be less infectious[124;176;177], it is possible that the annual risk of infection has indeed remained constant in this community. This theory is further supported by the stable TB notification rates in children over this time period (Chapter 5). A constant ARTI may reflect that younger children are not proportionately affected by the HIV-associated TB epidemic since their predominant social contacts are young peers, parents and teachers. However social interactions of adolescents may more closely reflect those of adults in the community, and ARTI also remained stable in the older study participants.

It could be argued that, despite the consistency across age groups, the ARTI may in fact be increasing in this community[274;275] (Figure 2.8b). There is evidence to suggest that, as in other African countries with functional Directly Observed Therapy, Short-course (DOTS) programmes[2], the risk of infection in South Africa may have been decreasing in the years preceding the HIV epidemics[258], with reports of 0.5-1.4% ARTI in South Africa in the 1970's[256;258]. It is possible that the HIV epidemic led to a reversal of this trend, and an increase in the risk of TB infection. We have reported a significant increase in pulmonary TB (PTB), including smear-positive PTB, among adults in this community, associated with the HIV epidemic (Chapter 5). This may support the argument of an increasing ARTI over time, especially in light of a recent study that reported increasing ARTI from 1998 to 2005 in another South African community, albeit a community with low HIV prevalence[264]. It should be noted the same study also reported a stable ARTI in a second, low HIV prevalent South African community, over the same time period[264]. Conversely, if the community experienced an HIV-associated increased risk of TB infection, then it stands to reason that the introduction of the high coverage antiretroviral therapy programme may have led to a decline in TB infection risk, as the HAART programme has been associated with a decline in TB notifications, including smear-positive TB, as well as a decline in TB prevalence (Chapters 5 and 6). However these changes would have occurred over a relatively short period in this community and may, therefore, be obscured by the "averaging" nature of the ARTI measure (Figure 2.8c).

As ARTI is an average measure of risk across all the years from birth to time of testing, it may not be possible to separate variations in risk with age from changes in risk trends within calendar years in a single cross-sectional analysis[1;274;275]. Subsequent mathematical modeling has shown that in a single survey even with constant ARTI across age groups, the risk of TB infection could in fact be increasing, decreasing, or indeed remaining constant over the calendar period[274].

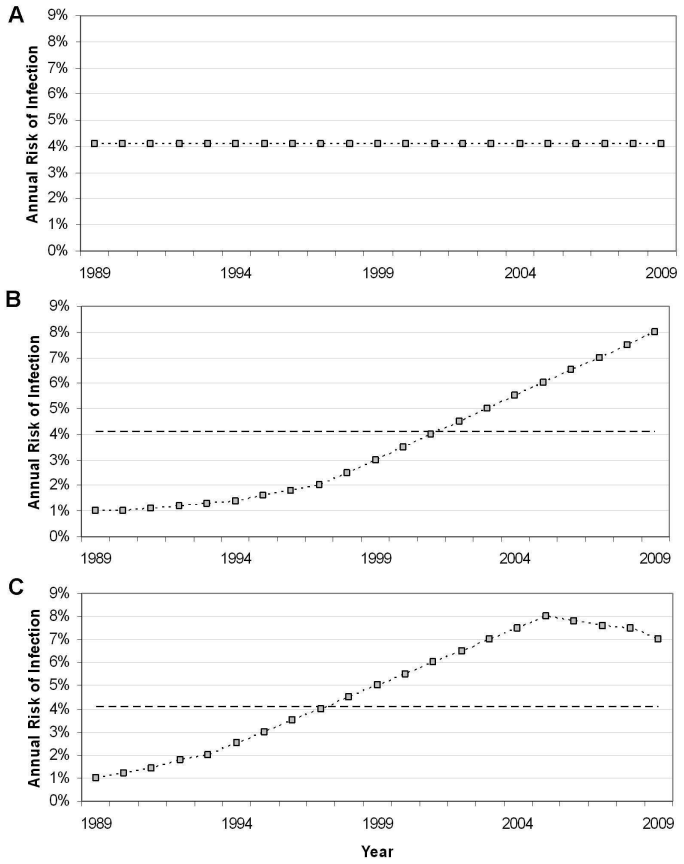


Figure 2.8: Hypothetical scenarios of risk of TB infection, resulting in the observed ARTI in Site M

Because ARTI is an averaged measure of the participants' life-time risk of infection, this measure most accurately reflects recent transmission risk in younger children. As such, the ARTI of 4.5-4.9% in children aged 5 to 6 years most likely provides a reasonably accurate estimate of risk of infection in these children over the past 5 years. When assessed with the low force of infection in children aged 5 to 10 years of age, it appears that the risk of transmission is high in very young children (<5 years), decreases as they enter early school years, and increases again as they approach adolescence.

Site M was established in 1994, and consequently many of the older children may have spent some time in other communities. Therefore this measure reflects the annual risk over a period of up to 20 years, as well as the risk encountered in different communities in which participants may have resided over that time. As a result the ARTI across the whole sample provides little information on the current transmission within the study

population. However, the ARTI does provide insight into transmission in South Africa generally, suggesting considerable TB infection rates over the past 20 years. Irrespective of the direction of the trend in risk of infection, an ARTI of 4% means that the national TB control programme is falling short of the goal of reducing the proportion of infected individuals necessary to achieve control of the TB epidemic[115].

2.5.2 Interpreting the Force of Infection

A more accurate measure of recent transmission is required in order to understand the current transmission dynamics in the study setting. The force of infection measure is not subject to the "averaging" nature of ARTI and provides just such a gauge of recent transmission.

In this study, we demonstrated a high force of infection that was positively associated with increasing age. These findings are consistent with those suggested by mathematical modeling[265]. The advance of this study on the mathematical paper was that of greater numbers of older adolescent participants recruited from the same community, and the ability to show that the force of infection continues to increase up to approximately 20 years of age, at which age force of infection may plateau.

Force of infection is a function of the probability of an effective encounter with an infectious TB case, and as such is the product of infectious TB disease prevalence and social mixing patterns. The correlation noted between force of infection and age may be due to increasing social mixing patterns, resulting from changing social interactions associated with age. In this community TB infection in primary school children was associated with the presence of an adult TB case on their residential plot (Chapter 3)[182], while TB transmission between adults was due to interactions off the residential plots (Chapter 4)[265]. Molecular epidemiological data from elsewhere have also confirmed that young children are significantly more likely to acquire TB infection from a household contact compared to adolescents[276]. Data from Europe show that the number of social contacts peak in adolescence[277], suggesting that the likelihood of contact with infectious persons may also peak at this time. The social interactions, and therefore risk of TB infection, of mid-teens may more closely resemble that of adults rather than younger children in the community. Social mixing data show that people tend to be in contact predominantly with others close to their own age[277] and molecular epidemiology has

suggested that index TB cases may preferentially transmit TB to people who are of a similar age[278]. Given that older patients are more likely to be sputum smear-positive compared to young children, these social interactions may, in part, explain the increasing force of infection noted in adolescents.

We have also shown a changing risk of TB infection by gender. In the primary school surveys, gender was not a risk factor for TB infection, but in the secondary school survey male gender was associated with increased TB infection. In other words, as males get older, their risk of TB infection out-strips that of females of similar age, and this is consistent with other reports in the literature[1;212]. Hypothetically, this increased risk may be due to a combination of increased biological susceptibility, differing immunological responses, or differences in socialization patterns of male compared to female adolescents. Further studies into the changing and diversifying socialization patterns by age and gender in this setting are needed.

Force of infection and HIV

While the HIV-associated TB epidemic undoubtedly contributes to the force of infection in this community, it is unlikely to be driving force of infection to a greater degree than the HIV-unassociated TB epidemic. The most significant risk factor for TB transmission is the sputum bacillary burden of the index case[55-58]. Up to 70% of TB patients in this community are co-infected with HIV, and HIV-infected TB patients have lower rates of smear-positive TB, compared to HIV-uninfected TB patients[18;118] (in this community 47% vs 67% smear-positive respectively). There is also evidence to suggest that HIV-associated smear-positive TB cases may be less infectious than their HIV-uninfected counterparts[124;176;177] (although it should be noted that there is also evidence suggesting no difference in infectivity between HIV-infected and HIV-uninfected smear-positive TB cases[181-186]). In addition, HIV-infected patients may contribute a shorter infectious period in the community due to rapid progression to TB disease and increased mortality[124;178]. It has been estimated that HIV-associated TB may be responsible for as little 7% of TB transmission[9] and is therefore unlikely to contribute significantly to the force of infection.

However, while the HIV epidemic may not be the primary driver of the force of infection, it is certainly adding fuel to the raging TB epidemic. The high force of infection in this community would result in significant rates of both primary and secondary TB infection:

by the time of school entry at 6 years of age, approximately 25% of children are latently infected with TB and the majority of adolescent are infected before the onset of sexual debut[205;279]. The prevalence of latent TB infection among young HIV-uninfected adults is as high as 64-88%[265;272;273]. Given the high HIV prevalence among adults in Site M[179;197] and the considerable increased risk of progression to TB disease in HIV-infected individuals, both from reactivation of latent infection and following recent infection[14;102], these findings could explain the substantial incidence of TB disease in this setting.

In order to reduce the force of infection, we need to decrease the effective contact between infectious cases and susceptible individuals. This could be achieved by decreasing the prevalence of infectious cases and the duration of infectiousness in the community, as well as by changing the conditions of social contact. Chapter 6 reports that a high coverage HAART programme reduces TB prevalence among HIV-infected participants, due to both improved immune function and the active TB case-finding among patients initiating HAART[197]. Extending active case-finding (ACF) to HIV-uninfected residents may substantially reduce the burden of infectious cases in the population as well[280]. ACF together with efficient initiation of TB treatment would also serve to decrease the infectious period of cases in the community. Social programmes that impact the environment in which individuals interact, such as improved housing with decreased crowding and improved ventilation, may also lead to a reduction in the incidence of TB infection.

2.5.3 Interpreting the Repeat TST Testing Results

The substantial incidence rate of TB infection in the subset of participants tested two years apart confirmed the current high force of infection, more than two-fold higher than the ARTI. The incident rate in this subset was greater even than that suggested by the force of infection calculations, and this is likely due to the small sample size (and therefore wide confidence intervals, which included the force of infection measure) as well as the boosting of the immune response noted with repeated TSTs[238;281-285].

Boosting is an increase in TST reaction size resulting from increased sensitization of the immune system to the tuberculin[2;238]. Skin sensitivity to the tuberculin may wane with age[213;220], resulting in a false-negative TST reaction on initial testing, and therefore boosting may be a desired effect of repeated testing: resulting in a positive TST

response due to immunity recall in an individual who previously tested TST negative[209]. However, the boosting phenomenon does complicate the ability to distinguish between a boosted positive response and a true conversion due to new infection[2].

Boosting is associated with increasing age[238;281;282;284;286]. While the boosting effect may be more common in older patients, it has been recognised in young children[269;283;285;286], although studies suggest it may not be as marked in children under 20 years of age[238;269].

The booster phenomenon is less frequent >60 days after the first negative TST result[211;238;269], but has been reported at low rates of 3-4% in adult studies up to 1 to 1.5 years following the first tuberculin test[209;238;269,287].

Boosting raises a key limitation of the tuberculin test: TST is dependent on immunological response. As a result, repeated testing to determine incident cases is difficult to interpret due to the ”booster” effect of enhanced allergy[238;269] and this is highlighted by the fact that participants with a repeat test in 2009 had larger median TST reaction sizes compared to first-time participants. Similarly, immune suppression can limit the sensitivity of this test, resulting in anergic responses to TST, such as have been noted in HIV-infected patients[98-101], and this is underscored by the smaller median TST reaction sizes in HIV-infected participants compared to HIV-uninfected participants.

2.5.4 HIV and TB Infection

The lack of association between HIV and TB infection is another key finding of this study. Adolescents infected with HIV did not appear to be at higher risk for acquiring primary TB infection. This is in keeping with findings reported from lower HIV and TB prevalent settings[14;97], and is consistent with studies that suggest that the establishment of TB infection is mechanistic, as opposed to immune-based[288]. Despite the use of a revised TST cut-off of 5mm in HIV-infected participants, this finding should be interpreted with caution for a couple of reasons. Firstly, the lack of statistical significance is border line, and a larger sample size may provide greater power to detect an association between TB infection and HIV. Secondly, as mentioned above, the interpretation of TST results is difficult when immune function may be compromised. No CD4 data were available for the adolescents who tested HIV-infected, nor was the mode

of transmission identified. If adolescents were recently infected, then it could be expected that CD4 counts were relatively normal, thus accounting for the lack of association between TB and HIV infection. In this scenario the findings among the adolescents may not be generalisable to the broader HIV population. However, if a substantial number of the HIV-infected adolescents had acquired their infection through vertical transmission[289], then their CD4 counts may be sub-optimal, impairing the participants' response to the TST. A larger study which includes CD4 count testing, or utilizes a non-immune based test for TB infection is required in order to determine the risk of infection in HIV-infected individuals versus HIV-uninfected individuals.

This limitation notwithstanding, adolescents are at increased risk of progression to TB disease in the first two years following TB infection[72], and the high TB infection rates in this community place adolescents at substantial risk of TB disease.

HIV infection was associated with female gender in this study and this is characteristic of the HIV epidemic among adolescents and young adults in sub-Saharan[13] and South African[290] settings.

2.5.5 Strengths and Weaknesses

These data are based on a sample of school-attending children from a single community and therefore they may not be generalisable to other regions. However, our findings have been confirmed by the subsequent reports of similar TB infection and ARTI rates in other South African communities[263;264]. This study had a very low refusal rate (<4%), and as such volunteerism is unlikely to have biased our results. However, it should be noted that the school-attending children may not be representative of all the children in the community, in particular with regards to risk of HIV infection[291;292].

In this study, only 1% of TST results were between 0 and 5mm, and therefore there is little evidence of the cross-reaction with environmental mycobacterium. This is in keeping with the findings of other studies in similar settings[205;263]. Given the high prevalence of TB in this community, the positive predictive value of the TST is likely to be high.

The size of reaction used to define TST positivity is the topic of much discussion in the literature[269;293-296], with a number of methods for determining the most epidemiologically appropriate cut-off suggested[99;216;223]. Epidemiologically-based methods include the mirror method or mixture analysis, and the most appropriate analytic method depends on the environmental context of the specific survey[223]. Due to the low rate of environmental mycobacterium cross-reaction noted in our study, we used the mean reaction excluding all non-reactors[223] method for determining the TST positivity cut-off in our sensitivity analyses in the primary school sample. These sensitivity analyses confirmed the main study findings, and this consistency of findings across these methodologies was also reported in another South African study[263]. Therefore we used the conventional, clinical cut-off of ≥10mm to define TB infection for the main analyses, and this is in keeping with other reported studies[263;283].

Despite the South African policy since 1960 to vaccinate all infants with the BCG vaccine[297], only 20% of the children in this study had an observable BCG scar. However BCG scarring following vaccination may be variable[285;298], and the absence of the scar does not necessary denote lack of immunity[293]. We found no difference in the distribution of TST results between those children with or without BCG scars, and we therefore included all children in the analysis. The lack of correlation between the presence of a BCG scar and tuberculin reaction has been shown in other studies[293;298;299], as has a waning of BCG-related reaction to TST with increasing time since vaccination[229;232;293;298;300]. However the lack of association between BCG scar status and TST result may imply that there is no immunological benefit of infant vaccination in the adolescent age group.

HIV testing was not performed for the primary school children in this survey as this may have adversely affected parental willingness to consent to their child's participation. Primary school study participants were born between 1990 and 2001, when the reported HIV prevalence at antenatal clinics ranged from 0.7 to 24%[301]. This was prior to the introduction of the prevention-of-mother-to-child-transmission programme. With a transmission rate of approximately 30%, we estimate that 0.2% of the older participants and up to 7% of the younger children may have been infected with HIV perinatally. However considering the high early mortality rate among infected children[302], it is probable that a maximum of 0.1 to 4% of children in this study sample might be HIV-

infected. This reasoning is supported by the 1% HIV prevalence found in 13 to 15 year olds in the secondary school survey. This is a low prevalence is unlikely to have had a large impact on the TB results. We were also able to exclude HIV-infected children from the TB analysis in the secondary school, thereby reducing the potential bias resulting from dual infections.

2.5.6 Conclusion

These data report an already considerable prevalence of TB infection, a staggering ARTI and a substantial force of infection among children and adolescents in a high HIV and TB prevalent setting. ARTI is high among very young children, followed by a force of infection that is lower among children aged 5 to 10 years, and subsequently escalating in adolescents. This association of force of infection, which provides a measure of recent transmission, with increasing age is most likely due to changing social mixing patterns among adolescents, which may result in increased contact with infectious TB cases.

Chapter 3

Transmission of Tuberculosis to Children: Risk factors for TB Infection and Disease

3.1 RATIONALE

Chapter 2 demonstrated the high rate of tuberculosis (TB) infection in children resident in Site M. The next step was to determine the risk factors associated with transmission of TB to children in the community, and to determine the role of the HIV co-epidemic in the risk of transmission.

Adult TB disease comprises a combination of recent infections and reactivation of latent TB, and therefore represents both recent and historic TB exposure. Childhood infection is acquired predominantly from adults (as child to child transmission is uncommon) and represents acquisition of TB some time from birth to chronological age[303]. Once infected, young children have a greater risk for progression to active disease compared to adults[69;304]. Therefore infection and disease in young children are the result of recent TB transmission, predominantly from adults to children[204].

This chapter will address the second objective of this thesis, namely to determine the risk of TB transmission to children residing in close contact with adult TB cases, and to determine the importance of adult HIV-associated TB in this transmission. Data from this chapter have been published in the second paper[182] listed in the preface on page *xi*.

3.2 BACKGROUND

3.2.1 Burden of Paediatric TB Disease

While the World Health Organization (WHO) estimates sputum-positive TB rates for children <15 years of age to be in the range of 29/100,000 in South Africa[305], these data grossly underestimate the total paediatric TB burden. Firstly, the difficulty in obtaining sputum specimens from young children results in a low proportion of confirmed smear-positive paediatric TB cases[306;307]. Secondly, childhood TB disease occurs most frequently in children <2 years, resulting in a numerator of disease for this small age group being diluted by the larger population denominator (0-14 year olds) used by the WHO.

Studies from the 1980's and early 1990's estimated that childhood TB accounted for 15-22% of TB cases in developing countries[117;308], and 7% of TB mortality[117]. More recently, analysis of TB notification data from two South African communities showed TB disease rates of over 400/100,000 in children <15 years of age[205;309] and >3,500/100,000 in children <6 years of age[310]. In these communities, paediatric TB may account for as much as 39% of community case load[310].

TB disease is a significant cause of mortality in children, especially in developing countries. The WHO estimated that 450,000 children died from TB in 1999[8]. More recent studies of mortality in developing countries have shown TB to be an important respiratory cause of death[311], especially among HIV-infected children[312].

3.2.2 Risk Factors for TB Transmission to Children

As prolonged time and proximity of contact with an infectious person increases a susceptible individual's risk of acquiring TB infection[60;66;67], it is not surprising that the risk of infection and disease for children has long been associated with household exposure to TB disease, in both developed and developing countries[54;57;313-316]. More recently, investigators have attempted to confirm this risk using molecular epidemiological studies. While insufficient statistical power, due to the low yield of sputum specimens and therefore genotyping data, has hampered this endeavour[317;318], the findings of at least one such molecular epidemiological study has confirmed the risk of household transmission to children[276].

In keeping with the general principles of TB transmission, household exposure to smear-positive disease carries the highest risk of transmission[57;59;61;319;320], although smear-negative disease in the household has also been associated with transmission to children[62]. Similarly, the risk of transmission is associated with intimacy of contact with the adult index case[56;57;61;313;321], as well as duration of contact[313;321;322]. Some studies have reported that index cases with close familial relationships are more likely to infect children in the household[322], in particular female index cases[54;319], presumably as a function of time spent with child contacts.

Other risk factors for TB infection and disease in young children include poor nutritional status[319;323-325], and living in crowded households[59;310;323;325;326].

3.2.3 Studies of Household Transmission to Children in Sub-Saharan Africa

Studies performed in sub-Saharan Africa have consistently reported increased risk of infection and disease in children exposed to infectious TB cases in the household.

Sub-Saharan Africa

In the 1960's, before the HIV epidemic, data analyzed from 9 African countries, including Swaziland and Lesotho (then called Basutoland), confirmed a higher prevalence of TB infection in children living in households with an infectious TB case, compared to children living in other households[316]. Two Kenyan studies also performed in the 1960's reported TB infection rates of 41-60% in children <15 years of age who were household contacts of sputum positive TB cases[54;327], and these infection rates were considerably higher than the background infection rates[54]. The children also had a substantially higher prevalence of disease compared to the general population[54].

More recently, studies performed in the past two decades, subsequent to the advent of the HIV epidemic, have consistently reported high prevalence of TB infection and disease in children who are household contacts of infectious adult TB cases. TB infection rates ranged from 35%-54% in children up to 15 years of age[184;185;313], and TB infection rates in contacts ≤5 years old were substantially higher than those in the background community (27% vs 10% respectively)[319]. The rate of TB disease varied from 1.7-23% in household contacts ≤5 years of age[319;326;328] and 6% in all household contacts[326], considerably higher than background TB rates in these countries[7].

South Africa

South African-based studies in the 1980's showed a background infection rate of 13% in the general child population[256;329], while children who were household contacts of confirmed TB cases had an infection rate as high as 30%[329], and a TB disease prevalence of up to 11%[308].

In a recent study in a community with high tuberculosis rates and relatively low HIV prevalence rates, 34% of children ≤5 years of age living in households with an infectious

adult TB had TB disease and a conservative estimate of an additional 14% had TB infection[323].

Most studies in relatively low to moderate HIV prevalence settings (adult prevalence <10%[330-332]) have reported that the HIV status of the adult source case did not impact on transmission to children[184;185;313], but in at least one study performed in a high HIV prevalence setting (HIV prevalence >10%[333]) HIV-infected status of the adult case was associated with less evidence of TB transmission[175].

3.2.4 Current Interventions to Reduce Childhood TB

It is clear that transmission of TB to children in the household remains a significant problem. Childhood TB infection not only results in childhood TB disease, with a substantial burden of morbidity and mortality, but also serves as a reservoir for future adult TB disease. Therefore, the WHO has recommended screening of children in close contact with adult pulmonary TB (PTB) cases, in particular sputum smear-positive cases, and the initiation of chemoprophylaxis or chemotherapy in these children as appropriate[334]. Although these guidelines have been incorporated into the national TB control programmes of many developing countries, due to limited resources[328] the tracing, screening and management of child contacts is often poorly implemented[319;335;336].

Given the high rates of TB infection among children in Site M and the paucity of data on transmission to children in high TB and HIV prevalent settings, we performed a retrospective study to examine the risk of TB transmission to children residing on residential plots with adult TB cases in Site M, and to explore the importance of HIV-associated TB in this transmission.

3.3 STUDY DESIGN

Adult TB notification data from Site M together with Geographic Information System (GIS) software were used to correlate the relationships between TB exposure, infection and disease in children with exposure to adult HIV-infected and HIV-uninfected TB disease on residential plots over time. The temporal association between adult cases and childhood disease was also explored.

3.3.1 Childhood TB Infection

TB infection data were obtained from primary school tuberculin skin test (TST) surveys performed in the community in 2006 and 2007, and described in Chapter 2[205]. Childhood TB infection was defined as an induration ≥10mm in response to two units of purified protein derivative (PPD) administered intradermally.

3.3.2 Childhood and Adult TB Disease

Childhood TB disease cases in the study community from 1997 to 2007 were extracted from the community TB register – this is the time period from which TB notification data are available for the study community, until the tuberculin surveys were performed. All adult TB cases notified by the community TB clinic between 1997 and 2007 were cross-referenced with both childhood TB cases and TST results from the school surveys. Children diagnosed with childhood TB disease during the study period were excluded from the TST database for this analysis. Adult TB cases were defined as patients ≥15 years and children were <15 years of age.

Site M consists of a formal sector with demarcated, and individually numbered, serviced plots and an informal sector of shacks sharing communal services. In the formal sector, residents use the assigned plot number as their address. However, in the informal sectors no official addresses are available and, due to the hazards of fire and flooding, shack numbers may be "recycled" for different abodes. Therefore, to ensure reliability of matched data, this analysis was restricted to residents in the formal sector (approximately 78% of the community, based on community census data). Demographic and clinical data including age, gender, TB clinical details and HIV status were collected from the TB register and clinic records.

3.3.3 Data Analysis

Data were analyzed using STATA 10.0 (StataCorp, College Station, Texas).
Bivariate analyses employed Student's t-, Wilcoxon sum rank and chi^2 tests, as appropriate. Rates of notified child disease were calculated using denominators derived from the population model described in Chapter 1 and Appendix A.

Childhood Infection: Children with positive TST results were compared to children with negative TST results, with regard to exposure to adult TB cases on residential plots.

Multiple logistic regression models were developed to determine characteristics of adult TB cases that were associated with positive TST results. In the analysis of the primary school TST data, age of the child was the only factor significantly associated with a positive TST[205] (Chapter 2), and therefore logistic regression models assessing factors associated with TST positivity included age of the child. Models were also adjusted for clustering effect on plots. Adult TB case characteristics were included as binary exposures (any exposure to a TB case with a particular characteristic) and as counts of exposures to a TB case with a particular characteristic, per child. Children who were included in the childhood disease analysis were excluded from the childhood infection analysis.

Childhood Disease: The characteristics of adult TB cases occurring on residential plots on which childhood TB disease cases were diagnosed were assessed. The time difference between adult and child cases was calculated as year of childhood TB case diagnosis minus year of adult TB case diagnosis. Analysis of childhood TB cases initially included all adult TB exposures on the same residential plot, followed by an analysis of the adult TB exposure with the closest temporal link to each childhood TB case. All statistical tests are 2-sided at $\alpha=0.05$.

Exposure was defined as a child living on the same residential plot as an adult diagnosed with active TB disease. This measure of exposure did not take into consideration the duration of contact with the adult case.
An exposure rate, defined as the average number of exposure to adult TB disease per year, was determined for childhood TB disease, infection and non-infection. This measure was calculated by dividing the percentage of children exposed to adult disease by the median age of the group of interest.

ArcMap 9.2 (Esri™) Geographic Information System was used to assess the spatial distribution of childhood TB disease, infection and exposure with linked adult cases in the community.

3.4 STUDY FINDINGS

Over the study period 1,708 TB cases were notified, of which 1,386 (81%) were resident in the formal sector. Of the 1,386 TB cases 1, 213 were adult cases and 170 were childhood cases, with 3 cases excluded from analysis due to no recorded age.

TB patients in the formal sector did not differ from those in the informal sector (Table 3.1) in terms of age (p=0.23), gender (p=0.62), site of TB disease (ie pulmonary vs extra-pulmonary TB [EPTB] disease; p=0.84) and HIV status (p=0.96). Patients in the formal and informal sectors did not differ in terms of TB mortality (p=0.78), nor in TB treatment completion rates (p=0.86) or treatment failure rates (p=0.13). However, TB cases from the formal sector were more likely to be transferred out of the community than those from the informal areas (p=0.01) and less likely to interrupt TB treatment (p=0.02).

Table 3.1: Comparison of demographics and TB disease characteristics between TB patients residing in the formal sector and those residing in the informal sector of Site M∞

	TB cases in formal sector (n=1,386)	**TB cases in informal sector (n=322)**	**p-value**
Age: Median (IQR)	31 (24-39)	30 (24-37)	0.23
Gender			
Male	746 (54%)	168 (52%)	
Female	640 (46%)	154 (48%)	0.62
TB category			
New TB case	1,062 (77%)	241 (75%)	0.50
Retreatment TB case	324 (23%)	81 (25%)	
Site of TB disease			
Pulmonary TB	1,062 (74%)	239 (74%)	0.84
Extra-pulmonary TB	365 (26%)	83 (26%)	
TB outcomes∞			
Cured/Treatment completed	1,013 (73%)	237 (74%)	0.86
Treatment interrupted	116 (8%)	41 (13%)	0.02
Treatment failure	10 (1%)	0 (0%)	0.13
Died	93 (7%)	23 (7%)	0.78
Transferred out of community	147 (11%)	19 (6%)	0.01
Tested for HIV			
HIV status known	973 (70%)	239 (74%)	0.15
HIV status*			
HIV-infected	645 (66%)	158 (66%)	
HIV-uninfected	328 (34%)	81 (34%)	0.96

∞ Some outcomes data were missing: analysis were run on available data

*Excluding those of unknown HIV status

3.4.1 Childhood TB Infection and Exposure to Adult TB Case on Residential Plot

Of the 831 children analyzed in the TST survey, 651 (78%) lived in the formal sector and 640 (77%) of those had not had childhood TB during the period from 1997 to 2007. The 640 children included in this analysis did not differ significantly from the total TST study sample in terms of age (p=0.96), gender (p=0.25), BCG status (p=1.00) or positive TST reaction (p=0.69).

Of the 640 children, 321 were TST positive, with a median age of 12 years (interquartile range [IQR]: 9-14). The remaining 409 were TST negative and had a median age of 10 years (IQR: 8-13).

Of the 640 TST survey participants, 359 (56%) had been exposed to an adult TB case on their plot. Of these 359 children, 316 (49%) were exposed to PTB, of which 265 (41%) were exposed to smear-positive PTB. Table 3.2 shows the characteristics of the adult TB case by the child participants' TST result.

Table 3.2: TST results and exposure to adult TB cases on the same residential plot:

Tuberculin Skin Test	Exposure to adult TB case n (%)	Exposure to adult PTB case n (%)	Exposure to adult smear positive PTB case n (%)	Exposure to adult smear negative PTB case n (%)
Total (n=640)	359 (65)	316 (49)	265 (41)	115 (18)
TST positive (n=231)	149 (65)	137 (59)	123 (53)	39 (17)
TST negative (n=409)	210 (51)	178 (44)	142 (35)	76 (19)
p-value*	0.001	0.001	<0.001	0.61

* Comparison of proportion exposed between TST positive and TST negative children.

Figures 3.1 and 3.2 show the distribution in the community of adult TB cases together with TST positive and TST negative children, respectively. The odds of a positive TST

result, compared to a negative TST result, were 71% higher in those children exposed to any TB case on their plot (odds ratio [OR]: 1.71; 95% confidence interval [CI]: 1.20-2.44), 88% higher in those exposed to PTB (OR: 1.88; 95% CI: 1.32-2.68), and 110% higher in those exposed to sputum smear-positive PTB disease (OR: 2.10, 95% CI: 1.46-3.01) compared to no plot-based exposure to adult TB. Exposure to sputum smear-negative PTB was not associated with a positive TST result (OR: 0.95; 95% CI: 0.59-1.54), and this finding persisted after adjusting for HIV status of notified adult TB cases (OR: 0.68; 95% CI: 0.21-2.18).

Figure 3.1: Distribution of children with TST positive results and of adult TB cases from 1997 to 2007 in the community

In this figure, the solid dark line denotes the border of the formal sector in Site M. Grey borders within the formal sector denote residential plots. Grey-shaded rectangles indicate one or more adult TB cases occurred on that plot over the study period. Green dots of increasing size indicate increasing numbers of children with TB infection occurring on the plot during the study period.

Among the 359 children exposed to an adult TB case on their plot, the number of adult exposures ranged from 1 to 7 per plot (mean number of exposures: 2.2). The number of

sputum smear-positive TB case exposures ranged from 1 to 6 cases per plot (mean: 1.6 cases).

The odds of a positive TST result increased by 31% for every additional case of TB that a child was exposed to (OR: 1.31; 95% CI: 1.05-1.24), by 43% for every additional case of PTB (OR: 1.43; 95% CI: 1.11-1.84), and by 60% for every additional case of smear-positive PTB (OR: 1.60, 95% CI: 1.21-2.11). The increasing association between positive TST result and exposure to smear-positive PTB cases persisted after adjusting for HIV status of the adult TB cases (OR: 1.62; 95% CI: 1.19-2.20).

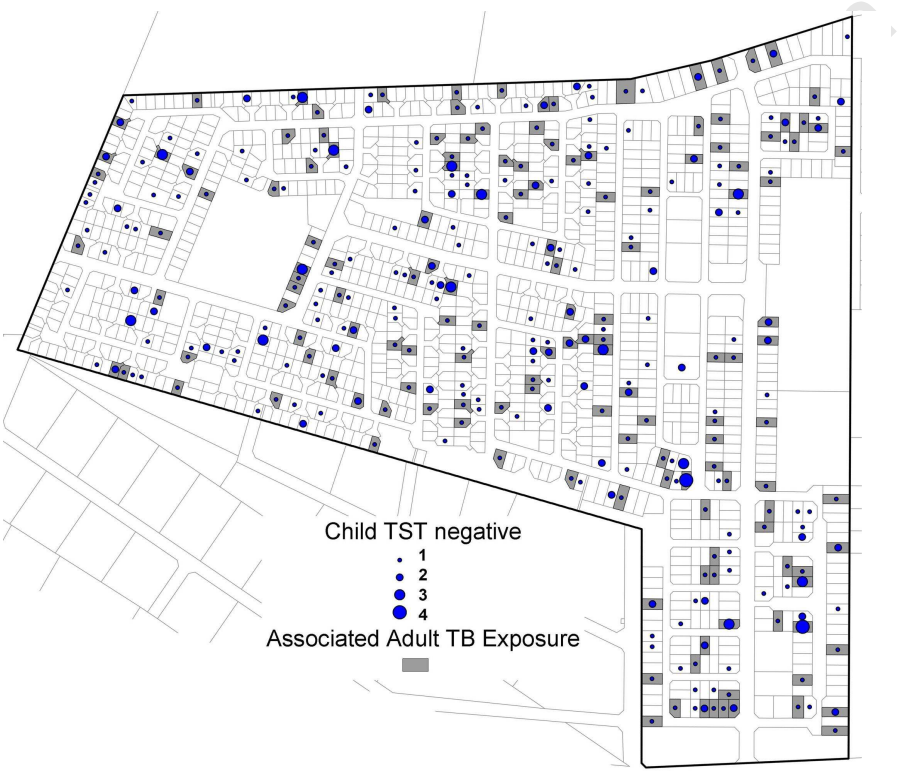


Figure 3.2: Distribution of children with TST negative results and of adult TB cases from 1997 to 2007 in the community

In this figure, the solid, dark line denotes the border of the formal sector in Site M. Grey borders within the formal sector denote residential plots. Grey-shaded rectangles indicate one or more adult TB cases occurred on that plot over the study period. Blue dots of increasing size indicate increasing numbers of children without TB infection on the plot during the study period.

HIV status of adult TB cases was not significantly associated with the child's TST result: the odds of a positive TST result if the adult case was HIV-uninfected was 1.02 (95% CI:

0.65-1.60) and the odds of a positive TST result if the adult case was HIV-infected was 0.88 (95% CI: 0.59-1.31). The TST result was not associated with age (p=0.73) or gender (p=0.28) of the adult exposure TB cases.

3.4.2 Childhood TB Disease and Exposure to Adult TB Case on Residential Plot

The annual rates of childhood TB disease in the community ranged from 443 to 1,062/100,000 over the study period (Chapter 5), with a mean annual rate of 728/100,000 for all TB. Childhood TB cases in Site M are described in more detail in Chapter 5. The median age of the 170 childhood TB cases from the formal sector was 2 years (IQR: 1-6 years) and 58% were female. Childhood TB cases occurred on 144 plots in the community, with a range of 1 to 3 cases per plot (mean of 1.19 cases per plot).

Of the 170 childhood TB cases in Site M from 1997 to 2007, 113 (66%) had one or more exposures to a notified adult TB case on their residential plot. In total, 92 (53%) were exposed to adult PTB cases. Childhood TB cases were exposed to significantly more smear-positive TB disease than smear-negative TB disease: 75 of childhood cases were exposed to smear-positive PTB and 17 to smear-negative PTB (44% of all child TB cases vs 10%; p<0.001). Figure 3.3 shows the distribution of child TB cases and adult TB cases in the community.

Among the 113 children exposed to an adult TB case on their plot, the number of adult exposures ranged from 1 to 11 per plot (mean number of exposures: 2.4). The number of smear-positive TB case exposures ranged from 1 to 6 cases per plot (mean: 1.9).

The 113 childhood TB cases that were exposed to TB cases on their residential plot did not differ significantly from the 57 childhood TB cases not exposed to TB on their plot in terms of age (p=0.23), gender (p=0.51) or HIV status (p=0.76). However, childhood TB cases not exposed on their residential plot are more likely to be retreatment TB cases than those exposed to TB on their plot (12% vs 4%; p=0.03).

Timing of Adult and Childhood TB Cases on Residential Plots

Among the 113 children with TB disease and with adult exposures on their plot, 47 (42%) of the adult cases were diagnosed in the same year as the childhood cases. Overall, 25 (22%) were diagnosed before the childhood case and 41 (36%) after the childhood case was diagnosed. The time interval between diagnosis of all adult

exposure cases and the child case ranged from 9 years prior to the child's TB diagnosis to 10 years after the diagnosis of the child TB case (median=1 year after child case; IQR: +1 year to -4 years after) (Figure 3.4).

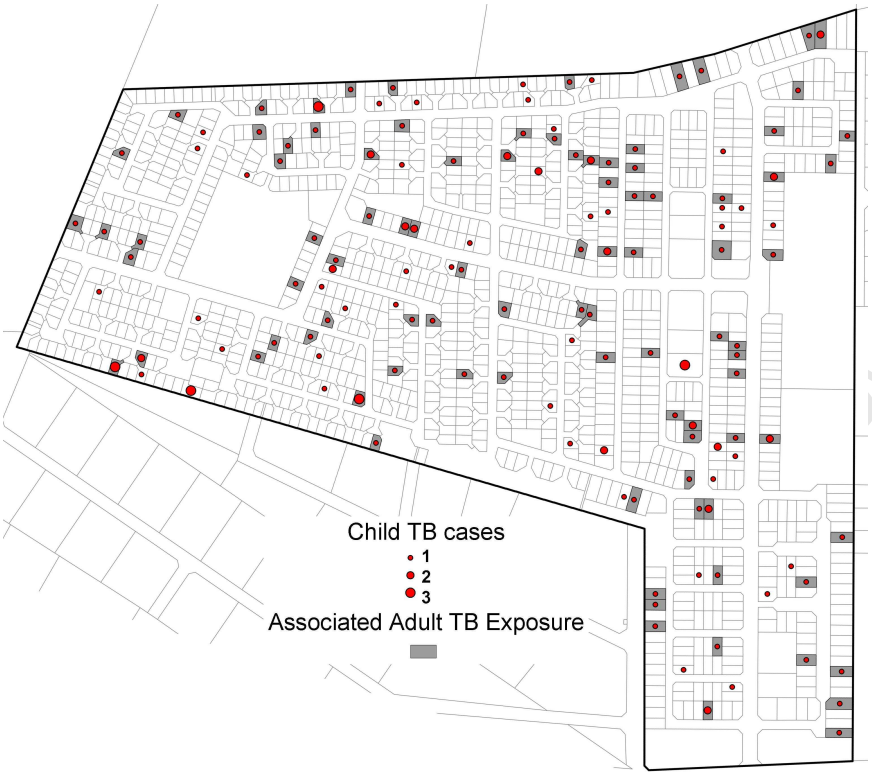


Figure 3.3: Distribution of childhood TB cases and of notified adult TB cases from 1997 to 2007 in the community

In this figure, the solid, dark line denotes the border of the formal sector in Site M. Grey borders within the formal sector denote residential plots. Grey-shaded rectangles indicate one or more adult TB cases occurred on that plot over the study period. Red dots of increasing size indicate increasing numbers of children with TB disease occurring on the plot during the study period.

When analysis was restricted to the adult TB case most closely linked temporally to each child case, the time interval between cases varied from 3 years prior to child TB case to 8 years after the child TB case (Figure 3.5). The adult cases which occurred in the same year, before the child TB case and after the child case did not differ statistically from each other with respect to age (p=0.91), gender (p=0.55), HIV status (p=0.87), TB category (ie new or retreatment TB: p=0.20) or TB treatment outcomes (p=0.40). The disease characteristics of the closest adult TB cases by time are shown in Table 3.3.

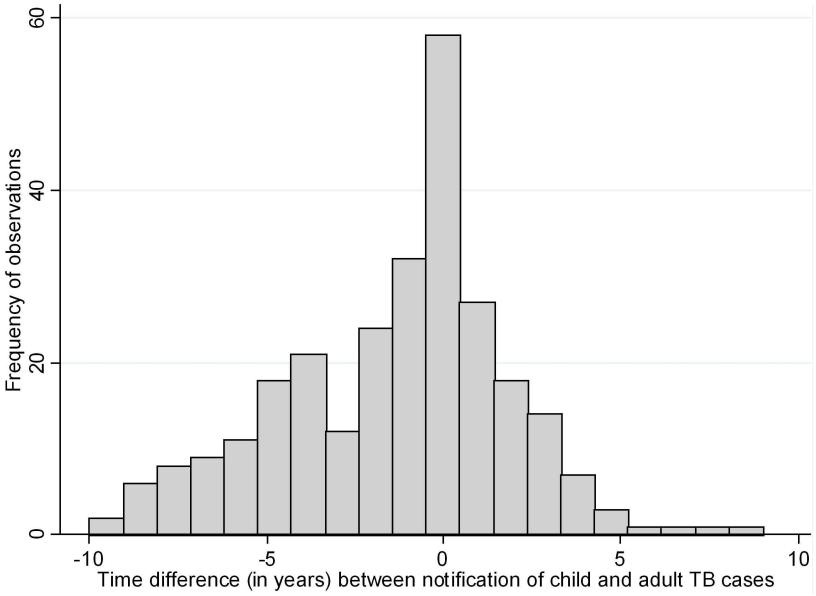


Figure 3.4: Distribution of time difference between all adult TB exposures and childhood TB case

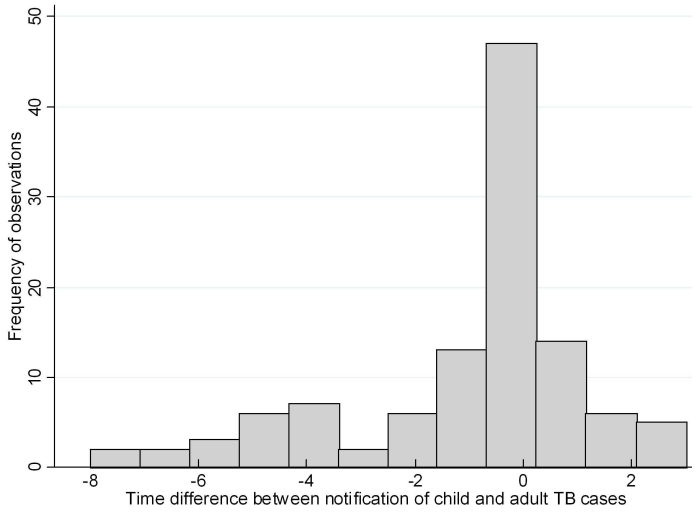


Figure 3.5: Distribution of time difference in years between temporally closest adult TB exposure and childhood TB case on the same residential plot in study community

Table 3.3: Temporal Associations between childhood TB cases and exposure to temporally closest adult TB cases on the same residential plot

	Exposure to adult TB case n (%)	Exposure to adult PTB case n (%)	Exposure to adult smear positive PTB case n (%)	Exposure to adult smear negative PTB case n (%)
Child TB cases (n=171)	113 (66)	92 (53)	75 (44)	17 (10)
Adult TB case notified before or in same year as child case	72 (64)	60 (83)	48 (67)	10 (14)
Adult TB case notified after child case	41 (36)	32 (78)	27 (66)	5 (12)
p-value*	<0.001	0.47	0.93	0.80

* Comparison of exposure proportions between adult cases notified before or in same year as child, to those notified after child case.

3.4.3 Childhood TB Disease, Infection and Exposure

The mean number of adult exposures per plot was lower in children with negative TST results compared to those with positive TST results (1.8 vs 2.2 respectively; p=0.03), and also compared to those with childhood TB disease (1.8 vs 2.4 respectively; p=0.02). There was no statistical difference between the mean number of adult exposures per plot for children with TB infection (TST positive) and those with TB disease (p=0.34).

The mean number of adult smear-positive exposures per child is higher in children with TB disease than those with TB infection (1.9 vs 1.6; p=0.06), as well as higher than those without infection (1.9 vs 1.0; p<0.001). Children infected with TB had a significantly higher number of mean exposures compared to those children not infected (1.6 vs 1.0; p<0.001).

3.4.4 Average Annual Exposure Rate

The average annual exposure rate on residential plots for children with disease was 33% per annum, for children with infection it was 5.4% per annum and for children without infection, 5.1% per annum.

3.5 DISCUSSION

Children in this community had an extremely high exposure to adult TB disease in their restricted social environment as defined by their residential plot, and child TB infection and disease rates were strongly associated with this exposure to adult TB.

The residential plots used as the unit for this analysis differs from the traditional household unit applied in other studies. Consistent with other peri-urban communities in South Africa, the residential plots in Site M are an average of 180m^2 in size and are often crowded, with from 1 to 22 dwellings built on a plot (mean of 4 houses per plot). Plots are frequently shared by extended families, and represent a broadened but intimate social environment with communal living, including shared child care responsibilities.

3.5.1 Risk Factors for TB Disease and Infection

Childhood TB infection and disease were associated with adult TB cases on their residential plot. The association was strongest with adult smear-positive PTB, rather than smear-negative PTB or extra-pulmonary forms of TB disease. A few studies have suggested that in endemic areas TB transmission to children frequently occurs outside the household[317;337]. However, in keeping with the bulk of published literature[57;310;313;319;323], our studies would suggest that, especially in younger children, the main transmission risk remains in the residential social environment.

Of note was the finding that the HIV status of the adult TB case was not an independent risk factor for infection or disease in children. The finding that smear-positivity and not HIV status of an adult TB index case is the main risk factor for TB infection in children exposed at home supports the suggestion in Chapter 2 that the HIV-associated TB epidemic alone is unlikely to be the primary driver of the force of infection in this community, but may indirectly fuel childhood TB disease and infection rates by

increasing the burden of adult smear-positive TB cases in their immediate social network[39].

Some studies have reported an association between female index cases and increased risk of transmission[54;319]. However, we found no association between the gender of the adult case and the risk of infection or disease for children, and this is in keeping with other study findings in published literature[276;320;322].

3.5.2 Annual Exposure Rates to TB Disease

Whilst TST surveys assess the rates and force of TB infection in children, this analysis allowed an assessment of annual rate of exposure to adult TB cases on children's residential plots. The average annual exposure to adult TB cases on residential plots was high for all participants living on serviced plots in this community, with >5% of infected and uninfected children being exposed to an adult TB case per year. The annual exposure rate was more than six-fold higher for children with TB disease. This substantial average annual exposure of children to TB on their residential plot was the product of the large burden of adult TB disease in this community together with overcrowding on plots and has resulted in high rates of both child TB infection and disease in Site M.

This analysis was restricted to exposure to TB on the child's residential plot, and while we have shown a strong association between exposure on plot and childhood TB infection and disease, we have not accounted for all transmission to children. There are several potential explanations for TB transmission to children in whom no adult TB case was identified on the residential plot. These could include an unrecognized primary source that subsequently moved off the plot, died prior to diagnosis, or remained subclinical, including so-called "chronic excretors". In addition, modeling has shown that, while the greatest risk of transmission to children was from infectious adults on residential plots, up to 25% of transmission to children may be due to interaction with non-resident adults[338].

3.5.3 TB Control Programme and Childhood Exposure to TB Disease

The high rates of child TB infection and disease are an indication of failure to control exposure to prevalent infectious adult cases.

The majority of childhood TB disease (64%) was diagnosed in the same year or soon after an index adult TB case was notified to the TB control programme. However, over a third of child TB cases in this study were notified prior to the temporally closest notified adult case on their plot. It was noted that adult cases were diagnosed as much as 8 years after the childhood case of TB. The more temporally distant cases most likely represent secondary or even tertiary cases due to ongoing adult-to-adult transmission.

Recent studies have illustrated that patient-initiated delayed presentation of TB cases to the health care services as well as provider-initiated delays in the diagnosis and the initiation of TB treatment are a considerable problem in resource limited settings[339-342]. These delays contribute to the infectious period of a case and therefore to TB transmission.

This study was only able to assess the role of diagnosed adult TB on transmission to children. However, TB case finding has been low in this community: a random, community-based survey in Site M found that 0.1% of the adult population had undiagnosed smear-positive TB, and an equal proportion had undiagnosed culture-positive TB, with an overall TB case detection rate of 37% in the community[179]. Undiagnosed and undetected TB would also contribute to TB transmission to children, and therefore the impact of adult TB cases on a residential plot was most likely underestimated in this study.

Since 1996, the national TB control programme has mandated screening (including tuberculin skin testing)[310], and provision of chemotherapy or chemoprophylaxis as appropriate, in all children <5 years who are close contacts of an infectious adult TB case[195]. The high rate of TB infection among children with household contacts suggests that this policy is not well implemented, and the failure to effectively implement this active child-contact tracing policy has been reporting in another South African study[336]. While children in this study are older than 5 years of age and as such may not have been included by the TB control programme, 25% of 6 year olds are already infected with TB. An evaluation of the contact tracing and screening component of the TB control programme is required in order to inform interventions for improved screening and management of paediatric TB contacts.

3.5.4 The Role of Active Case-Finding

Given the substantial annual exposure rate and risk of re-infection among children in this setting, in addition to improved contact tracing, a reduction in the annual exposure rate is also required. In order to achieve this reduction in exposure, enhanced and active case-finding (ACF) may be needed to supplement the directly observed therapy, short-course (DOTS)-based programme. The yield of ACF varies in different settings. The yield of house-to-house active case-finding surveys in developing country settings has ranged from 0.1% to 0.2%[179;343;344]. However ACF in household contacts of diagnosed TB cases, both adult and paediatric cases, have shown substantial yield in diagnosing previously undetected cases in both high and low incidence settings[54;184;345-347], especially among symptomatic household contacts[328;348]. A recent meta-analysis of ACF among household contacts of TB cases showed high yields in low-income countries: 7% active TB disease identified, and 40% latent TB infection. The yield of active TB disease was highest in children <5 years of age: 8.5% active TB disease cases identified[349]. This analysis, which included studies from 13 sub-Saharan African countries, strongly supports the value of targeted active case-finding among TB contacts.

Targeted ACF is, therefore, likely to have a higher yield than random cross-sectional surveys and may decrease the prevalence of infectious cases, resulting in a lower rate of transmission to children. This study would suggest that in communities such as Site M, targeted ACF should include a patient's residential plot, rather than only household. In addition to the TB case detection yield, efficacy of such a targeted intervention over time could be assessed through repeated TST surveys in children, to ascertain the impact on childhood infection rates.

3.5.5 Strengths and Weaknesses

In Site M, residential plots contain a mean of 4 households which share communal water and sanitation services, and constitute a geographically defined unit for social interaction between adults and children outside of the immediate household. This study was restricted to the formally serviced sector of the community in which 78% of the community was resident. We were unable to include adults and children living in the informal sectors as shack identifiers in this sector were not constant over the study period. However, TB patients in the formal and informal sectors did not differ significantly in terms of demographics or TB characteristics, with the exception that TB cases from

the formal sector were more likely to leave the community and less likely to default from TB treatment. These factors may mitigate the risk to children residing on the same plot as the adult TB cases, in which case our study would have underestimated the impact of exposure to adult TB cases on transmission to children.

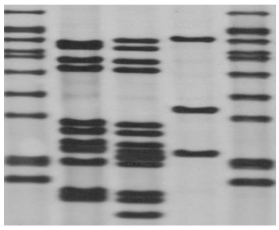
For the purposes of this analysis we assumed that the children had all lived in the community since 1997 or from birth. Some children may have immigrated into the community subsequent to these dates; however this would also have decreased the strength of association between adult-child TB exposures and resulted in an underestimation, rather than overestimation, of contact rate between adult and children with TB infection and disease.

Children with TB disease were excluded from the analysis of TB infection. This conservative approach may have also resulted in an underestimation of the TB transmission.

Molecular epidemiological studies to confirm the links between adult index cases and childhood cases would greatly strengthen these findings. Unfortunately, due in part to the minimal use of sputum testing in the investigation of childhood TB and in part to the low proportion of smear-positive cases in children[306], few RFLP data are available for paediatric cases. Such studies would be of value in furthering our understanding of these dynamics.

3.5.6 Conclusion

TB transmission within households and close geographic social networks is an important component of both childhood infection and disease in this community. Transmission to children was strongly associated with sputum smear-positive PTB cases, and was not independently associated with the HIV status of the adult index case. Childhood TB disease is a sentinel of infectious adult prevalence and therefore childhood infection and disease rates need to be monitored in these high prevalence settings in order to ascertain the true burden of infection and disease. Active contact tracing and screening of children exposed to adult disease needs to be improved in these settings. Furthermore, targeted ACF, aimed at close social networks of both child and adult TB cases may be an appropriate intervention in this setting.



Chapter 4

Transmission of Tuberculosis to Adults

4.1 RATIONALE

Chapter 3 reported on the risk factors associated with transmission of tuberculosis (TB) to children in the study community. While childhood TB disease is predominantly due to recent infection, adult disease may be due either to reactivation of previously acquired latent infection or rapid progression from recent TB infection. This chapter will explore TB transmission and the risk factors associated with transmission between adults in Site M.

While reactivation of latent infection is responsible for most of TB disease in the developed world[350-355], in sub-Saharan Africa studies have reported that recent infection is responsible for a greater proportion of TB disease[356-360]. Reactivation of latent infection is a manifestation of historical transmission, while recent infection is due to current, ongoing transmission. In settings in which progression from recent infection, rather than reactivation of latent infection, is responsible for the majority of TB disease, an effective intervention to decrease transmission may have the greatest impact on TB control[129].

An improved understanding of current TB transmission in high TB and HIV settings, as well as the impact of the HIV epidemic on this transmission, is required. Furthermore, understanding the impact on TB transmission of an intervention targeted at HIV, such as a highly active antiretroviral therapy (HAART) programme, could inform the development of effective adjunctive TB control strategies in high TB and HIV prevalent communities.

This chapter will address the third objective of this thesis, namely to describe the molecular epidemiology of TB in the study community, and to utilize molecular epidemiology to investigate the extent of clustering, as a measure of recent transmission, of *Mycobacterium tuberculosis* (*Mtb*) disease, to assess the impact of the HIV-associated TB epidemic on transmission, and to identify risk factors, including

demographics and household location, associated with transmission. Data from this chapter have been published in the third paper[361] listed in the preface on page *xi*.

4.2 BACKGROUND

4.2.1 Introduction to Molecular Epidemiology

Molecular epidemiology has been defined as "using the analysis of nucleic acids and proteins in the study of health and disease determinants in human populations"[362]. More simply stated molecular epidemiology is a multi-disciplinary approach that merges molecular methodologies and tools, including the ability to genotype organisms such as *Mtb*, with conventional epidemiological, statistical and clinical techniques. The result is a greatly increased ability to investigate and understand infectious disease epidemics, including those of TB and HIV.

The applications of molecular epidemiology in the research of infectious diseases are many and various[363]. Molecular epidemiology has been used to identify circulating *Mtb* strains in communities[357;364-367] and has enabled identification of clinical characteristics associated with specific strains[350;361;368-372]. Examples include the association of certain strains with drug resistance[373;374], and the differing host immune responses to different strains, with potential implications for pathogenicity and/or virulence and clinical outcomes[86;370;375-379]. One study has even reported differing impacts of *Mtb* strains on HIV-replication in infected cells[380].

Molecular epidemiology has also enabled the study of the spread of *Mtb* strains within and between communities[102;350;351;353;359;381-383]. Despite intensive contact investigation, conventional methodologies often identify few epidemiological links between TB cases[351;358;372;381;382;384;385]. Molecular epidemiology has greatly expanded our ability to identify *Mtb* outbreaks and clusters[350-352;354-360;381;386], including nosocomial transmission[374;387-389], and to distinguish between endogenous re-activation and recent exogenous infection in TB disease[127;351-353;355;356;360;390]. This information has provided important insights into the performance of TB control programmes[391;392], as well as aiding spatio-temporal investigations of transmission dynamics[351;361;384], and the identification of associated risk factors and high-risk groups or locations[337;350;351;354;382;393-399].

The W-Beijing strain is one of the most extensively studied *Mtb* strains, and is an excellent example of insights gained by molecular epidemiology. The W-Beijing strain appears to have originated in Asia, but has subsequently spread around the world[400;401], and is an extremely common, emerging strain in South Africa[317;361;366;367;402;403]. The ubiquitous nature of W-Beijing suggests that the strain has a selection advantage, but the possible mechanisms for this are not well understood. It has been proposed that certain sub-lineages of W-Beijing may have increased transmissibility[404], as supported by high bacillary loads[272;375;376] and TB outbreaks[367;401] associated with this strain, or that infection with this strain may progress more rapidly to disease[402;404;405]. The marked virulence of W-Beijing *Mtb* strains, shown in mice and rabbit models of infection[86;375;406], support this hypothesis.

4.2.2 Genotyping Technology

While the genome of *Mtb complex* is highly conserved[407], the pathogen does have polymorphic genomic regions[363;396], and it is these regions that are the focus of genotyping methods. There are a number of different genotyping techniques, each with their own strengths and weaknesses.

Restriction Fragment Length Polymorphism (RFLP) analysis was considered to be the gold standard genotyping technique for *Mtb* molecular epidemiology for many years[363;373;382;408;409]. While it is a complex and laborious method, requiring viable mycobacterial cultures, the procedure for RFLP analysis has been standardized[410], allowing for comparison across laboratories[411]. In addition, RFLP is reproducible[411] and remains one of the most discriminatory of all the genotyping techniques available for *Mtb*[363].

Insertion sequences (IS) are small mobile genetic units, which occur commonly in bacteria[412]. IS*6110* is unique to *Mtb complex*[413], and is, for the most part, distributed randomly throughout the genome[414]. The variability of the number and chromosomal positions of IS*6110* enables the differentiation between *Mtb* strains[408].

The methodology for RFLP has been well described[410;415]. Following *Mtb* culture and DNA extraction, restriction endonuclease enzyme PvuII (which cleaves IS*6110* at a single location) is used to digest the *Mtb* DNA, generating chromosomal fragments.

These fragments are separated on an agarose gel by electrophoresis, transferred to a DNA membrane (Southern-blot) and labeled with a DNA probe mixed in a hybridization buffer. The resulting Southern Blot hybridization of the restricted fragments and IS*6110* probe is highlighted by a chemiluminescence reaction. Computer-assisted analysis of images is performed, with the position and number of DNA bands used to identify the strain[416]. The nomenclature for classifying IS*6110* RFLP patterns has been well documented and described[401].

Other techniques include *single-nucleotide polymorphisms* (SNP), which is particularly useful in the study of microevolution of strains[363;396], but does require extensive sequencing of multiple chromosomal targets[363]. *Spacer oligonucleotide typing* (or spoligotyping) is based on analysis of 43 interspersed spacer sequences in the direct repeat (DR) region of the *Mtb* genome[417].This polymerase chain reaction (PCR)-based analysis is the simplest technique for strain genotyping but is less discriminatory than RFLP analysis[363;401;418]. *Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat* (MIRU-VNTR) typing is based on the analysis of 12 or 15 loci comprising major polymorphic tandem repeats[409;419]. When more than 12 loci are used, this method is as discriminatory as RFLP analysis, and indeed more so in mycobacterial strains with few IS6110 bands[420;421]. However, this technique may not be appropriate for the study of endemic strains, due to the slow rate of mutations in the combined loci[422].

Given the number of molecular techniques available, it is critical to choose the appropriate tool for the research question of interest. The key considerations are usually the stability of the biomarker used, and the diversity of the strains in the community[363]. The stability of the biomarkers is sometimes referred to as the “molecular clock” of the biomarkers: the biomarker must be stable enough to reliably link epidemiologically related cases, but change at a rate sufficient to distinguish non-related or distantly related strains[363;423].

For this thesis, IS*6110* Restriction Fragment Length Polymorphism was used to genotype *Mtb*. The biomarker IS6110 has been shown to have both a high degree of stability (with a transposition half-life of 2-4 years)[423-425] and sufficient variability[358;356;359;365] to ensure an excellent tool for identifying transmission at a population level. Furthermore, RFLP analysis is reproducible, allowing for robust

comparison between different laboratories' findings[408;420]. It has been widely used in *Mtb* molecular epidemiology, and as such there is much published RFLP data, allowing for comparison across different settings.

One limitation of the RFLP technique for transmission analysis is that it has lower discriminatory power in strains with 6 bands or less. In this situation a second typing technique, utilizing different biomarkers, such as SNP or spoligotyping, may be of value in identifying genetically distinct strains[422;426;427]. Conversely, strains with identical patterns of more than 6 bands are most likely to be clonal[363].

4.2.3 Molecular Epidemiology and Understanding TB Transmission

Based on the principle that organisms with similar or identical genetic fingerprints are epidemiologically related, clustering of identical *Mtb* strains is often interpreted as evidence of recent transmission, while unmatched or unique strains are considered to represent reactivation of previously acquired infection[128;350;372;416;428]. As mentioned above, studies have reported that recent infection is responsible for as much as 42-61% of TB disease in sub-Saharan Africa[356-360].

Population-based molecular epidemiological studies have provided insights into risk factors associated with TB transmission, including urban residence[353], prior imprisonment[357] and locations including bars[382;351;357] and churches[357] have been identified in different settings. However, there are also conflicting findings as to whether gender[353;357;359;429;430] or age[351;353;356;357;359;430] are associated with clusters.

While many of the studies in the developed world have reported an association between HIV infection and clustering[350;351;353;431;432], countries with generalized epidemics have conflicting findings on whether HIV infection is associated with clustering of *Mtb* strains[356-358;391;430;433-435]. Few studies from African countries with high TB incidence have reported on the association between HIV infection and transmission, and most of these studies have reported that HIV-infection is not associated with transmission[357;358;391;430]. One study found an association between TB disease due to recent infection and HIV-infection among older HIV-infected patients[356]. No studies have reported on the impact of a high coverage HAART programme on TB transmission.

4.3 STUDY DESIGN

4.3.1 Study Participants

Sputum specimens of acid-fast bacilli (AFB) smear-positive TB or smear-negative and *Mtb* culture-positive TB were collected from 2001 through 2008 from patients resident in the community, for genotypic analysis. Demographic and clinical data including age, gender, details of clinical diagnosis, disease management and clinical outcome, HIV status and location of residence of each patient were extracted from the clinic TB register and patient clinic folders.

4.3.2 Sputum Specimens

Sputum specimens were obtained from TB suspects in accordance with the National TB Control Programme diagnostic protocol[195], and were labeled as study specimens at the clinical site, for identification by laboratory personnel. All sputum specimens were assessed for the presence of *Mtb* bacilli by fluorescent (auramine) microscopy. AFB positive samples were cultured on Lowenstein-Jensen (LJ) slants, as were smear-negative specimens for which culture was clinically indicated (for example, retreatment patients). From 2001 to end 2005 isoniazid and rifampicin susceptibility testing was performed on retreatment patients or patients still AFB positive after two months treatment, in accordance with the National TB Control Programme[195]. From 2006 to end 2008, isoniazid and rifampicin susceptibility testing was performed on all *Mtb* specimens, and second line sensitivities were performed on all multi-drug resistant (MDR)-TB (resistant to at least isoniazid and rifampicin) specimens. *Mtb* culture-positive isolates were inoculated in duplicate into 7H9 liquid medium supplemented with oleic acid, albumin, dextrose and catalase (OADC) and 15% Glycerol at the research laboratory at the Institute of Infectious Diseases and Molecular Medicine at the University of Cape Town, and stored at -70ºC.

4.3.3 Molecular Analysis of *M. tuberculosis* Strains

Frozen duplicate culture stock was shipped to the Public Health Research Institute (PHRI) Tuberculosis Center at the University of Medicine and Dentistry of New Jersey. Culture stocks were sub-cultured on LJ slants and DNA extracted from each isolate. IS*6110*-based RFLP analysis was performed on each isolate as described elsewhere[436]. RFLP patterns were analyzed using BioImage pattern matching software (BioImage, MI).

Mtb isolates with DNA fingerprints with an identical hybridization banding pattern were considered to be the same strain and assigned a strain code following the previously described nomenclature system[381]. In addition, *Mtb* strains were assigned to one of nine (I – VIII and II.A) discrete synonymous single nucleotide polymorphism (sSNP)-based phylogenetic lineages, as determined by RFLP patterns and previous analysis of PHRI *Mtb* clinical isolates[437]. Strain patterns that were only represented once in the PHRI database (ie no matching strain found in the database of over 10,000 strains collected from eight USA sites, three South African sites and over 11 other countries[381]) and did not qualify for a family assignment were considered orphan strains and given a default assignment (001).

4.3.4 Definitions

A unique strain is an isolate with an RFLP pattern that occurs only once within the study dataset, ie a singleton strain. Strain family is a group of strains that exhibit similar, but not identical, IS*6110* hybridization profiles, suggesting common recent ancestry (e.g. W-Beijing family, CC family, BM family) as described elsewhere[363;437]. Strain clusters were defined as more than one occurrence of a specific strain in the study period. The first case diagnosed with a specific clustered strain was defined as the index case in each cluster (temporally-based definition), and subsequent patients in the clusters were referred to as secondary cases. A couplet was a cluster comprising of two patients with the same *Mtb* strain. In addition, we also identified temporally-related couplets: these were two patients with the same *Mtb* strain that occurred within 12 months of each other and did not have another case with the same strain within 6 months on either side of the pair.

Patients with unique strains (ie non-clustered strains) and index patients were considered likely to have TB disease due to reactivation of latent infection. Secondary cases in clusters were interpreted as having TB disease due to recent infection.

The pre-HAART era was defined as the years 2001 to end 2004, and the post-HAART era as years 2005 to end 2008. We a priori chose 2005 as the cut-off year of HAART availability, as this was the first year that an appreciable proportion of patients were receiving HAART (>5% of HIV-infected patients).

4.3.5 Data Analysis

Data were analyzed using STATA 10.0 (StataCorp, College Station, Texas). Bivariate analyses employed Student's t-, Wilcoxon rank sum tests and chi^2 tests, as appropriate. The adjusted number of strains per year (corrected for the differing sampling rates) was calculated as the product of the number of strains per patient (based on available data) and number of sputum-positive patients each year. Family diversity was calculated as the number of families divided by the number of isolates, and strain diversity was calculated as the number of strains divided by the number of isolates.

The proportion of patients and strains occurring in clusters was determined as the total percentage of patients and strains in clusters ("n" method), and the proportion of patients arising from recent transmission was calculated using the ("n-1") method[351]. The probability that a unique strain is in fact a couplet was determined by the following equation:

$P = \binom{B}{1}\left(\frac{1}{A}\right)^{1}\left(\frac{A-1}{A}\right)^{B-1}$, where A is the number of isolates in the community that were analyzed, and B is the number of isolates in the community that were not analyzed.

Changes in unique strains and clustering over time were assessed using Cox-Stuart test for trend[267;268]. Multivariate logistic regression models were developed to examine factors associated with specific *Mtb* strains, as well as with clustering of strains and with the index cases of clusters.

Patients were divided into age groups as follows, children (<15 years of age), 15-24 years, 25-34 years, 35 to 49 years and ≥50years of age. The proportion of reactivation and recent infection disease was compared across these age groups. The age difference between cases occurring in couplets was calculated as the age of index case (in years) - age of the secondary case (in years). The random probability that two cases with the same strain would occur on the same plot was determined by the Poisson distribution.

ArcMap 9.2 (Esri™) Geographic Information System (GIS) was used to illustrate the spatial distribution of *Mtb* strains occurring in pairs during the course of the study.

4.4 STUDY FINDINGS

4.4.1 Study Population

From 2001 to 2008, there were 1,681 TB cases (excluding 30 patients transferred into the community already on treatment), of which 1,240 were pulmonary TB (PTB). Of these PTB cases, 862 were smear-positive cases and 1,048 were smear-positive and/or culture-positive.

Sputum specimens were collected from 633 (64%) of the smear-positive cases, and 716 (68%) of the smear- and/or culture-positive cases. Of these specimens, 521 (73% of collected specimens) had RFLP patterns available. Reasons for missed specimens and unavailable RFLP data are shown in Figure 4.1. The sampling rate from each year is tabulated in Table 4.1.

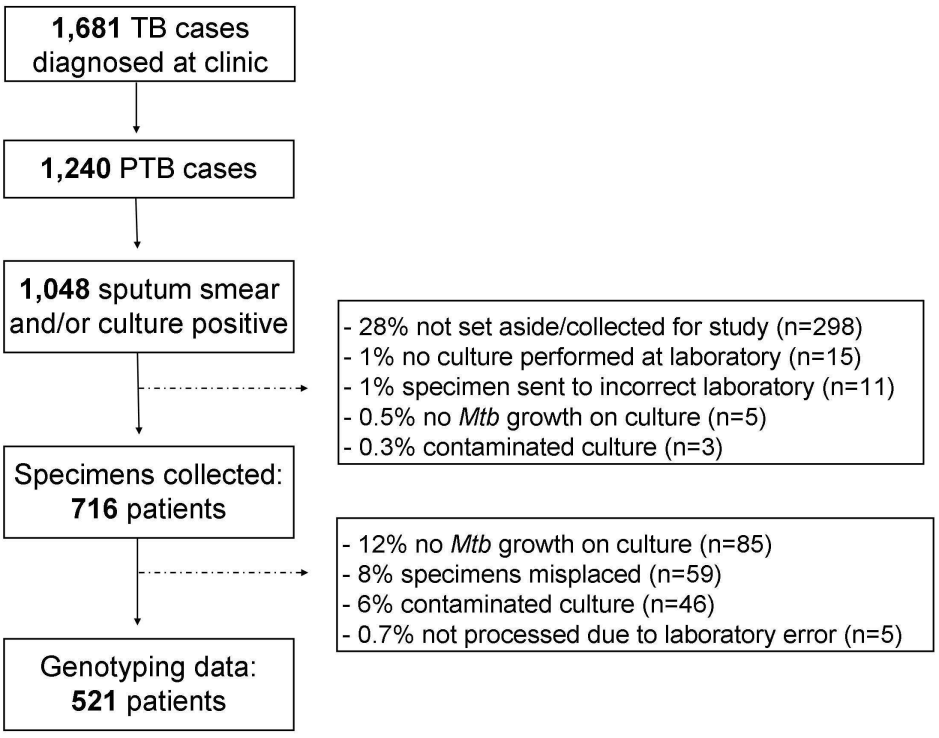


Figure 4.1: Consort diagram of the TB cohort in the study community from 2001 to 2008

Table 4.1: Number of smear and culture positive PTB patients, number of specimens retrieved, and number of patients for whom genotyping data is available, per year

Year	Smear and/or culture positive PTB	Specimen collected	RFLP pattern obtained
2001	72	20 (28%)	18 (25%)
2002	83	44 (53%)	32 (39%)
2003	98	63 (64%)	41 (42%)
2004	115	63 (55%)	34 (30%)
2005	191	97 (51%)	34 (18%)
2006	162	128 (79%)	109 (67%)
2007	164	150 (92%)	116 (71%)
2008	163	151 (93%)	137 (84%)
TOTAL	**1,048**	**716 (68%)**	**521 (50%)**

Patients for whom we obtained RFLP patterns did not differ from those for whom we did not obtained RFLP patterns, with regard to age (p=0.22), gender (p=0.92) and HIV status (p=0.73). There was no difference in new and retreatment cases between the two groups (p=0.27). We were less likely to obtain RFLP data for TB patients who died (p=0.004), and more likely to obtain RFLP data for patients who failed treatment (p=0.03). There were no differences in the other TB outcomes between patients for whom RFLP data was obtained and those for whom RFLP data was not available. We were more likely to obtain RFLP data on patients receiving HAART (Table 4.2).

The 521 patients with RFLP results ranged in age from 1 to 68 years (median age: 32 years, interquartile range [IQR]: 26-39) and 12 (2%) of the patients were children. Overall 299 (57%) of the patients with genotyping data were male. In total, 456 (88%) patients tested for HIV, and 61% of those 456 patients tested were HIV-infected. Of the 521 patients, 20 (4%) had confirmed MDR-TB and no cases of extensively-drug resistant TB were identified. TB treatment was completed by 82% of the 521 patients and there were 18 (4%) deaths among these patients.

While RFLP data are available for 521 patients, in total 530 RFLP patterns are available, as nine patients had a dual infection.

Table 4.2: Comparison of patients for whom sputum specimens were and were not collected, and of patients with and without genotyping data

	Specimens missed (n=332)	**Specimens received (n=716)**	**p-value***	**Specimens with RFLP result (n=521)**	**p-value****
Age: Median (IQR)	33 (27-42)	32 (26-40)	0.09	32 (26-39)	0.22
Gender					
Male	183 (55%)	420 (59%)		299 (57%)	
Female	149 (45%)	296 (41%)	0.28	222 (43%)	0.92
TB category∞					
New TB case	228 (69%)	516 (72%)	0.26	378 (72%)	0.27
Retreatment after previous cure	48 (14%)	83 (12%)	0.19	60 (11%)	0.34
Retreatment after previous completion	43 (13%)	73 (10%)	0.19	50 (10%)	0.13
Retreatment after treatment interruption	10 (3%)	40 (6%)	0.07	29 (6%)	0.23
Retreatment after treatment failure	3 (1%)	4 (1%)	0.52	4 (1%)	0.69
MDR TB					
Confirmed MDR TB	3 (1%)	24 (3%)	0.02	20 (4%)	0.01
TB outcomes∞					
Cured/Treatment completed	249 (75%)	523 (74%)	0.75	379 (74%)	0.84
Treatment interrupted	24 (7%)	78 (11%)	0.05	59 (12%)	0.07
Treatment failure	2 (1%)	9 (1%)	0.32	9 (2%)	0.03
Transferred out of community	27 (8%)	65 (9%)	0.57	45 (9%)	0.94
Died	29 (9%)	29 (4%)	0.002	18 (3%)	0.004
Tested for HIV					
HIV status known	288 (87%)	629 (88%)	0.62	456 (88%)	0.98
HIV status†					
HIV-infected	181 (63%)	377 (60%)		280 (61%)	
HIV-uninfected	107 (37%)	252 (40%)	0.40	176 (39%)	0.73
Receiving HAART	21 (26%)	61 (74%)	<0.001	46 (75%)	<0.001

* Specimens missed compared to specimens received

**Specimens with RFLP result compared to all specimens (ie sum of missed specimens and received specimens without genotyping data)

∞ Comparison of specified category or outcome compared to all other categories/ known outcomes.

† Excluding those of unknown HIV status

4.4.2 Strain Diversity

From 2001 to 2008, 243 genotypes were identified including 7 orphan strains.

SNP clusters

Figure 4.2 shows the phylogenetic tree for 529 isolates present from 2001 to 2008 (one strain was not assigned to an SNP cluster). Overall, strains from all nine SNP clusters were identified in the community. SNP clusters II and VI were the largest clusters with 152 and 231 isolates in each cluster, respectively.

On average there were 26 strains per year from 2001 to 2005, and an average of 75 strains per year from 2006 to 2008. Following adjustment for different sampling rates in each year, there were on average 96 strains per year, with no significant trend found in the number of strains by year (p=0.17).

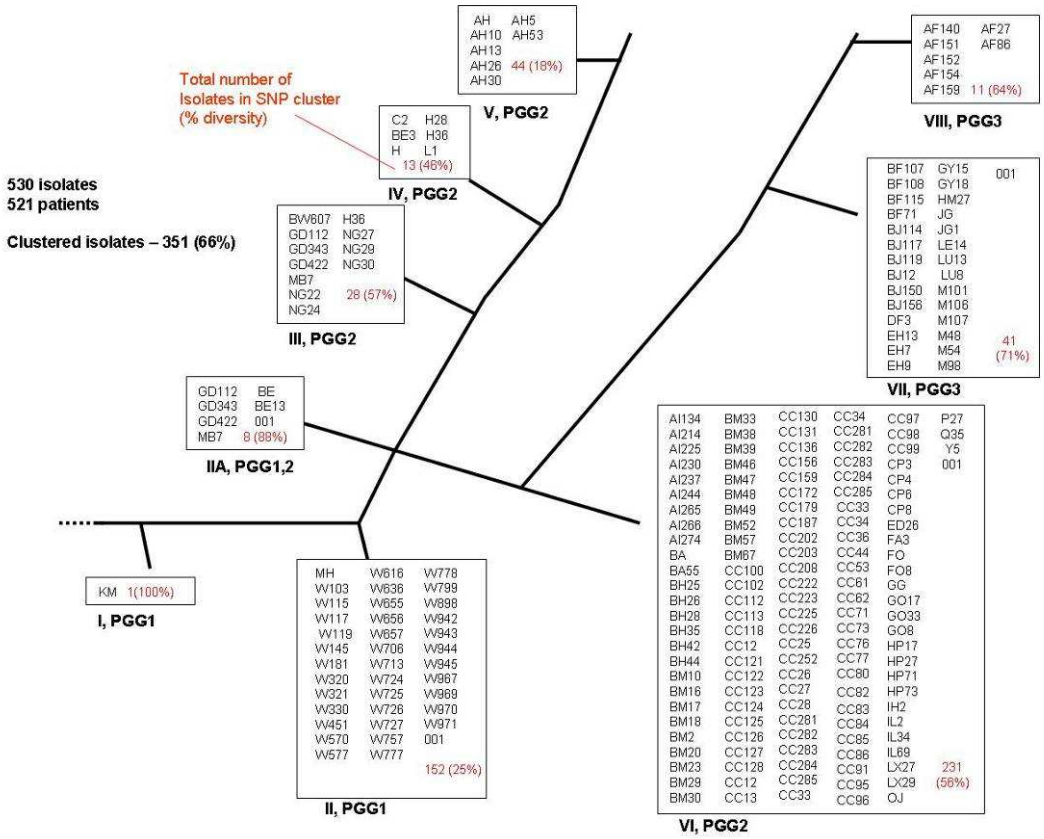


Figure 4.2: Phylogenetic tree of strains identified in the community from 2001 to 2008

Families and Strains

The 530 isolates were divided into 52 strain families and 243 strains. Overall strain family diversity was 10% and strain diversity was 46%. The dominant strain families are W-Beijing (28%), CC-related (25%), AH (8%) and BM (5%). These four families comprise 66% of the strains in the community. The strain diversity within the W-Beijing family is 24% (36 strains among 150 isolates), and 49% in the CC-related family (64 strains among 130 isolates).

In a multivariate logistic regression model adjusting for age, gender and sputum smear-positive status, the W-Beijing family was associated with HIV-infected patients in the pre-HAART era (odds ratio [OR]: 6.2; 95% Confidence interval [CI]: 1.8 – 21.3; p=0.004). This association was no longer statistically significant in the post-HAART era (OR: 1.6; 95% CI: 0.9 – 2.7; p=0.06). Overall, the W-Beijing strain was not associated with sputum smear-positive PTB (p=0.21) and, among HIV-infected patients, W-Beijing was not associated with receiving HAART (p=0.88). In a multivariate logistic regression model adjusting for age, gender and sputum smear-positive status, the CC-related, AH and BM families were not associated with HIV status in either the pre-ART (p=0.49, p=0.58 and p=0.14 respectively) or post-ART era (p=0.86, p=0.19 and p=0.10 respectively).

The W-Beijing family was associated with MDR-TB (p=0.01), and this association persisted after adjusting for HIV status (p=0.02). Of the 20 patients with MDR-TB, 7 (35%) had the same W-Beijing strain.

In the 46 patients on HAART at the time of TB diagnosis, 15 families were represented (33% family diversity) and 37 strains (80% strain diversity). The strain with the greatest representation was W451, with four (9%) patients on HAART infected with this strain.

4.4.3 Unique versus Clustered Cases

Of the 243 strains, 179 (74%) were unclustered, ie unique, and 64 (26%) occurred in clusters of two or more patients. Given the incomplete sampling rate, the mathematical probability that a unique strain was in fact a couplet (ie, that we missed a second specimen of the same strain), was 37%.

Of the 521 patients, 174 (33%) had strains that were unique. Of the 347 patients who had strains that occurred in clusters (two patients with dual infections had strains in both unique and clustered groups), 63 (12%) were index cases for the clusters (one patient with a dual infection was the index case for two clusters), and the remaining 284 (55%) were secondary cases in clusters (Figure 4.3). The proportions of unique cases and clustered patients did not change significantly over the study period (p=0.50 and p=0.81 respectively) (Figure 4.4), nor did clustering proportions change between the pre- and post- HAART implementation periods (p=0.62). In addition, the proportion of clustered patients among HIV-infected and HIV-uninfected patients did not change over the study period (p=0.29 and p=0.91 respectively).

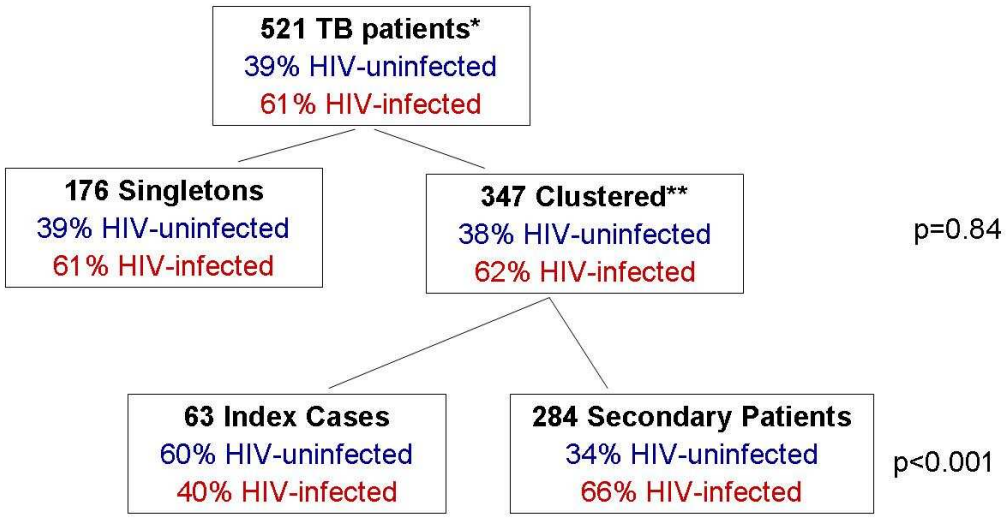


Figure 4.3: Distribution of unique and clustered TB cases, and index and secondary TB cases overall and by HIV status

* Percentages of HIV-uninfected and HIV-infected are based on individuals of known HIV status (456 of 521)
**Two patients with dual infections had strains in both singletons and clustered groups.

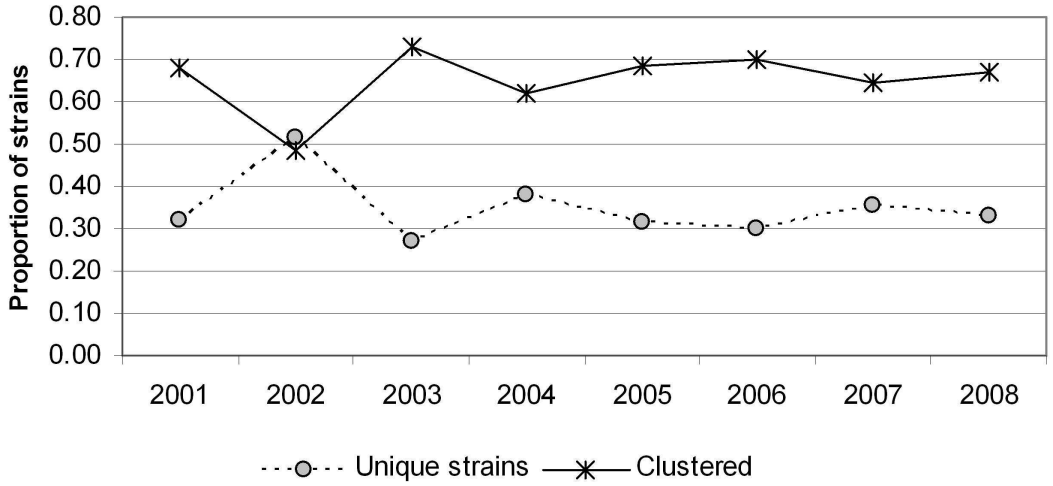


Figure 4.4: Proportion of strains that were unique and clustered, by year from 2001 to 2008

The proportion of unique strains did not change over the study period (p for trend=0.50) and the proportion of clustered strains did not change over the study period (p=0.81).

In multivariate logistic analysis, patients with unique strains did not differ from those with clustered strains in terms of age (OR: 0.99; 95% CI: 0.97-1.01; p=0.47), gender (OR: 0.9; 95% CI: 0.6-1.4; p=0.85), TB category (OR: 1.2; 95% CI: 0.8-1.8; p=0.47) or HIV status (OR: 1.0; 95% CI: 0.7-1.5; p=0.99). Among HIV-infected patients, those patients with unique strains did not differ from patients with clustered strains in terms of HAART status, and this finding persisted after adjusting for age, gender and TB category (OR: 1.3; 95% CI: 0.6-2.6; p=0.47).

4.4.4 Unique and Index Cases versus Secondary Cases

Overall, 53% of HIV-uninfected patients were unique or index cases compared to 47% which were secondary cases (p=0.11). Among HIV-infected patients, significantly more disease was due to secondary cases (58% vs 42% unique and index cases; p<0.001), and this was also true for HIV-infected patients on HAART (67% vs 33% respectively; p<0.001). HIV-infected patients were 1.6 times more likely to be secondary cases, rather than unique or index cases (OR: 1.6; 95% CI: 1.1- 2.3; p=0.02). When comparing HIV-infected patients on and off HAART, there was no difference in distribution of unique and index cases, and secondary cases (p=0.17).

In multivariate logistic analysis, index cases did not differ from secondary cases in clusters in terms of age (OR: 0.99; 95% CI: 0.96-1.02; p=0.36), gender (OR: 0.8; 95% CI: 0.4-1.6; p=0.56) or TB category (OR: 0.87; 95% CI: 0.4-1.6; p=0.48). Index cases were less likely to be HIV-infected compared to secondary cases (adjusted OR: 0.3; 95% CI: 0.2-0.6; p=0.001). Among HIV-infected patients, index cases did not differ from secondary cases in terms of HAART status (OR: 0.2; 95% CI: 0.02-1.4; p=0.09).

HIV-uninfected index cases were more likely to be sputum smear-positive compared to HIV-infected index cases (97% smear-positive versus 81%; p=0.01). This was also true for all PTB cases in the community over the study period, regardless of whether RFLP data was obtained (79% of HIV-uninfected PTB cases were smear-positive versus 64% of HIV-infected cases; p<0.001). The association between index cases and HIV-uninfected patients persisted in multivariate analysis adjusting for age, gender, TB category and sputum smear-positive status (OR: 0.3; 95% CI: 0.2-0.6; p=0.001).

4.4.5 Clustering

Cluster size

Cluster sizes ranged from 2 to 53 patients, with a mean cluster size of 5.5 patients. While clustering was not associated with HIV status, the average cluster size in HIV-infected patients was significantly larger than the average cluster size for HIV-uninfected patients (10 vs 5 respectively, p=0.002).

Figure 4.5 shows the distribution of clustered strains over time, by HIV status. Overall, 78% of clusters were comprised of both HIV-infected and HIV-uninfected patients. Smaller, temporally associated clusters were noted among HIV-infected patients within the larger clusters of the W451 (in 2003, 2007 and 2008) and W330 (in 2008).

Couplets

In total, there were 26 clusters comprising of two identical strains (ie couplets). The age difference between adult patients in couplets ranged from the index case being 15 years older to 27 years younger than the secondary case. Overall 44% of the index cases were 10 years older or younger than the secondary case. In temporally defined clusters, the index case ranged from being 28 years older to 26 years younger than the

secondary case, with 57% of index cases being 10 years older or younger than the secondary case.

Age and clustering

As reported above, age was not linearly associated with unique cases, index cases or secondary cases. Figure 4.6 illustrates the distribution of unique, index and secondary cases by age groups, overall (Figure 4.6a), and by HIV status (Figures 4.6b and 4.6c). Overall there was no statistical difference in the distribution of unique and index cases versus secondary cases across the age groups (p=0.75), and this persisted when adjusted for HIV status (p=0.70). However, there was a trend for older patients to have a greater proportion of unique and index cases among HIV-uninfected patients (56% due to reactivation) and to have a greater proportion of secondary cases among HIV-infected patients (60% disease due to recent infection).

There was no statistical difference in the distribution of index cases versus secondary cases between the age groups by HIV status (p=0.97), and this persisted when adjusted for HAART status (p=0.42).

Retreatment TB and clustering

Of the 521 patients, 143 (27%) were retreatment cases, of which 98 (68%) were clustered (p=0.57). Retreatment cases were not associated with HIV status (64% HIV-infected; p=0.52). For 12 patients we obtained RFLP specimens from both the first and second TB episodes. Five of these patients interrupted TB treatment in the first episode and had the same *Mtb* strain in their second TB episode. Three of the patients completed treatment in the first episode, but had no bacteriological confirmation of TB cure. These three patients all had the same *Mtb* strain in their second episode. The remaining four patients all had bacteriologically confirmed cures in their first TB episode, and all four patients had a different *Mtb* strain in their second episode.

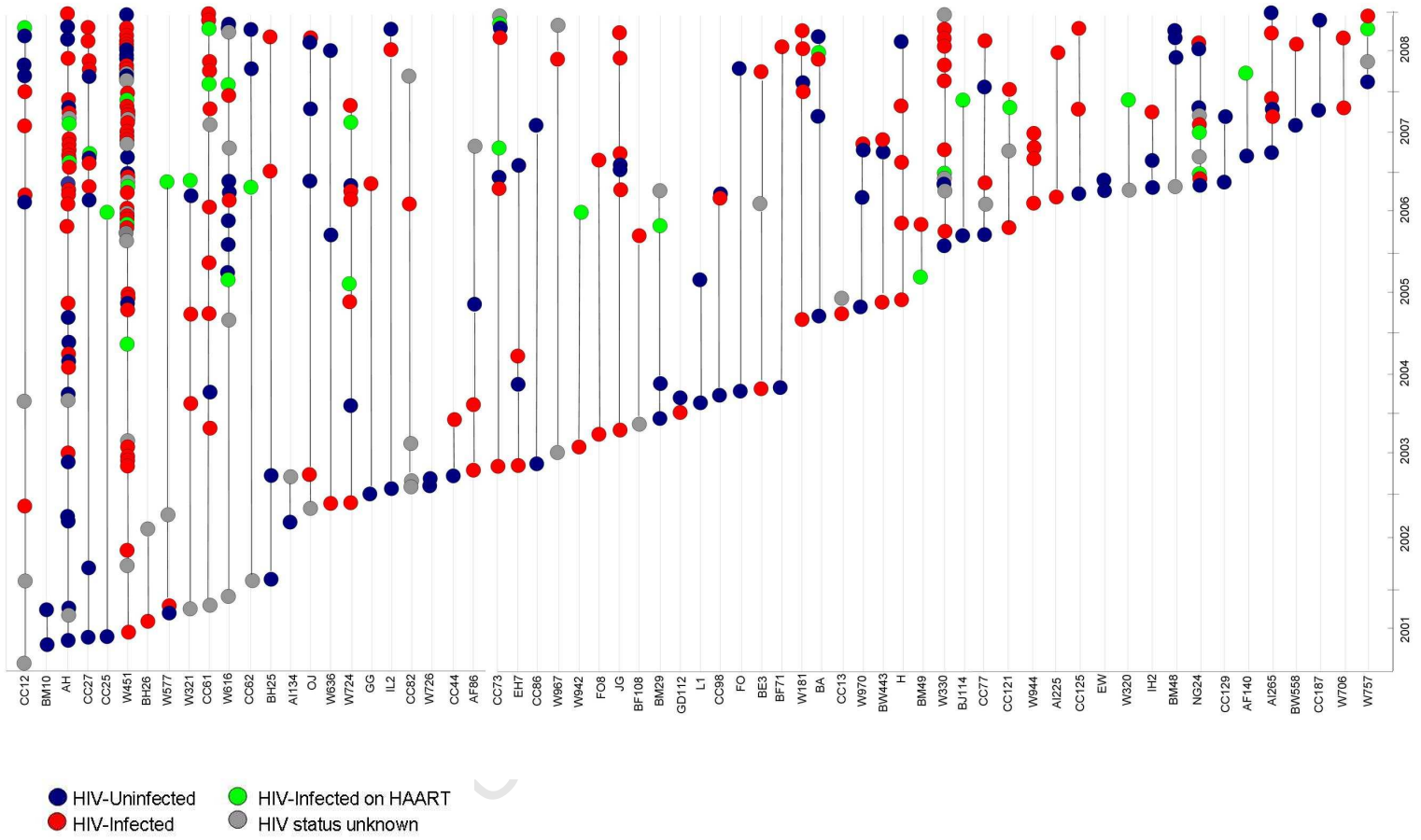


Figure 4.5: Clusters by strain, HIV status and ordered by start date

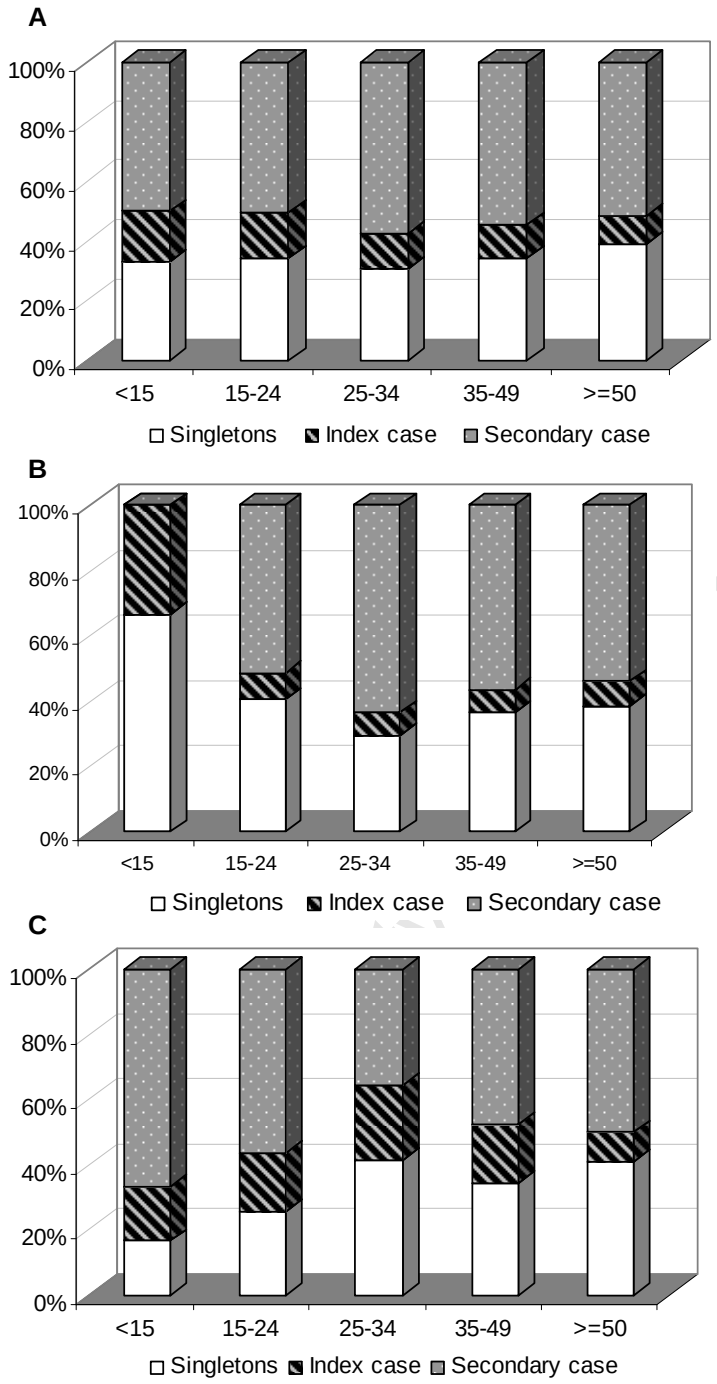


Figure 4.6: *Figure A:* Distribution of patients with unique strains, and patients who are index cases and secondary cases in clusters; *Figure B:* Distribution of HIV-infected patients with unique strains, and HIV-infected patients who are index cases and secondary cases in clusters; and *Figure C*: Distribution of HIV-uninfected patients with unique strains, and HIV-uninfected patients who are index cases and secondary cases in clusters

Geographical distribution of clusters

On average 10% of adults in all clusters shared a residential plot with another adult infected with the same strain. Among couplets, both adults occurred on the same plot for 3 of the clusters (12%). Similarly, among temporally defined couplets, 3 of the 28 couplets occurred on the same plot (11%). Random distribution of couplets across all plots in the community would have resulted in 1.9% of couplets occurring on the same plot, significantly fewer than noted in the study ($p<0.001$). Figure 4.7 shows couplets distributed throughout the community.

Figure 4.7: Couplet strains in the community

Thick red borders denote both patients with same strain on the same plot.

4.5 DISCUSSION

This study described the molecular epidemiology of the TB epidemic in a high TB and HIV prevalent community, and assessed the impact of the HIV epidemic and a HAART programme on TB transmission in this setting. The study community is geographically well-defined, has substantial TB and HIV burden and has a high degree of *Mtb* strain diversity. These factors have enabled us to assess clustering and transmission in the community, both overall and by HIV status.

4.5.1 Strain Diversity

The study has demonstrated a broad diversity of *Mtb* strains, consistent with findings in other studies in sub-Saharan Africa[357;438]. This may be compatible with the community's relatively recent establishment (circa 1992) with immigration from a number of different areas of Southern Africa. The high degree of genotypic diversity within the large CC-related family may indicate that it is an endemic *Mtb* family in this population. The W-Beijing family also shows a high degree of diversity, although to a lesser degree than the CC family, suggesting W-Beijing strains may be emerging and diversifying in the community. However, chromosomal location of IS*6110* insertions may impact on the movement of the IS elements and therefore, genetic diversity as determined by IS*6110* RFLP may be independent of strain endemicity.

There was a relatively constant number of strains in the community in each year (approximately 96), and the proportion of both unique and clustered strains also remained constant, suggesting a steady state of *Mtb* strain movement in and out of the community.

4.5.2 TB Disease due to Recent Infection versus Reactivation

In interpreting molecular epidemiological data, single strains and the index cases of clusters are often considered to result from reactivation of previously acquired infection, and secondary patients in clusters represent disease due to recent infection. While there are a number of limitations to this interpretation, the approach is widely used[128;350;372;416]. Under these assumptions, at least 55% of TB disease in this community resulted from recent transmission of *Mtb*. Up to 45% of the TB disease was due to reactivation of previous latent infection, of which at least 12% resulted in transmission.

However, the proportion of reactivation is most likely over-represented in this analysis, resulting in an underestimation of the proportion of recent infection. Of note is that 50% of children <15 years of age had unique strains or were index cases for a cluster. As TB disease in young children is predominantly the result of rapid progression of infection acquired from recent adult transmission[204], these data suggest that the source case for these children was missed in this study. Social interaction data from this community reported that at least 50% of casual indoor contacts occurred outside of the study

community [S. Robertson; unpublished data], and this may partially explain unidentified source cases.

Due to incomplete sampling rates, as much as 37% of single strains may in fact belong to clusters. Moreover, unique strains may be strains that do not transmit effectively, or are less pathogenic and, as such, result in latent infection rather than rapid progression to disease. If the latter is true, we may expect to see such strains re-appear in the community at a later date. This may explain clustered strains that have a wide time difference between strain appearances.

Censoring due to the data collection period may have resulted in some strains being mistakenly classified as unique, that is, patients with the same strains may have been missed prior to 2001 and post 2008. In addition, specimens were only collected from sputum-positive TB patients. Transmission of *Mtb* does not always lead to sputum-positive TB, but may be responsible for sputum-negative PTB or extra-pulmonary TB. We were unable to assess transmission resulting in these forms of TB.

Therefore, recent transmission is most likely responsible for a greater proportion of the TB disease than estimated in this study. This argument is further supported by the evidence high rates of recent infection among key groups in this study population, including patients with MDR-TB and retreatment patients who had previously completed TB treatment and had bacteriological confirmation of cure. The high rates of disease due to recent infection are also in keeping with the substantial force of infection reported in Chapter 2.

4.5.3 TB Transmission and HIV

HIV-infected patients had a greater proportion of disease due to recent infection compared to HIV-uninfected patients. The majority of clusters contained both HIV-infected and HIV-uninfected patients, suggesting that the HIV-associated and HIV-unassociated epidemics are not independent of one another. However, within clusters, index patients were more likely to be HIV-uninfected and secondary cases were more likely to be HIV-infected. This indicates that HIV-uninfected patients may be responsible for a greater proportion of transmission in the community.

The association of HIV-uninfected patients with index cases persisted after adjustment for sputum smear status, and this suggests that HIV-infected patients are less infectious. This finding is consistent with studies that report lower infectivity of HIV-infected patients, irrespective of smear or culture status[124;176;177].

An alternative explanation for the greater representation of HIV-uninfected patients among index cases could be that HIV-infected patients have slower progression to TB disease, and therefore present to health services later than HIV-uninfected patients. Contrary to this hypothesis, available data report that, following TB infection, HIV-infected patients progress more rapidly to TB disease compared to HIV-uninfected counterparts[14;102;111], although there is inconsistency in the published literature as to whether HIV infection is associated with rapid or delayed presentation and diagnosis of TB[339]. The more rapid progression of HIV-infected patients to TB disease may also account for the lower rate of transmission from these patients.

Our study showed that TB disease among HIV-infected patients was more likely to be due to recent infection, and that this was also true in patients receiving HAART. This suggests that ongoing transmission is still an important cause of disease among patients receiving HAART. However, the sample size for patients on HAART was small, and therefore further observation of this group is required.

4.5.4 TB Transmission and Demographics

No statistical association was found between patient age and clustering, or age and evidence of recent transmission. These findings suggest that transmission between adults is not age assortative in this community, and this is supported by the wide age range between index cases and subsequent secondary cases in couplets. However there was a trend for TB disease among older HIV-uninfected patients to be the result of reactivation of latent infection, and this is in keeping with findings elsewhere[351;353;356].

Despite the increased risk of infection reported among adolescent males in the secondary school tuberculin skin test survey (Chapter 2), there was no association between gender and TB disease due to recent infection in this study.

4.5.5 TB Transmission and Geographical Location

The spatial analysis, utilizing GIS, enabled us to determine that clusters did not occur in the same residential plots within the community. This was also true for temporally defined couplets. This finding suggests that adult transmission does not occur predominantly on residential plots, but that most adult to adult transmission is occurring outside of the households. This is consistent with the published literature[337;392;394], as well as with social interaction data, which report that the majority of both close and casual contacts among adults occur off the residential plot [S Robertson; unpublished data].

Other studies have reported hospitalization to be a risk factor for TB transmission[374;387]. Given that HIV primary care, HAART treatment and TB treatment are all managed at the same clinic in this community, assessing the possibility of nosocomial spread at the clinic was important. The high degree of both family and strain diversity in patients on HAART, as well as the lack of association of clustering in HAART patients suggests that nosocomial transmission of TB is not a substantial problem in the local HAART clinic. However, temporally-related clusters among HIV-infected patients were also identified within larger clusters, such as the W451 and W330 strain clusters. These sub-clusters may reflect nosocomial transmission at the general HIV community clinic. Similar smaller, temporal clusters were noted in the AH family, but due to poor differentiation of strains with few bands, such as AH, conclusions can not be drawn from this observation. Further analyses are required in order to determine possible high risk locations for transmission in this setting.

4.5.6 W-Beijing Family

An association was noted between W-Beijing and HIV-infection in the first period of the study. However, later in the study period this association weakened.

The association between HIV and W-Beijing may suggest that the W-Beijing strains differ functionally from the other strains so as to make them more transmissible to HIV-infected patients compared to HIV-uninfected patients, or it may suggest an increased susceptibility of HIV-infected patients to the strain. Alternatively, studies have reported that W-Beijing elicits a decreased[86;375;376] and non-protective immune response[376;379] in the infected host, with one study specifically noting decreased immune responses in the

presence of HIV-infection[380], and W-Beijing strains may therefore progress more rapidly to clinical TB disease in the presence of HIV co-infection.

The combination of a pathogenic strain together with an already compromised immune response may explain the strong association noted between W-Beijing and HIV in the early part of the study. Further, the weakening of this association following the wide-scale implementation of the HAART programme may be due to the improvement of immune function among the HIV-infected population as a result of HAART. However, no negative association was found between W-Beijing and HAART. This may be due to small numbers and the short follow-up period of the post-HAART era, and further observation in this community may help to clarify this relationship.

An alternative explanation for this initial association of W-Beijing and HIV infection may lie in the differing incubation periods (infection to disease) among HIV-infected and –uninfected patients. This study has shown evidence to suggest that W-Beijing is an emerging strain in this community. The faster progression to TB disease among HIV-infected patients[14;102;111] may result in a strain appearing to be associated with HIV in the shorter term. It is possible that the W-Beijing epidemic established itself more quickly among HIV-infected patients, with the HIV-infected population serving to amplify this emerging strain. The subsequent weakening of the association between HIV and W-Beijing may be due to "catch-up" of W-Beijing-associated disease in the HIV-uninfected population.

Ongoing observation, with larger numbers of patients, is required for further study of this possible association between HIV and W-Beijing.

Despite low MDR rates in this community (3% of PTB cases), we were able to show an association between W-Beijing and MDR-TB, and this is in keeping with the published literature[401;439-442]. Given the possible association between W-Beijing and HIV infection, this finding has serious implications for the spread of MDR-TB in these patients.

4.5.7 Strength and Weaknesses

This study linked genotyping data with clinical information, including HIV and HAART status, and with GIS technology.

There are a few potential sources of selection bias in this study. Sputum samples and RFLP data was not collected on all the TB patients in this community over the study period. Although the incomplete sampling was, for the most part, random in nature, there were some clinical differences between those PTB patients for whom we did and did not obtain genotyping data. Patients for whom we did not obtained genotyping data had a higher mortality rate, and patients for whom genotyping data were available were more likely to have MDR TB or to be receiving HAART compared to those patients with missed specimens. The increased mortality in those patients without RFLP data may reflect a lower sputum retrieval rate from the local hospital, where sicker patients were initially diagnosed. The increased yield in genotyping data on MDR patients was likely due to the increased number of sputum-positive specimens obtained from these patients during clinical management. Due to the incomplete sample collection from the study population the number of circulating strains, number of clusters and size of clusters will have been underestimated[428;443]. However, using mathematical modeling we were able to provide a corrected estimate of the number of circulating strains, as well as the proportion of unique strains that were most likely part of clustered couplets. Furthermore, the possibility that HIV-infected patients may have an increased risk of TB-related mortality prior to TB diagnosis, may result in a length-bias between HIV-infected and uninfected individuals.

We inferred recent transmission of TB from clustered strains. Despite the traditional interpretation of identical strains as epidemiologically linked, it is important to recognize that clustering is not automatically synonymous with recent transmission. In areas of high incidence, the proportion of clustered cases is influenced by factors such as the background strains, annual risk of infection, the age of the population (with clustering tending to be underestimated in younger patients and overestimated in older patients)[444], geographical distribution[351;385], as well as study duration[444;445], sampling strategies and percentages[428;443] and the genotyping method utilized. The interpretation of clustering as evidence of transmission is enhanced in our study by the long study duration of eight years[402;444], the substantial force of TB infection and the high diversity of strains in the community. In addition, IS*6110* fingerprinting is one of the most discriminatory typing techniques for isolates with >6 IS*6110* bands, such as the CC and W-Beijing families[363].

While the temporally-based definition of index cases (as the first case that was diagnosed with TB disease in a cluster) is a relatively simply approach, it is recognized that the identification of true index or source cases is extremely difficult[351;363;372;384] and no standardized method is available. Nonetheless, it is important to note that our approach does not take into account secondary transmission, and mathematical modeling may provide further insights into the transmission dynamics in this community

4.5.8 Conclusion

This study showed that recent transmission is responsible for the majority of TB disease in a high HIV and TB prevalent setting. This finding was particularly marked among HIV-infected patients, possibly due to this group's increased risk of rapid progression to disease following infection. Of importance is the finding that the HIV-associated and HIV-unassociated TB epidemics were not independent of one another, and that HIV-uninfected patients may be responsible for most of the transmission in this community. Age and gender did not appear to be associated with transmission, and the household was not the key location for adult to adult transmission. This study provided reassurance that the HAART clinic was not a location for TB transmission to vulnerable patients in this community.

These findings have important implications for TB control, highlighting the necessity of identifying the factors and locations associated with transmission and the need to interrupt transmission patterns in these settings. Our attention and interventions need to expand beyond HIV-infected patients, and include reducing transmission from HIV-uninfected patients.

Chapter 5

Impact of an Antiretroviral Treatment Programme on TB Disease Incidence and Outcomes

5.1 RATIONALE

Incidence is the number of new cases of active tuberculosis (TB) disease occurring in a specified period of time[3], and serves as the primary measure of the success or failure of TB control progammes[446].

A key target of the Millennium Development Goals (MDG) is the reduction of the current global incidence of active TB of >1,300 cases/million population/year to an incidence of <1 case/million population/year by 2050[7;11]. However, the current World Health Organization (WHO)-recommended Directly Observed Therapy, Short-course (DOTS)-based TB control strategy is failing to contain the TB epidemic in high HIV prevalence countries, and adjunctive strategies are required in order to meet the MDG TB goals[7]. Understanding the impact of alternative interventions on TB incidence in different settings is necessary for the development of evidence-based adjunctive TB control strategies. However, incident TB encompasses recognised (notified) and unrecognised TB disease, as well as TB patients who die before diagnosis. Therefore, incidence is difficult to measure directly, and even the long term follow-up of large cohorts may not fully identify all incident cases. As a result, TB incidence is often estimated from other data, such as prevalence or mortality data[7]. Alternatively, TB notification rates can be used as a surrogate measure of TB incidence.

This chapter will address the fourth objective of the thesis, namely to describe the changes in TB disease incidence (as determined by TB notification rates) and outcomes in both the overall community population and in the HIV-infected and HIV-uninfected subsets of the population. These changes will be assessed over time and following the scale-up of highly active antiretroviral therapy (HAART) access in the community. Data from this chapter have been published in the fourth paper[447] listed in the preface on page *xi*.

5.2 BACKGROUND

5.2.1 Historical Perspective on TB epidemics

In industrialised countries, TB incidence and mortality rates had begun to decline by the late 1800's, and this decline persisted throughout most of the 20th century[49;448-453]. The decrease in TB rates have been well recorded in the United Kingdom[448-450], Western and Eastern Europe[49;450;452] and the United States of America[7;453]. Figure 5.1A shows the decline in TB notification rates in Western European countries since 1980[452], and Figure 5.1B shows TB cases in the United States of America in the second half of the 20th century[49].

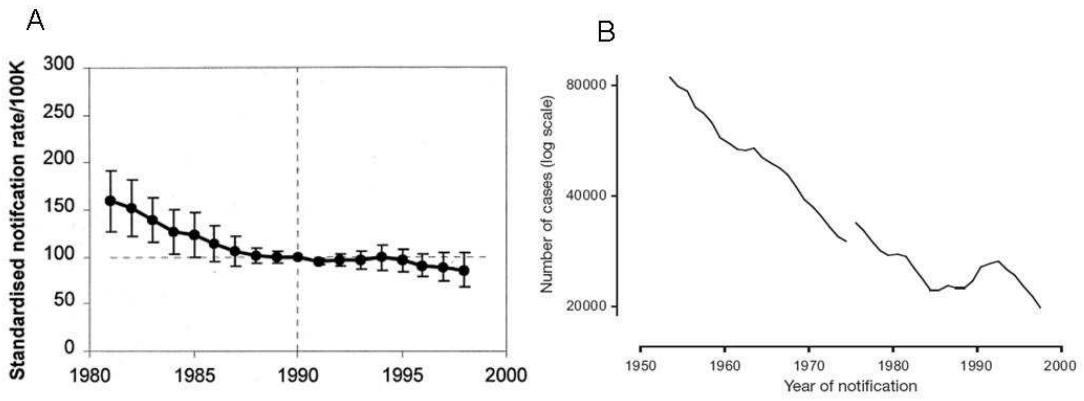


Figure 5.1: Figure A: TB notification rates from 12 Western European countries from 1980 to 2000 (Figure A obtained from Dye, 2000[452]). Figure B: TB cases in the United States of America in the second half of the 20th century. (Figure B obtained from "Epidemiological Basis of Tuberculosis Control"[49])

As shown in Figure 5.2, these declines in TB rates occurred prior to the availability of such intervention strategies as chemotherapy and vaccination. This has led some scientists to argue that the reduction in TB rates was primarily due to improved living and working conditions as well as improved nutrition[448;449;454]. This theory is supported by the temporary increase in TB rates noted in England, Europe and America during the two World Wars[450;453;455;456]. Other theories as to the cause of the decline include decreased infection due to segregation of TB patients[457], and natural selection[458]. More recently, a modelling paper has shown that a major factor in the decline in TB rates was a decrease in the effective contact numbers of infectious cases, resulting in a decline in the annual infection rate[459].

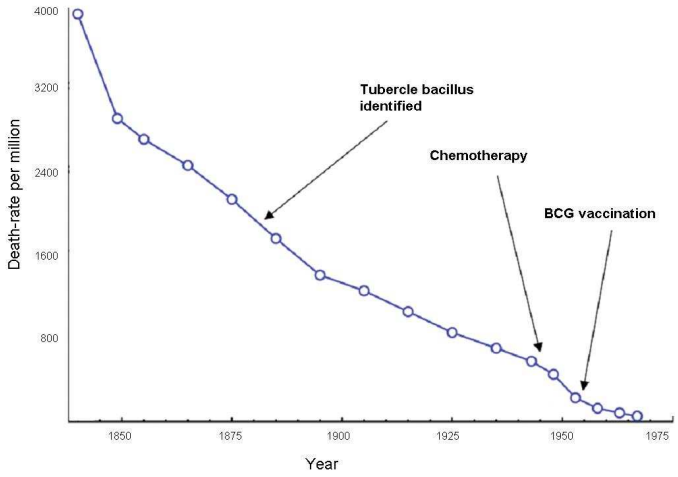


Figure 5.2: Pulmonary tuberculosis mortality rates in England and Wales (Figure obtained from Selgelid, Public Health Ethics[451;460])

5.2.2 Impact of HIV Epidemic

Whatever the reasons for the decline in the TB infection rates and the subsequent decline in TB rates, in 1950 it was believed that TB would soon be eradicated. However, the rapid spread of HIV has had a profound and deleterious impact on TB control. While the impact of the HIV epidemic has been moderate in high income countries, the effect has still been evident. Since the escalation of the HIV epidemic, a deceleration of TB decline occurred in Western Europe in the late 1990's[7] and a small increase in TB cases was noted in America in the 1980's and 1990's[49]. TB rates in America have subsequently decreased once again, and the downward trends have been sustained into the 21st century.

The impact of the HIV epidemic has been far more dramatic in low and middle income countries, especially where HIV prevalence is high. HIV has resulted in a substantial and sustained escalation of TB epidemics in a number of the global regions, including Eastern Europe and Africa (Figure 5.3)[9;452]. TB rates started increasing in Africa in the 1980's[452] followed by increases in Eastern Europe in the 1990's[452] and this escalation of TB rates continues to-date in both regions[9]. It is estimated that 9% of all new TB cases are directly attributable to HIV globally, and as many as 31% of TB cases in Africa[9].

It should be noted that improved reporting of notified TB cases[7] may also have contributed to escalating TB incidence over the past two decades, as well as social

changes such as rapid urbanization with immigration to the cities and resulting crowded living conditions[37].

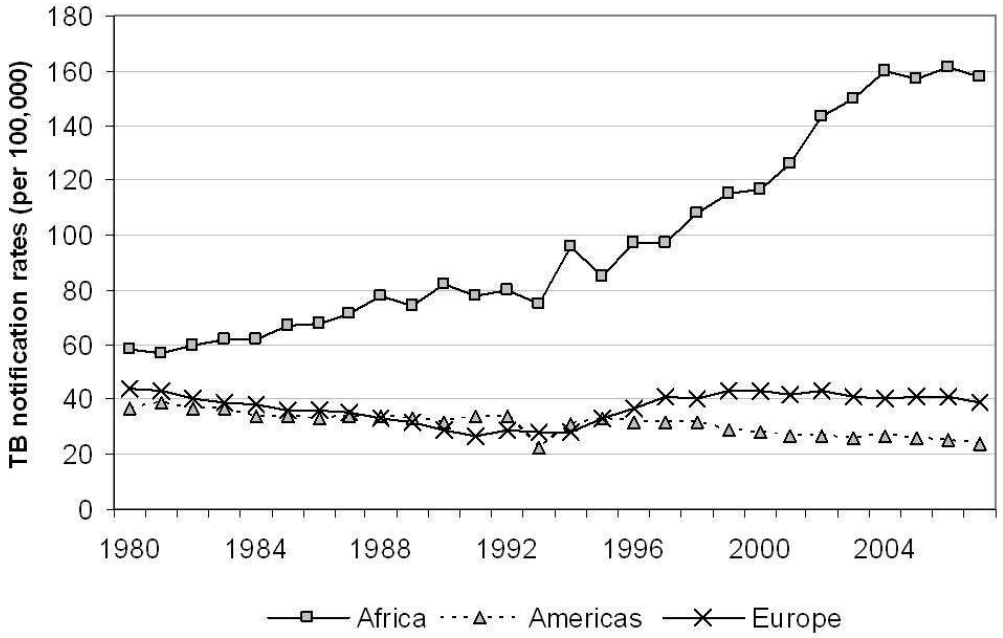


Figure 5.3: TB notification rates in three WHO regions from 1980 to 2007[7]

The impact of HIV on TB epidemics is due to the substantially increased risk of TB disease in HIV-infected patients compared to HIV-uninfected individuals[14-16;55;69;70]. HIV-infected patients have an increased risk of both reactivation of latent TB infection[14;112] and rapid progression to disease following recent infection[14;112102;111].

As previously mentioned, the current WHO-recommended TB control strategy is failing to contain the TB epidemic in high HIV prevalence countries[7]. In South Africa the overall incidence rates of all forms of TB were estimated to have increased from 360/100,000 in 1997, to 925 in 2005 and 948 in 2007[7].

The STOP TB Partnership has proposed adjunctive strategies to address this problem in low and middle-income countries with generalised HIV epidemics[461], including the intensified case-finding, isoniazid preventative therapy, infection control[462] and antiretroviral therapy (ART). Of these strategies, only provision of HAART has been extensively implemented, with substantial progress in patients' access to HAART over the past five years[29].

5.2.3 Impact of HAART on Population TB Notification Rates

While it is well documented that the incidence of active TB disease in HIV-infected patients is reduced by 54-92% by HAART[36;45;169], the impact of HAART on TB rates at a population level remains uncertain. HIV-infected patients on HAART have a substantial reduction in TB risk[45;46], but they still have a 5-10 times higher risk of TB disease 3 years into HAART treatment compared to HIV-uninfected individuals[35;165]. The combination of prolonged survival and residual increased risk of TB incidence in HIV-infected patients on HAART will result in an increased number of highly susceptible individuals in the population. Therefore even substantial population coverage with HAART may have a limited impact on TB incidence at a population-level. However, empirical data addressing this issue are sparse and most evidence comes from mathematical modelling[190].

Despite the well-described TB benefits of HAART use for HIV-infected individuals, there are no population level studies describing the impact of increased access to HAART on community TB rates in areas with generalised HIV epidemics. Therefore we assessed the impact of increasing antiretroviral provision on TB notification rates in a community with high HIV prevalence.

5.3 STUDY DESIGN

5.3.1 Study Population

As described in Chapter 1, Site M is served by a single primary care clinic that follows the national TB control programme guidelines[463], based on WHO-recommended DOTS programme. The clinic manages all TB patients resident in the community and the protocol for diagnosis and management of TB patients did not change significantly from 1997 to 2008. The main change to the national TB protocol has been the addition, since 2004, of active TB screening for patients initiating HAART. This screening was based on an initial symptom screen, followed by sputum investigation, and did not include testing for latent TB infection. Isoniazid preventative therapy has not been implemented in this community. Despite an apparently well-functioning TB programme, with treatment completion rates of approximately 80%[464], we have previously reported escalating TB notification rates in this community prior to rapid, high coverage HAART availability[39]. HAART provision began in 2003 with patients in the community accessing antiretroviral treatment at the local clinic or local hospital, but the HAART programme was only scaled-up in 2005.

5.3.2 Data Analysis

TB and HAART data

TB notification data were obtained from the study community TB clinic from 1997 to 2008. HIV status, HAART status and CD4 count data were obtained from TB register, clinical folders as well as clinic and hospital HAART databases. Children were defined as patients ≤14 years of age, and adults were defined as patients ≥15 years of age. All TB analyses were restricted to TB patients resident in the study community.

Population Model

Denominators for rate calculations were derived from the community population and HIV model, described in Appendix A. The population model, used to calculate overall and age-specific TB rates, was derived from the 1996 South African national census, and community household censuses performed in 2002, 2004, 2006 and 2008. The community HIV prevalence model, used to calculate TB rates among HIV-infected and -uninfected population, was estimated using the Actuarial Society of South Africa (ASSA) 2003 AIDS and Demographic model for the African population[198;465]. This model was adjusted based on the 2005[179] and 2008[197] community-based, random cross-sectional HIV prevalence surveys among adults ≥15 years of age in the study community. This model is described in more detail in Appendix A.

Numbers of patients on HAART in each year, used as denominators for TB notification rates on HAART, were derived from the antiretroviral programme registers at the community clinic and local hospital. HAART coverage was calculated as the proportion of the adult HIV-infected population in the community receiving HAART in each year.

HIV and TB rates calculations

All TB rates were reported as cases/100,000. Age-specific TB rates were calculated in 5-year intervals for patients ≤19 years of age, and in 10-year intervals from 20 to >50 years of age. Direct standardisation method was used to calculate the age-standardised annual TB rates for the population using the 1997 population as the reference population[466].

HIV testing was routinely offered to TB patients from 2002, and therefore HIV-associated rates were only available from that year. HIV testing uptake was not complete among TB

patients and the analyses in HIV-infected and HIV-uninfected strata were restricted to patients with known HIV status. To account for missing HIV test results, a sensitivity analysis was performed in which extreme case scenarios for HIV-associated and non HIV-associated TB rates were calculated assuming 100% of patients with unknown HIV status were HIV-infected and HIV-uninfected, respectively.

We a priori chose 2005 as the first year of HAART availability, as this was the first year that an appreciable proportion of patients were receiving HAART (>5% of HIV-infected patients). Mean changes in TB rates and significance of trends prior to large-scale HAART implementation (pre-2005) and following large scale HAART implementation (post-2004) were assessed using linear regression models. An autoregressive model with a one year lag was used to account for the autocorrelation of the data, and the impact of HAART was examined through an interaction term. The median baseline CD4 counts of patients initiating HAART in each year were calculated from the most recent CD4 count in the 3 month period prior to HAART initiation.

Direct standardisation method[466], using HIV-infected population as the reference population, was used to calculate age-standardised rate ratios (RR) of TB in HIV-infected adults versus HIV uninfected adults, as well as HIV-infected adults off HAART versus adult patients on HAART.

Notification rates were assessed over time for different categories of TB disease: namely, pulmonary TB (PTB), sputum smear-positive PTB, extra-pulmonary TB (EPTB) and retreatment TB. Patients with concurrent PTB and EPTB diagnoses were counted twice: once in each category. Retreatment TB was defined as a new TB diagnosis in a patient who had previously completed TB treatment.

TB outcomes (including treatment completion, treatment interruptions and all cause mortality) were reported as a proportion of TB patients initiated on TB treatment in each year, excluding those who were transferred out of the community during the treatment course. TB completion was defined as TB treatment completion or cure.

Trend analysis for proportion of smear-positive PTB and TB outcome rates over time were assessed using chi^2 test for trend[467].

Data were analysed using Stata Version 10·0 (StataCorp, College Station, Texas). All statistical tests were 2-sided at alpha=0·05.

5.4 STUDY FINDINGS

5.4.1 TB cohort

Over the 12 year study period, 1,973 TB cases were notified in the study community. Of these, 232 of these cases were children, with a median age of 2 years (interquartile range [IQR]: 1 – 5 years) and 56% were female. In total, 1,736 were adult cases, the median age of which was 32 years (IQR: 26 – 40 years), and 45% were female. Age data were not available for five patients, and they were therefore excluded from age-based analyses.

Overall TB notification rates in this community increased from 630/100,000 in 1997 to a peak of 2,132/100,000 in 2005 and decreased to 1,816/100,000 in 2008 (Table 5.1). TB notifications rates increased by an annual average of 134/100,000 cases from 1997 to end 2004 (95% confidence interval [CI]: 111 to 157; p<0.001), followed by a reduction of 92 cases per year from 2005 to 2008 (-295 to 111; p=0.37).

Overall, HIV testing was performed in 78% of adult cases, of which 67% were HIV-infected. From 2002, 89% of adult TB cases tested for HIV, of which 68% were HIV-infected. In 2003, 1% of the total HIV-infected population were receiving HAART. By the end of 2005 this proportion had increased to 13%, and by the end of the study period, 22% of the total estimated HIV-infected population were receiving HAART (Figure 5.4). Overall 21% of the estimated adult HIV-infected population was receiving HAART by the end of 2008.

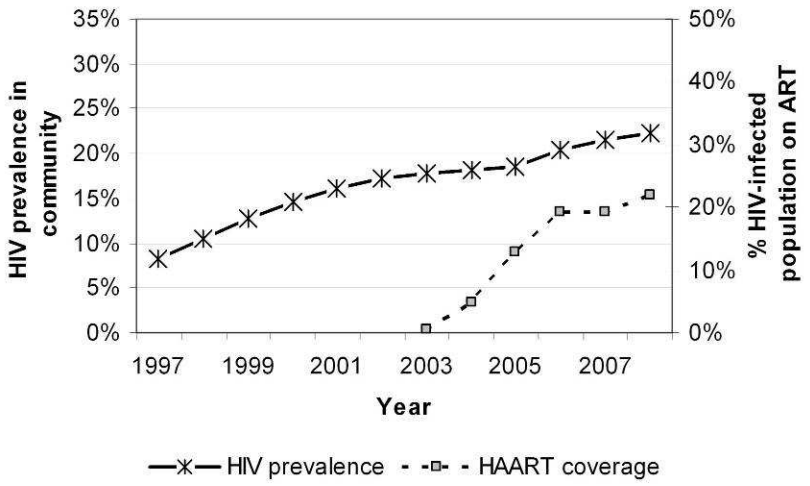


Figure 5.4: Overall HIV prevalence and HAART coverage in Site M

5.4.2 TB Notification by Age

Table 5.1 shows TB notifications and rates overall, and in children and adults from 1997 to 2008.

Figure 5.5 shows TB notifications by age strata over the study period. TB notification rates remained relatively stable in children over the study period. These trends are discussed in more detail under “Childhood TB Notification". In most adult age categories, TB notification rates steadily increased from 1997-1998 to 2003-2004, and appeared to stabilise from 2005 to 2008, while rates decreased in the 20-29 year olds, in the same period. The annual age-standardised rates confirmed these trends, with an increase in annual rate from 1998 to 2005, followed by a decreasing trend to 2008 (Table 5.1).

5.4.3 Childhood TB Notification

Total childhood TB notification rates have remained relatively stable from 1997 to 2008 (p=0.38; Table 5.1). Overall childhood TB rates did not show a significant trend either prior to 2005 (average annual decrease of 32 cases/100,000; 95% CI: -70 to 6; p=0.10) or subsequently (average annual increase of 75 cases; 95% CI: -499 to 650; p=0.79).

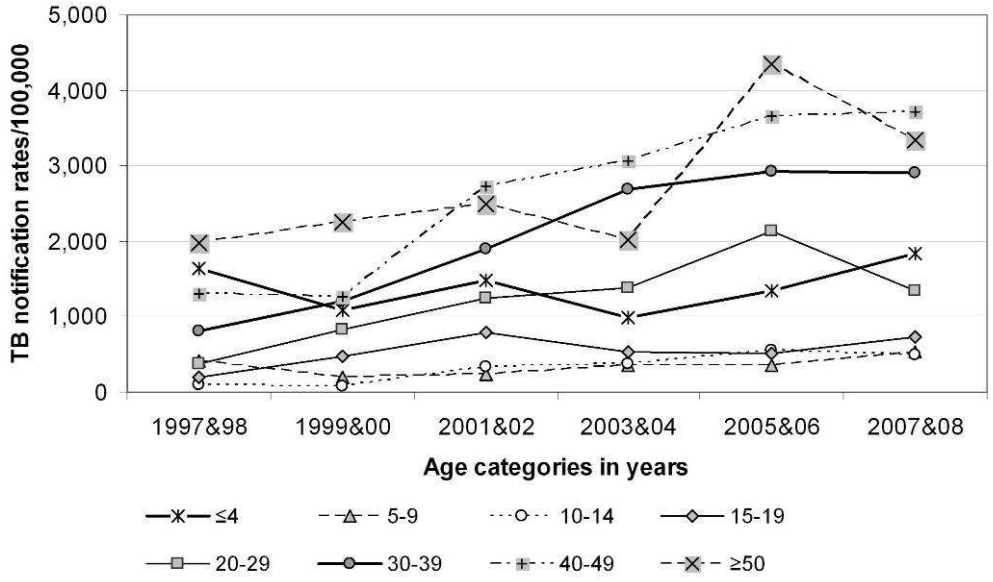


Figure 5.5: TB rates by age stratification and year in the study community, from 1997 to 2008

The population numbers in each age category were used as the denominators for the age-specific rates calculations.

In summary, overall there were no significant trends in notification rates in the 5-year age groups, with the exception of initially increasing TB notification rates in young adolescents (10-14 years). This was followed by a subsequent stabilising of TB rates in this age group (Figure 5.6).

TB notification rates (per 100,000) in the ≤4 year old age group ranged from 1,164/100,000 in 1997 to 1,579 in 2008, with the lowest rate recorded in 2003 (565/100,000) and a peak of 2,095 in 2007. There were no significant trends in notification rates in this age group: from 1997 to 2004 there was an average annual decrease of 84 cases/100,000 (95% CI: -177 to 6; p=0.07) and from 2005 to 2008 there was an average annual increase of 152 cases (95% CI: -428 to 732; p=0.61). Similarly, the 5 to 9 year old age group showed no significant trends in notification rates, with an average decrease of 13 cases/100,000 per year (95% CI: -37 to 10; p=0.26) followed by an average annual increase of 86 cases/100,000 (95% CI: -650 to 822; p=0.82).

Table 5.1: Total, childhood and adult TB notification numbers and notification rates in the study community, from 1997 to 2008

Year	Number of TB cases			Population			TB Rates/100,000			Age-standardised TB rate/100,000
	Total TB	Childhood TB*	Adult TB*	Total	Children	Adult	Total	Childhood	Adult	
1997	40	10	29	6,353	1,658	4,695	630	603	618	Reference population
1998	66	20	46	7,188	1,883	5,305	918	1,062	867	882
1999	71	10	61	8,023	2,107	5,916	885	475	1,031	1,060
2000	85	13	71	8,858	2,331	6,527	960	558	1,088	1,117
2001	138	26	111	9,693	2,555	7,138	1,424	1,018	1,555	1,582
2002	144	13	130	10,502	2,780	7,722	1,371	468	1,684	1,762
2003	173	13	160	11,651	2,937	8,714	1,485	443	1,836	1,876
2004	198	22	176	12,801	3,095	9,706	1,555	711	1,813	1,914
2005	277	25	252	12,990	3,055	9,935	2,132	818	2,536	2,784
2006	255	24	231	13,180	3,015	10,165	1,935	796	2,273	2,386
2007	261	30	231	13,886	2,824	11,062	1,880	1,062	2,088	2,171
2008	265	26	238	14,592	2,634	11,958	1,816	987	1,990	2,183

*5 patients with missing age data were excluded from age-based analyses

TB notification rates in young adolescents (10-14 years) initially increased from 1997 to end 2004, with an average annual increase of 49 cases/100,000 (95% CI: 5 to 92; p=0.03). From 2005 to 2008, adolescent TB notification rates declined non- significantly by an average of 28 cases/100,000 per year (95% CI: -116 to 59; p=0.53).

Sputum smear-positive TB rates in all children ranged from 53/100,000 in 1998 to 152/100,000 in 2008. While there was no statistically significant trend noted in childhood smear-positive TB rates from 1997 to end 2004 (p=0.78) or from 2005 to end 2008 (p=0.71), across the entire study period there was an increasing trend in smear-positive rates among children (p=0.05).

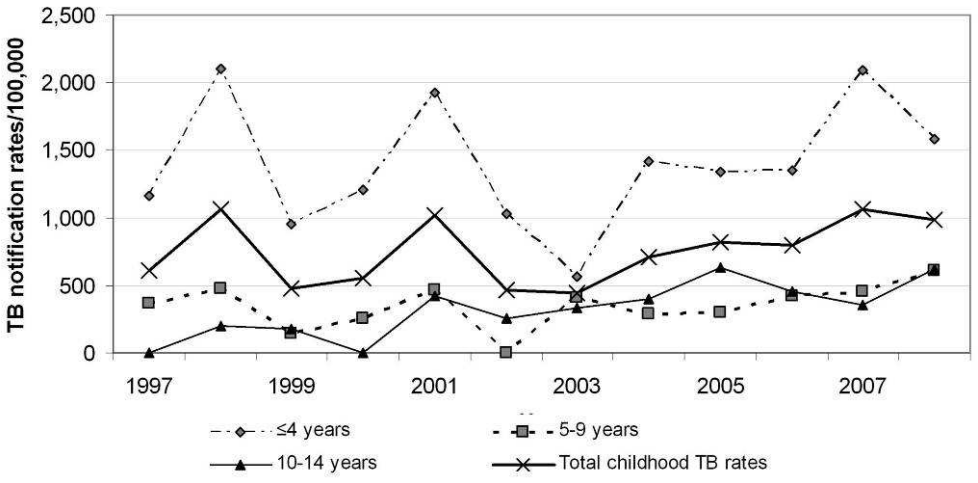


Figure 5.6: Childhood TB notifications rates in the study community, by 5 year age-strata, from 1997 to 2008

Analysis by HIV and HAART status is not possible in these age groups, as uptake of HIV-testing is very low, with HIV status unknown for 53% of all childhood TB patients, and 66% of TB patients in the 10-14 year age group. Furthermore, only 3 of the childhood TB patients were receiving HAART at time of TB diagnosis, all of them under 9 years of age.

HAART coverage was higher in children, compared to adults. By 2008, an estimated 35% of HIV-infected children in Site M were receiving HAART, significantly more than the 21% of the adult HIV-infected population (p=0.001). Based on the HIV model, it is

estimated that over 50% of the HIV-infected young adolescents (10-14 years of age) were receiving HAART.

5.4.4 Adult TB Notification

As shown in Figure 5.7, from 1997 to end 2004 adult TB notification rates (per 100,000) increased by an average of 95 cases a year (95% CI: 31 to 159; p=0.004). From 2005, the rate of adult cases decreased by an average of 182 cases per year (95% CI: -295 to -70; p=0.02).

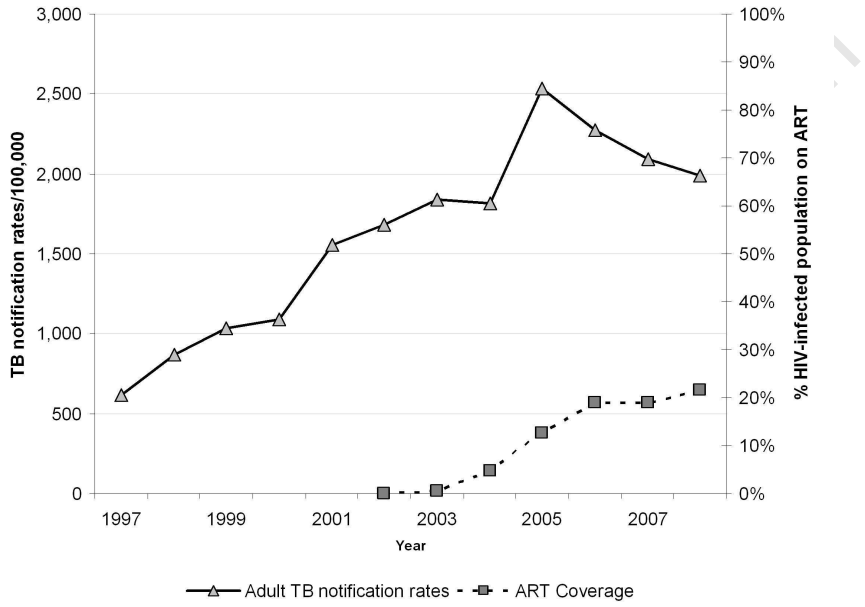


Figure 5.7: Adult TB notifications rates and antiretroviral coverage in study community, from 1997 to 2008

This figure shows the rapid scale-up of HAART coverage and the increasing TB notification rates from1997 to 2004 (p=0.004), followed by a decrease in TB notification rates from 2005 to 2008 (p=0.02).

5.4.4.1 HIV-Infected and HIV-Uninfected TB Notification

Table 5.2 shows TB notification data for HIV-uninfected patients, as well as HIV-infected patients not on HAART and those patients on HAART at time of TB diagnosis from 2002 to 2008. Adult TB rates in HIV-infected and HIV-uninfected TB patients are shown in Figure 5.8A, including extreme case scenarios for HIV-associated and non HIV-associated TB rates assuming 100% of patients with unknown HIV status were HIV-infected and HIV-uninfected respectively.

In summary, TB rates among HIV-uninfected patients remained relatively stable, showing only a modest decline after 2004. In contrast, TB rates in HIV-infected patients increased significantly from 2002 to 2004, followed by a significant decline.

TB rates (per 100,000) in patients known to be HIV-uninfected did not change substantially from 2002 to 2004 (with an average annual increase of 58 cases/100,000; p=0.59), but declined by 82 cases per year from 2005 (95% CI: -139 to -25; p=0.01). From 2002 to the end of 2004, overall TB rates in known HIV-infected adults increased by an average of 432 cases per year (95% CI: 109 to 755; p=0.01), after which rates decreased significantly by 578 cases per year (95% CI: -697 to -459; p<0.001).

5.4.4.2 TB Notification and HAART

The number of HIV-infected patients initiating HAART in each year is shown in Table 5.2. Figure 5.8B shows TB rates (per 100,000) in HIV-infected patients stratified by HAART status. In summary, TB rates among HIV-infected patients not on HAART increased from 2002 to 2004, followed by a significant decline from 2005, while TB rates in patients on HAART showed a dramatic decline from 2004.

TB rates in HIV-infected patients not on HAART increased by an average of 367 cases per year from 2002 to end 2004 (95% CI: -392 to 1126; p=0.34). From 2005, there was a significant average annual decrease of 409 TB cases in HIV-infected patients not on HAART (95% CI: -518 to -300; p<0.001). TB rates in HIV-infected patients on HAART decreased significantly from 2004 by an average of 1,155 cases per year (95% CI: -1246 to -1064; p<0.001).

Table 5.3 shows the age-standardised rate ratios of TB in adult HIV-infected patients off HAART compared to those on HAART. After standardizing for age differences across annual populations, HIV-infected patients not on HAART had a lower rate of TB compared to HIV-infected patients on HAART early in the HAART programme (RR=0.41 in 2004). However, as the period of the HAART programme increased, the RR of TB in patients off HAART increased to nearly twice that of the patients receiving HAART (RR=1.98 in 2008).

Table 5.2: TB notification rates among adult HIV-uninfected and HIV-infected patients in the study community, from 1997 to 2008

Year	Number of Adult TB cases			Adult HIV-infected Population	Adult HIV-infected population on HAART	Adult TB Rates/100,000		
	HIV-uninfected	HIV-infected off HAART	HIV-infected on HAART			HIV - uninfected	HIV-infected off HAART	HIV-infected on HAART
2002	38	70	0	1,737	0	635	4,030	0
2003	50	85	0	1,993	0	744	4,265	0
2004	52	100	8	2,233	104	696	4,697	7,692
2005	81	130	20	2,303	290	1,061	6,458	6,897
2006	56	120	28	2,591	491	739	5,714	5,703
2007	59	126	25	2,877	547	721	5,408	4,570
2008	68	129	21	3,164	680	773	5,193	3,088

Table 5.3: Age Standardised Rate Ratios (RR) for TB disease in HIV-infected patients on and off HAART in the study community, from 2002 to 2008

Year	HIV-infected off HAART		HIV-infected on HAART		Crude RR	Age Standardised RR
	Crude Rate	Age Standardised Rate*	Crude Rate	Age Standardised Rate*		
2004	4,697	4,750	7,692	11,616	0.61	0.41
2005	6,458	6,772	6,897	6,712	0.94	1.01
2006	5,762	6,014	5,499	5,883	1.05	1.02
2007	5,408	5,760	4,570	4,497	1.18	1.28
2008	5,193	5,762	3,088	2,897	1.68	1.98

*****HIV-infected population used as reference population for age standardised rates

Table 5.4: Adult TB site of infection and retreatment rates (per 100,000), overall and by HIV-status, in the study community from 1997 to 2008

Year	Pulmonary TB			Extra-pulmonary TB			Retreatment TB		
	All TB cases	HIV-infected cases	HIV-uninfected cases	All TB cases	HIV-infected cases	HIV-uninfected cases	All TB cases	HIV-infected cases	HIV-uninfected cases
1997	554			64			256		
1998	735			151			151		
1999	862			220			304		
2000	965			123			276		
2001	1,247			308			350		
2002	1,178	2,533	551	531	1,554	100	376	979	167
2003	1,469	3,211	655	390	1,154	89	505	1,204	238
2004	1,381	3,403	629	474	1,523	94	556	1,478	227
2005	2,164	5,384	943	523	1,650	144	664	1,867	249
2006	1,849	4,554	673	502	1,428	106	580	1,775	119
2007	1,718	4,101	660	452	1,390	86	497	1,425	98
2008	1,581	3,603	648	443	1,233	136	585	1,233	273

Note: 1) Patients of unknown HIV status are excluded in the HIV-stratified analyses
2) Patients with concurrent PTB and EPTB diagnoses were counted twice: once in each category.

Table 5.5: Adult TB treatment outcomes, overall and by HIV-status, in the study community from 1997 to 2008

Year	TB Treatment Completion			TB Treatment Interruption			TB Mortality		
	All TB cases	HIV-infected cases	HIV-uninfected cases	All TB cases	HIV-infected cases	HIV-uninfected cases	All TB cases	HIV-infected cases	HIV-uninfected cases
1997	17 (63%)			5 (19%)			2 (7%)		
1998	32 (80%)			7 (18%)			1 (3%)		
1999	53 (98%)			0 (0%)			1 (2%)		
2000	46 (77%)			4 (7%)			7(12%)		
2001	75 (81%)			8 (9%)			11 (9%)		
2002	93 (79%)	49 78%)	29(81%)	13 (11%)	6 (10%)	4 (11%)	10 (8%)	8 (13%)	1 (3%)
2003	122 (79%)	61 (74%)	42 (86%)	16 (10%)	8 (10%)	5 (10%)	17 (11%)	13 (16%)	2 (4%)
2004	125 (82%)	75 (80%)	41 (87%)	11 (7%)	7 (7%)	2 (4%)	18 (11%)	12 (13%)	3 (6%)
2005	176 (79%)	106 (78%)	60 (86%)	26 (12%)	12 (9%)	8 (11%)	20 (9%)	17 (13%)	1 (1%)
2006	170 (78%)	103 (75%)	45 (83%)	29 (13%)	19 (14%)	7 (13%)	16 (7%)	12 (9%)	2 (4%)
2007	181 (85%)	116 (85%)	52 (91%)	17 (8%)	9 (7%)	4 (7%)	13 (6%)	10 (7%)	1 (2%)
2008	171 (78%)	110 (78%)	50 (79%)	25 (11%)	16 (11%)	8 (13%)	12 (5%)	6 (4%)	3 (5%)

Note: Patients of unknown HIV status are excluded in the HIV-stratified analyses

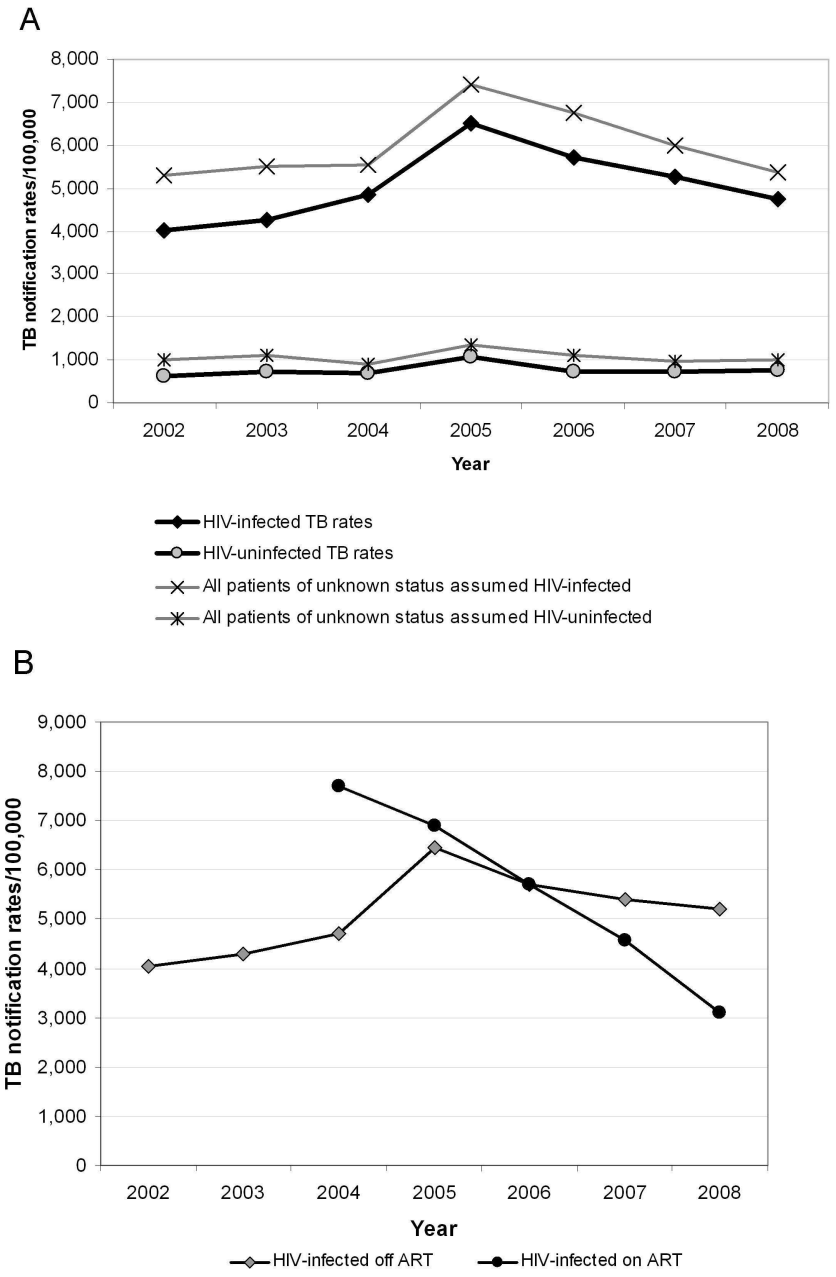


Figure 5.8: *Figure A*: TB rates in adult HIV-infected and HIV-uninfected patients in the study community, from 2002 to 2008. *Figure B*: TB rates in adult HIV-infected patients receiving HAART and adult HIV-infected patients not receiving HAART, over the same time period

Figure A shows the increase in TB notification rates among HIV-infected patients from 2002 to 2004 ($p=0.01$), followed by a decrease in notification rates from 2005 to 2008 ($p<0.001$). This figure also shows the stable TB notification rates among HIV-uninfected patients from 2002 to 2004 ($p=0.59$), followed by a moderate decline from 2005 ($p=0.01$). Extreme case scenario sensitivity analyses are also shown.
Figure B shows the increase in TB notification rates from 2002 to 2004 among HIV-infected patients not receiving HAART ($p=0.34$), followed by a decrease in notification rates from 2005 to 2008 ($p<0.001$). This figure also shows the decreasing TB notification rates from 2004 to 2008 among HIV-infected patients receiving HAART ($p<0.001$).

5.4.4.3 Baseline CD4 Count

The number of HIV-infected patients initiating HAART in each year is shown in Table 5.2.The median baseline CD4 counts in patients commencing HAART increased from 15 cells/µl in 2003, to 86 cells/µl in 2004, 129 cells/µl in 2005, peaking at 153 cells/µl in 2006, and then stabilising at 122 cells/µl in 2007 and 141cells/µl in 2008.

5.4.4.4 TB Categories

Pulmonary and Extra-Pulmonary TB

Pulmonary and extra-pulmonary TB rates (per 100,000), overall and by HIV-status, are reported in Table 5.4.

In summary, both overall PTB and smear-positive PTB rates increased significantly from 1997 to 2004, and then declined from 2005. Among HIV-uninfected patients, PTB and smear-positive PTB rates were stable from 2002 to 2004, decreasing moderately from 2005. Among HIV-infected patients PTB and smear-positive PTB rates increased from 2002, followed by a significant decline from 2005. In both PTB and smear-positive PTB, the decline in rates was greater in HIV-infected patients receiving HAART. PTB and smear-positive PTB rates by HIV and HAART status are illustrated in Figures 5.9A and 5.9B respectively.

Total PTB rates increased by an annual average of 131 cases/100,000 from 1997 to 2004 (95% CI: 117 to145; p<0.001), followed by a significant decline from 2005 (average annual decrease of 171 cases; 95% CI: -240 to 102; p<0.001). In HIV-uninfected patients, PTB rates were stable from 2002 to end 2004 (p=0.34), and declined moderately from 2005, with an average decrease of 83 cases per year (95% CI: -124 to -41; p<0.001). In HIV-infected patients, PTB rates increased from 2002 by an average of 452 cases per year (95% CI: 256 to 648; p<0.001), followed by a significant decline from 2005, with an average decrease of 561 cases per year (95% CI: -694 to -428; p<0.001). The decline in PTB rates was significant in both HIV-infected patients not on HAART (average decrease of 441 cases per year; 95% CI: -579 to -302; p<0.001) and in patients receiving HAART (average decrease of 762 cases per year; 95% CI: -1191 to -334; p<0.001).

Overall sputum smear-positive PTB rates (per 100,000) increased significantly from 1997 to end 2004, by an annual average of 81 cases/100,000 (95% CI: 52 to 109; p<0.001). Sputum smear-positive rates then declined after 2005, by an annual average of 109 cases (95% CI: -333 to 114; p=0.34). No significant increase was detected in either HIV-uninfected or HIV-infected patients from 2002 to end 2004 (p=0.93 and p=0.67 respectively). From 2005 smear-positive PTB declined in both HIV-uninfected patients (average annual decrease of 48 cases; 95% CI: -94 to -1; p=0.04) and in HIV-infected patients (overall p=0.004). The decline in HIV-infected patients was noted in both patients not on HAART (average annual decrease of 192 cases; 95% CI: -368 to -16; p=0.03) and in HIV-infected patients receiving HAART (average annual decrease of 430 cases; 95% CI: -765 to -94; p=0.01) (Figure 5.9B).

There was no change over time in the proportion of PTB cases that were sputum smear-positive, either in HIV-uninfected patients (p-value for trend=0.27) or HIV-infected patients (p=0.31), including HIV-infected patients not receiving HAART (p=0.23). HIV-infected patients had significantly lower odds of smear-positive PTB compared to HIV-uninfected patients, and this finding persisted when adjusted for age and gender (odds ratio [OR]: 0.46; 95% CI: 0.34 – 0.61; p<0.001). However, HIV-infected patients did account for 52% of the overall burden of smear-positive disease. There was no difference in sputum smear-status among HIV-infected patients on or off HAART (p=0.40).

In summary, total EPTB rates and HIV-uninfected EPTB increased significantly to 2004, after which the EPTB rates stabilised. Among HIV-infected patients, the increase in EPTB from 2002 to 2004 was modest, followed by a decrease that was most marked in those patients receiving HAART. EPTB rates by HIV and HAART status are illustrated in Figures 5.9C.

Overall EPTB rates (per 100,000) increased significantly from 1997 to 2004, by an average annual rate of 62 cases per year (95% CI: 17 to 107; p=0.01), and then stabilised from 2005 (95% CI: -617 to 561; p=0.93). In HIV-uninfected patients, EPTB rates (per 100,000) increased modestly from 2002 to end 2004 (average annual increase of 2 cases per year; p<0.001), but were stable from 2005 to end 2008 (p=0.49). In HIV-infected patients, EPTB rates had a non-significant increase from 2002 to end

2004 by an average of 36 cases/100,000 per year (95% CI: -156 to 223; p=0.73), followed by a significant decline from 2005, with an average decrease of 138 cases per year (95% CI: -243 to -34; p=0.01). The decline in EPTB rates was not significant in HIV-infected patients off HAART (p=0.97) but was significant in patients receiving HAART (average decrease of 477 cases per year; 95% CI: -785 to -169; p=0.002).

Based on the TB register data, EPTB cases were comprised of 51% TB of "other respiratory organs" (eg pleural effusions) 38% "other organs" (including pericardial TB, hepatic TB etc), 5% military TB, 2% TB lymphadenitis, 1% peritoneal or intestinal TB, 1% TB meningitis, 1% TB of the bones or joints and 1% TB of the genito-urinary tract

Retreatment TB

In summary, retreatment TB rates overall, and among HIV-uninfected and HIV-infected patients, increased over the first part of the study period. From 2005, retreatment rates overall and in HIV-uninfected patients stabilised. Retreatment rates in HIV-infected patients declined from 2005, with the greatest decline occurring in those patients receiving HAART. Retreatment TB rates by HIV and HAART status are illustrated in Figures 5.10.

Overall retreatment TB rates (per 100,000) increased significantly from 1997 to 2004, at an annual average increase of 53 cases/100,000 (95% CI: 31 to 75; p<0.001), after which retreatment rates stabilised (annual average decrease of 44 cases; 95% CI: -91 to 2; p=0.06). Retreatment TB rates increased in HIV-uninfected patients from 2002 to end 2004, with a modest average annual increase of 48 cases (95% CI: 47 to 48; p<0.001), and were subsequently stable from 2005 to end 2008 (p=0.95). In HIV-infected patients not receiving HAART, retreatment TB rates increased by an average of 138 cases per year (95% CI: 138 to 139; p<0.001) from 2002 to 2004, and then decreased by an annual average of 188 cases (95% CI: -238 to -137; p<0.001). Annual retreatment TB rates in HIV-infected patients on HAART decreased significantly from 2004 by an average of 784 cases/100,000 per year (95% CI: -1406 to -163; p=0.01).

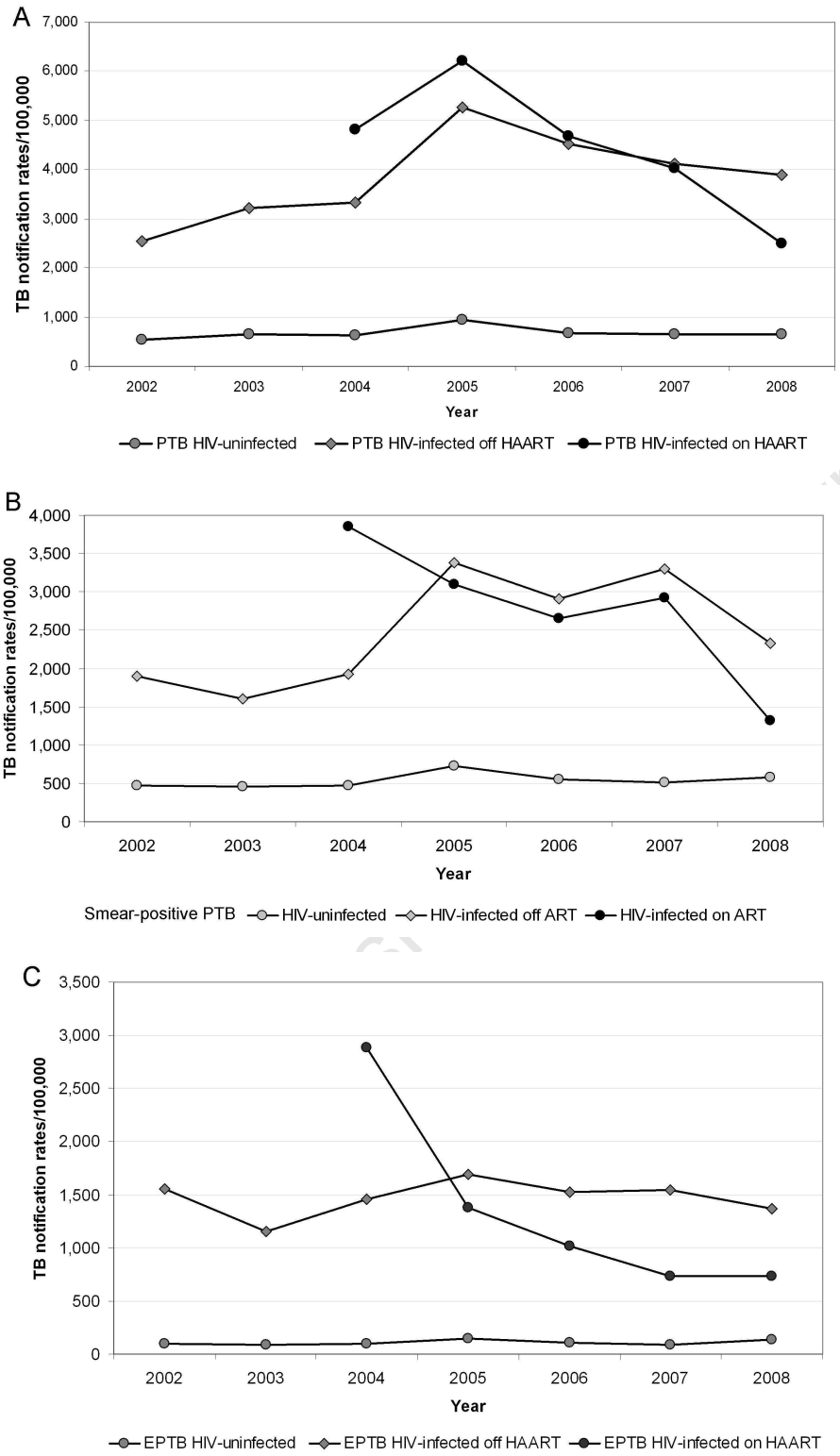


Figure 5.9: *Figure A:* Adult PTB rates by HIV and HAART status; *Figure B:* Adult sputum smear-positive PTB rates by HIV and HAART status; and *Figure C:* Adult EPTB rates by HIV and HAART status, in the study community from 2002 to 2008

Figure A shows the stable PTB notification rates among HIV-uninfected patients from 2002 to 2004 (p=0.34), followed by a moderate decline from 2005 (p<0.001). This figure also shows the increase in PTB notification rates among HIV-infected patients not receiving HAART from 2002 to 2004 (p<0.001), followed by a decrease in notification rates from 2005 to 2008 (p<0.001). The decreasing PTB notification rates among HIV-infected patients receiving HAART is also illustrated (p<0.001).

Figure B shows the stable smear-positive PTB notification rates among HIV-uninfected patients from 2002 to 2004 (p=0.93), followed by a moderate decline from 2005 (p=0.04). This figure also shows the smear-positive PTB notification rates among HIV-infected patients not receiving HAART from 2002 to 2004 (p=0.67), followed by a decrease in rates from 2005 (p=0.03). The decreasing PTB notification rates among HIV-infected patients receiving HAART is also illustrated (p=0.01).

Figure C shows the increasing EPTB notification rates among HIV-uninfected patients from 2002 to 2004 (p<0.001), followed by a stabilizing of rates from 2005 (p=0.49). This figure also shows the relatively stable rates of EPTB notification rates among HIV-infected patients not receiving HAART from 2002 to 2004 (p=0.73), and from 2005 (p=0.97). The decreasing EPTB notification rates among HIV-infected patients receiving HAART is also illustrated (p=0.002).

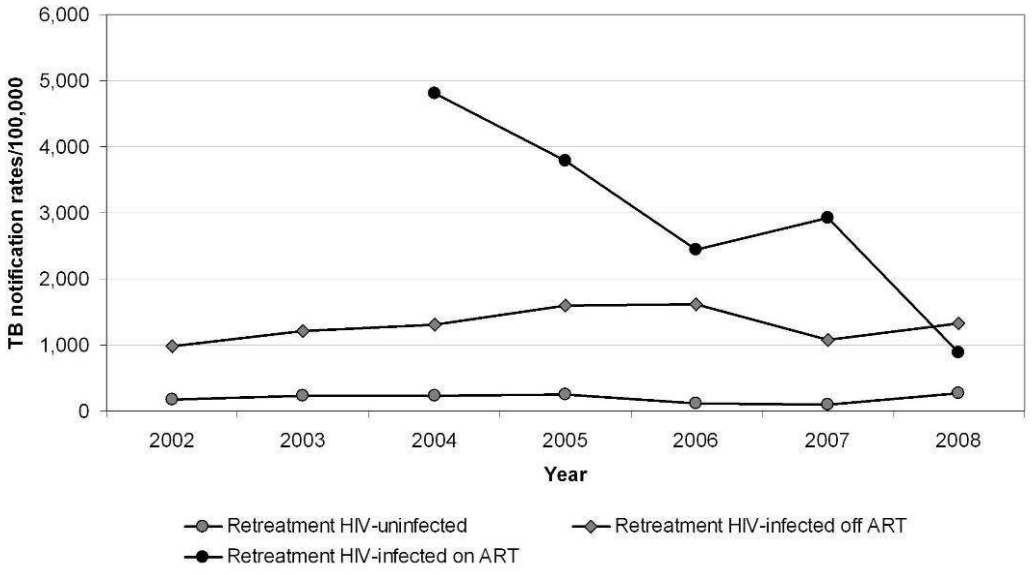


Figure 5.10: Adult retreatment TB rates by HIV and HAART status, in the study community from 2002 to 2008

This figure shows the retreatment TB notification rates among HIV-uninfected patients, increasing modestly from 2002 to 2004 (p<0.001), and stabilising from 2005 (p=0.95). This figure also shows the increase in retreatment TB notification rates among HIV-infected patients not receiving HAART from 2002 to 2004 (p<0.001), followed by a decrease in rates from 2005 to 2008 (p<0.001). The decreasing retreatment TB rates among HIV-infected patients receiving HAART is also illustrated (p=0.01).

5.4.4.5 TB Outcomes

TB outcomes, specifically treatment completion, mortality and treatment interruptions, both overall and by HIV status, are tabulated in Table 5.5. There were 14 patients over the 12 year period for which outcomes were not recorded (11 were multi-drug resistant (MDR)-TB patients who were transferred to an MDR treatment facility). These 14 patients have been excluded from the outcome analysis.

In summary, there were no significant changes in TB treatment completion or interruption rates among HIV-uninfected and HIV-infected patients. TB mortality decreased significantly from 2002, and this decrease was predominantly in HIV-infected patients.

TB treatment completion rates averaged 80% over the study period, with no significant change over that time (p-value for trend=0.94). TB treatment completion rates did not change significantly for HIV-uninfected or HIV-infected patients from 2002 to 2008 (average completion rate in HIV-uninfected: 85%, p=0.93; and average completion rate in HIV-infected: 78%, p=0.39). HIV-uninfected patients were more likely to complete treatment compared to HIV-infected patients (p=0.002), although this difference did not persist when adjusted for mortality (p=0.32).

Overall treatment interruption rates averaged 10% from 1997 to 2008, with no significant change over that time (p-value for trend=0.69). TB treatment interruption rates did not change significantly for HIV-uninfected or HIV-infected patients from 2002 to 2008 (average completion rate in HIV-uninfected: 10%; p=0.69 and average completion rate in HIV-infected: 10%; p=0.61). There was no difference in the interruption rates between HIV-uninfected and HIV-infected patients (p=1.00).

Overall mortality during TB treatment decreased over the study period from 8% in 2002 to 5% in 2008 (p-value for trend=0.02; Figure 5.11). While TB mortality rates remained relatively constant in HIV-uninfected individuals (p=0.93), TB mortality dropped significantly in HIV-infected patients from 13% in 2002 to 4% in 2008 (p=0.001).

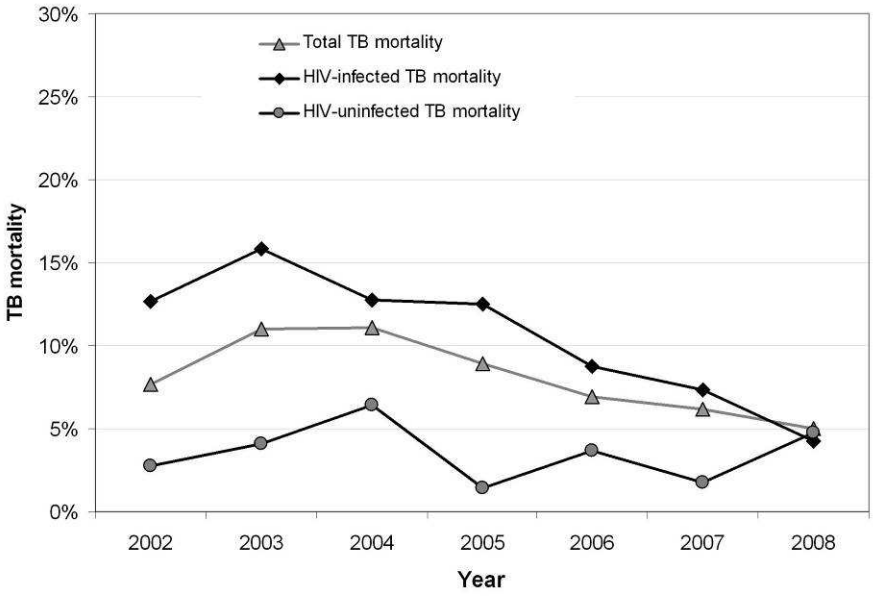


Figure 5.11: Adult TB mortality rate overall and by HIV-status, in the study community from 2002 to 2008

This figure shows the decline in all cause TB mortality rates overall (p=0.02) and among HIV-infected patients (p=0.001). TB mortality rates remained stable in HIV-uninfected patients (p=0.93).

The proportion of patients that were transferred out of the community to another health facility remained constant, both from 1997 to end 2004 (p-value for trend=0.35) and from 2005 to 2008 (p=0.31). Similarly, the transfer-out rates remained constant for HIV-uninfected patients (p=0.96) and HIV-infected patients (0.83). There was no statistical difference in the transfer-out rates between HIV-uninfected and HIV-infected patients (p=0.07).

5.5 DISCUSSION

This study demonstrated an association between the implementation of a high coverage HAART programme and TB notification rates in a community with high burdens of both TB and HIV. In this community, adult TB notification and mortality rates decreased with the rapid and high coverage implementation of a HAART programme. Overall TB rates in children remained relative constant over the study period.

In Chapter 2 we noted the exceptionally high risk of TB infection and re-infection in this community. We hypothesized that the high adult HIV prevalence in Site M[179;197], coupled with the considerable increased risk of progression to TB disease in HIV-infected

individuals, from both reactivation of latent infection and recent infection[14;102], could explain the substantial incidence of TB disease in this setting. This analysis has shown that HIV-uninfected TB rates have remained relatively stable in this community over the time period, and HIV-infected adult TB notification rates are at least six-fold higher than those in HIV-uninfected patients. This emphasises that HIV-associated TB rates have been responsible for the burden of TB disease in this community.

5.5.1 Childhood TB Notifications and Outcomes

In keeping with historical data on the natural history of TB[69;304], children under 5 years had the highest rates of paediatric TB disease, followed by substantially lower rates in children between 5 and 9 years and a slow increase once again in children greater than 10 years of age.

According to the WHO childhood smear-positive TB rates in South Africa are an estimated 29/100,000[7]. However, in the last two years of this study, the smear-positive TB rates in children in this community have been 2.5 to 5 fold higher than that estimated rate. As discussed in Chapter 3, sputum-positive TB rates for children grossly underestimate the total paediatric TB burden, and this is highlighted in this study, with only an average of 8% of the overall paediatric disease reflected in the smear-positive rates.

Of note is that children under 4 years of age had TB rates comparable with the adults in the community, reflecting a substantial burden of morbidity in this age group. However, the TB rates in children under 10 years of age were stable over the study period, with no statistical evidence of a direct benefit of the HAART programme. Children in this community are subjected to a substantial force of TB infection and re-infection and have a high annual exposure rate (Chapter 2 & 3). In addition, young children are at an increased risk of progression to disease following infection[69;304], even among HIV-uninfected children. The high burden of childhood disease may be more attributable to this considerable, combined risk of infection and progression to disease than to paediatric HIV infection.

Childhood TB is a reflection of the adult TB epidemic. While the adult TB rates have begun to decline in this community, it is possible that there is a time lag to observing the

impact of this decrease on childhood TB rates, and the four years of follow-up post-HAART implementation may be insufficient time to detect evidence of possible reduction in TB transmission and disease in this vulnerable group. Furthermore, while there are conflicting findings with regards to this hypothesis, HIV-uninfected adults may be more responsible for transmission to children. HIV-uninfected TB rates have not declined as dramatically as the rates among HIV-infected patients and this may also contribute to the relatively stable rates in children under 10 years of age.

Prior to HAART programme TB rates in young adolescents of 10 to 14 years of age were increasing over time. However, subsequent to the availability of HAART TB rates appear to have stabilised in this group. HAART coverage is high among HIV-infected adolescents and therefore the stabilising of TB rates in young adolescents may reflect the benefits of the HAART programme.

5.5.2 Adult TB Notifications and Outcomes

The decrease in adult TB notification rates in the study community occurred against a background of increasing national TB notification rates, as shown in Chapter 1[7]. TB rates in HIV-uninfected adults have remained relatively stable from 1997 to 2004, with HIV-associated TB driving the escalating epidemic in the study population. While HIV-uninfected rates showed a decline from 2005, the reduction in community TB notification rates in this study was predominantly due to a decrease in TB rates in HIV-infected patients, and more specifically, in patients receiving HAART. This finding was consistent for overall adult TB notification rates, as well as for PTB, EPTB and retreatment TB rates.

Although the HIV prevalence in this community was reasonably stable from 2002 to 2008, the adult TB notification rates in HIV-infected patients continued to escalate until 2005. Following the rapid scale-up of HAART availability from 2005, the TB rates in HIV-infected patients not on HAART stabilised and then decline moderately. However, there was a dramatic, almost 3-fold declined in TB rates in those HIV-infected patients on HAART. Of note is the increasing reduction in risk of TB for patients on HAART compared to patients off HAART, as illustrated by the age-standardised RR increasing from 0.41 in 2004 to nearly 2 in 2008. Similarly, there were high initial EPTB and retreatment TB rates in patients receiving HAART compared to those patients not on

HAART, followed by a substantial reduction in EPTB and retreatment TB in HIV-infected patients on HAART. These findings are most likely due to the immune system recovery associated with antiretroviral treatment and the consequent reduction in TB risk in HIV-infected patients on treatment[45;46;152;170].

The levelling-off and subsequent decline of TB notification rates (overall TB rates as well as EPTB and retreatment rates) in HIV-infected patients not on HAART may be due to the removal of those with the highest TB risk (lowest CD4 counts)[46] from this group into the group on HAART. This stabilising of notification rates suggests that the rate at which people are removed from the susceptible pool approximates the rate at which HIV-infected patients not on HAART are progressing into a high-risk state of immune compromise. This finding suggests that the rate at which a HAART programme is implemented in a community might be an important variable determining the impact of this intervention on overall HIV-associated TB rates. The importance of the rate of HAART implementation is emphasised by the fact that TB control may rely on reducing TB incidence rapidly in order to reduce transmission effectively[452].

Although TB does occur at all CD4 strata, the highest risk of TB in HIV-infected patients occurs at low CD4 counts (<200cells/µl)[34;75]. The South African National Antiretroviral programme recommended initiating patients on HAART at CD4 count <200cells/µl, or WHO clinical stage IV [199], although new guidelines will allow for the earlier initiation of HAART treatment for TB patients and pregnant women (at CD4 count <350cells/µl). Patients initiating HAART have a high risk of TB in the first months of treatment compared to later in treatment, due to the risks associated with low baseline CD4 counts and with possible unmasking of sub-clinical TB[76;152;166]. The overall increase in median baseline CD4 count in our study reflects the initiation of patients with more advanced disease onto treatment in the early stages of the HAART programme. With increasing duration of the HAART programme, the pool of severely immune-compromised patients off treatment decreased, patients were started on HAART at higher baseline CD4 counts. However, despite the overall increase in median baseline CD4 count, by 2008 the median CD4 count at initiation of HAART was still well below 200cells/µl and TB risk prior to HAART initiation remained high, as evidenced by the nearly 2-fold higher standardised RR in HIV-infected patients off HAART compared to those patients on HAART in 2008.

HIV-infected patients off HAART accounted for 64% of TB disease in 2008, and thus contribute a large portion of the TB burden. By 2008 the HAART coverage in this community was high, at 90% of the estimated community need as defined by the national HAART guidelines[199;468]. If similar coverage rates and impacts were achieved nationally, a greater than 20% reduction in TB rates might be attained, as seen in this community. But this impact could be more substantial if treatment was initiated earlier in the HIV-disease process, before the higher TB risk associated with advanced immune suppression, thus also reducing TB burden prior to HAART initiation and during the early months on HAART.

Alternative explanations of changes in notification rates

While there was a strong temporal association between the decline in TB notification rates and the implementation of a high coverage HAART programme, and there is substantive biological plausibility to support this association, it is worth considering alternative explanations for the decrease noted in TB notification rates.

Prevalence of TB is a function of TB incidence and period of infectivity in the community. Period of infectivity can be decreased by earlier diagnosis and treatment of cases. The key change to case-finding activities was the introduction of active case-finding for TB in patients initiating HAART. This practise may have resulted in a decrease in TB prevalence, thus indirectly contributing to the decrease in notifications over time. In addition, in 2005 a community-based cross-sectional survey was performed in 10% of the study community, investigating participants for active TB disease[179]. A sensitivity analysis, excluding those participants diagnosed in that survey from the notification data, showed no change in the study results, including the peak noted in 2005 (Appendix B). Therefore this survey did not appear to have had a direct effect on the notification rates in this community. The increased TB screening of HAART-eligible patients (through symptom screen and sputum investigation) that was associated with the scale-up of the HAART programme in 2005 may, in part, explain the 2005 peak in notification rates. This theory is strengthened by the observation that the peak is considerably more marked in PTB, specifically smear-positive PTB disease. This increased screening together with the survey may have increased community awareness of TB, and potentially contributed

indirectly to the moderate increase in TB notifications also noted in HIV-uninfected patients.

Other possible explanations for the study findings included improved TB treatment completion rates, which may have resulted in decreased TB transmission, in turn reducing TB incidence. However TB completion rates remained stable over the study period, and this was consistent for both HIV-infected and HIV-uninfected patients. Period of infectivity, and thus prevalence could be decreased by increased TB-associated mortality; however, TB mortality has decreased over the period of observation. There were no changes in the infection control policies within the clinic over this period, and therefore decreased nosocomial TB transmission is unlikely to explain the study findings.

This community showed substantial population growth over the study period, and a changing age distribution in the population may have impacted the TB notification rates. However, age-standardised rates were calculated using the population age distribution in 1997 as the reference population, and these results confirmed the trends in TB notification rates. Although changing social conditions may also impact TB transmission in communities, this community has remained one of extremely poor socio-economic status since its establishment in 1994. The unchanging social impact on TB rates is reflected by the stable TB notification rates among young children.

Sputum Smear-Positive PTB

HIV-infected patients have a reduced likelihood of sputum smear-positive TB disease, due to impaired immune responses, and as a result are thought to contribute less to TB transmission[9]. Therefore it is reasonable to hypothesise that patients on HAART, with improved immune functioning, may have an increased smear-positivity rate, and consequently contribute more to transmission than their HIV-infected counterparts not on HAART. However, in this study we reported that smear-positive rates declined in patients receiving HAART, although that decline was less than noted in all PTB, or in EPTB and retreatment TB. This is in keeping with the findings of another recent study that reported no increase in smear-positivity rates in HIV-infected patients receiving HAART[469]. In addition, smear-positive PTB rates in HIV-infected patients off HAART also remained higher than those of patients on HAART.

TB Outcomes

TB is a major cause of mortality in HIV-infected patients in sub-Saharan Africa[7;17]. A key finding in this study is the significant reduction in TB mortality rates in HIV-infected patients. The use of HAART has been associated with a reduction in TB-associated mortality among individuals and in treatment cohorts[470;471]. In our study the implementation of a community HAART programme was also associated with a decrease in overall TB mortality, predominantly due to the decrease in HIV-infected patients. In 2002, the mortality rate in HIV-infected TB patients was four times greater than that of HIV-uninfected patients, but had decreased to the same rate as HIV-uninfected patients by 2008. This suggests that HAART programmes may greatly assist in achieving the Stop TB Partnership goal to halve TB mortality rates by 2015. As mortality measures may more quickly reflect the impact of an intervention compared to incidence measures[452;472], this result supports the finding of decreasing notification rates. While overall reduction in TB notification rates has a potential public health benefit, the finding of decreased mortality risk reflects substantial benefit for the individual patient.

No change was noted in TB treatment completion or interruption rates among HIV-infected and -uninfected patients over the study period. In particular, this study did not demonstrate improved adherence to TB treatment among HIV-infected patients after the implementation of the HAART programme. Numbers of patients interrupting treatment were too small to provide a statistical comparison between HIV-infected patients on and off HAART.

5.5.3 Strengths and Weaknesses

While the adult TB notification rate analyses are robust (Appendix B), it should be noted that the absolute numbers of paediatric TB cases were relatively small and this may have impacted the statistical analyses of this group. Furthermore, rates of HIV testing were extremely low among paediatric patients, hampering a HIV-stratified analysis.

HIV testing uptake was also not complete among adult TB patients, although with 78% of adults tested, testing rates were substantially higher than among children. In order to explore the potential biasing influence of those patients with unknown status we performed an extreme case scenario analysis among adult patients. The result of this analysis showed that our findings were robust and did not alter the findings of the study

(Figure 5.8A). The population denominators were derived from community census data with the assumption of linear growth between censuses and HIV–infected denominators were obtained from a mathematical model fitted to local HIV data. Sensitivity analyses were run for models assuming different trends of population growth and different assumptions for HIV prevalence. These analyses also did not show substantive changes in study inferences (Appendix B). This community is typical of many recently urbanised populations in South Africa, but there is a need for further investigation to confirm the generalisability of our findings in other high prevalence settings. In addition, a longer period of observation is required to confirm persistence of these changes.

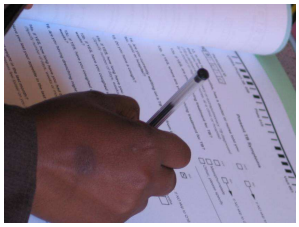
This study was performed in a well-demarcated community, with population data derived from frequent community censuses. All residents receive their TB treatment at a single clinic, and therefore TB notification data is likely to be a complete representation of TB notifications in the community. Similarly, residents obtained HAART from the clinic or the local referral hospital and access to both these databases ensured an accurate description of the HAART programme in this community. These analyses are dependent on the fidelity of the relevant data records, and therefore the databases underwent a 10% quality control assessment.

While TB notification rates are frequently used as a surrogate measure for TB incidence, there are limitations to this method particularly in settings in which the TB control programme relies on passive case-finding (ie self presentation by patients when ill)[179;473;474]. The accuracy with which notification data reflect TB disease burden is dependent on the case detection rate of the TB programme[179;344;475;476]. The case detection rate, in turn, is affected by the patients' knowledge and beliefs regarding TB disease, recognition of TB symptoms, subsequent health seeking behaviour and access to medical facilities[341;342;474;477-479]. The timely and accurate diagnosis of the patient at the health care facility will also influence the precision of notification data as a measure of disease burden[341;342;478] as will the quality and accuracy of data reporting. Case detection rates in this community were unknown for the earlier years of the study, but ranged from 48% in 2005 to 60% in 2008 (Chapter 6)[179;197]. Therefore the notification data reported in this study underestimated the true incidence of TB in this community, and represents a conservative estimate of this measure of disease burden. The increasing case detection rates in the second part of the study were predominantly found in HIV-infected patients,

and should have resulted in increasing notification rates. Therefore this observation supports the robust nature of the study finding of decreasing trends.

5.5.4 Conclusion

In conclusion, against a background of increasing TB notifications nationally, we have shown that a rapidly implemented, high coverage HAART programme can reduce the TB notification rates and TB mortality within a community heavily affected by both HIV and TB epidemics. This reduction in TB was due predominantly to the decrease in adult TB rates in HIV-infected patients receiving HAART, and may be the result of both active TB screening and improved immune function in these patients. The overall reduction in TB notification rates has a potential public health benefit, while decreased mortality rates reflect benefit for the individual patient.



Chapter 6

Impact of an Antiretroviral Treatment Programme on TB Disease Prevalence

6.1 RATIONALE

While tuberculosis (TB) notification rates are often used as a measure of disease burden, there are limitations to this methodology, particularly in settings in which the TB control programme relies on passive case-finding (ie self presentation by patients when ill)[179;473;474]. The accuracy with which notification data reflect TB disease burden is dependent on the case detection rate of the TB programme[179;344;475;476], as discussed in Chapter 5.

Once receiving TB chemotherapy, TB patients are considered to be less infectious[60], and it is this group of patients that is reflected in notification data. Untreated or inadequately treated TB disease, in contrast, is often unrecognised and is an important driver of transmission in a population[49;60;65;114]. TB prevalence is the proportion of a population with active TB disease, both treated (diagnosed) and untreated (undiagnosed), at a given point in time. Measuring the prevalence of TB in a population, therefore, provides information on the case detection rates of a TB programme as well as a useful measure of the reservoir of transmittable TB. In addition, the impact of interventions on disease burden is usually reflected in disease prevalence before it is noted in incidence measures[452].

This chapter aims to address the final objective of the thesis, namely to determine the prevalence of microbiologically-confirmed pulmonary TB disease in both the HIV-infected and HIV-uninfected subsets of the population and describe changes in the prevalence of PTB disease since the scale-up of antiretroviral therapy (ART) access in the community. Data from this chapter have been published in the fifth paper[197] listed in the preface on page *xi.*

6.2 BACKGROUND

As noted in Chapter 1, a key target of the World Health Organization (WHO) and the Stop TB Partnership is to reduce TB prevalence by 50% from 1990 to 2015[7]. Based on

TB prevalence estimates for the African region, we are failing to achieve this target and the WHO has called for national surveys in high burden countries, such as South Africa, in order to more accurately monitor prevalence trends[7]. However, evaluating population TB prevalence is logistically challenging: cross-sectional surveys are costly due to the large sample sizes required, particularly in low prevalence settings[480], and require well-trained staff and reliable laboratory services[480;481]. As a result, few population surveys have been preformed in resource poor countries[344;473;476;482;483], with surveys more often limited to high risk groups such as TB contacts[308;326-328;484;485], prisoners[486-490], miners[178;491;492] or HIV-infected patients[35;157;492-500]. Thus, due to the paucity of population data, TB prevalence described by the WHO for most countries is not measured directly, but is an indirect estimate based on related parameters[11].

In the particular example of South Africa, prevalence data has been calculated from TB incidence and trends in TB mortality[7]. Based on these calculations the WHO estimated the TB prevalence in South Africa was 707/100,000 in 2005, and 692/100,000 in 2008, with 50% of the TB prevalence occurring in HIV-infected individuals[7].

6.2.1 Community-Based Cross-Sectional Surveys

Few community-based surveys have been implemented in resource poor settings[343;501-503], including sub-Saharan Africa[280;344;473;475;476;482;483;504;505].

Sub-Saharan Africa

Community-based studies performed in sub-Saharan Africa in the last decade have reported TB prevalences among symptomatic participants ranging from 0.2% to 6%[344;473;476], while overall community TB prevalences have been reported in the range of 0.4% to 4.5%[280;475;505;506]. These surveys have also consistently reported case detection rates well below the WHO target of 85%[344;474;476;505]. Despite the significant impact of HIV on TB epidemics in Africa, only three studies have reported HIV status linked to TB results[280;475;506], and all reported a higher TB prevalence among HIV-infected patients. No studies have reported the highly active antiretroviral therapy (HAART) status of HIV-infected participants or assessed the impact of HAART programmes on TB prevalence.

Comparison of the findings of these surveys is complicated by differing inclusion criteria (from all randomly selected participants to symptomatic participants only, as well as

different participant age ranges), varied study procedures (such as sputum specimens and/or chest X-rays), and different definitions as to what constitutes a prevalent TB case. Despite these difficulties, a recent review and meta-analysis of population-based active case-finding (ACF) surveys reported an overall median prevalence of previously undiagnosed TB of 0.7% in sub-Saharan Africa communities[507].

South Africa

A small number of community-based prevalence surveys have been performed in South Africa. Studies performed in the 1970's and early 80's, prior to the escalation of the HIV epidemic, reported prevalences of sputum-positive TB from 1.5-2.1%[256;329;508] in South African communities.

Since the escalation of the HIV epidemic, few community TB surveys have been reported in South Africa[179;482;483]. In 2007, the prevalence of pulmonary TB (PTB) in a rural sub-district was reported to be 0.16%[474]. While in 2009, in an urban community with relatively low HIV prevalence (<12% among women accessing antenatal care[359;509]), sputum positive TB prevalence of 1% was reported among adults older than 14 years of age, compared to adult sputum positive TB prevalence of up to 3% in a high HIV prevalent community (HIV prevalence of 23%)[179]. Only one South African study has reported HIV data linked to the TB results[179], and consequently there is little information on the impact of the HIV co-epidemic on the TB burden in these communities.

6.2.2 TB prevalence in Site M

In 2005, prior to the extensive implementation of the HAART programme, we performed a community-based, TB prevalence cross-sectional survey in Site M. The survey reported a 3% overall TB prevalence in this community, of which just over half was previously undiagnosed TB. In particular a high rate of untreated, laboratory-confirmed PTB was reported among HIV-infected individuals[179], with a 44% case finding proportion among these participants compared to 57% among HIV-uninfected participants.

In order to assess the impact of the subsequent high coverage HAART programme on TB prevalence in this community, we repeated the cross-sectional survey in 2008, measuring the prevalence of both TB disease and HIV infection.

6.3 STUDY DESIGN

This study was performed from June to December 2008, and the same methodology as the 2005 survey was used[179]. A house-to-house enumeration of the community provided a database of 14,592 residents, of whom 1,500 residents ≥15 years of age were randomly selected for study participation (10% of the community).

All participants completed a structured questionnaire, investigating participant demographic characteristics, TB history, TB symptoms (cough, night sweats, loss of appetite and loss of weight), risk factors for TB (including housing, alcohol use, smoking and recreational drug use, prior imprisonment, and employment history) and risk factors for HIV infection. Questionnaires were interviewer-administered in the participants' home language.

Two sputum specimens were collected from each participant: an early morning sputum produced at home, and a second, induced-sputum collected at the site using saline nebulisation. Both sputum specimens were tested at the same laboratory for acid-fast bacilli (AFB) by microscopy and for *Mycobacterium tuberculosis* (*Mtb*) growth by culture. An oral transudate specimen was collected for anonymous HIV testing, with HIV results linked to TB results.

6.3.1 Laboratory Procedures

Sputum specimen smears were examined for AFBs using an auramine-O stain. Sputum sediments were cultured in the Mycobacterial Growth Indicator Tubes (MGIT) automated system and incubated for 6-8 weeks before being reported as negative. Positive cultures were examined for the presence of AFB by Ziehl Neelsen staining and were identified as *Mtb complex* using a polymerase chain reaction assay. The oral mucosal transudate specimen for HIV testing was collected using the Orasure® oral fluid collection device (Orasure Technologies, Bethlehem, PA). The Vironostika Uni-Form II HIV-1 and HIV-2 plus 0 ELISA test (bioMérieux SA, Marcy l'Etoile, France) was used to test for HIV-1 and HIV-2 antibodies.

6.3.2 Case Definitions

Following on the 2005 methodology, participants who reported on the questionnaire that they were currently receiving TB treatment were classified as “treated TB cases”. “Untreated TB cases” were defined as participants without a prior known TB diagnosis, but with laboratory confirmed infection, as identified by two positive AFB smear results or two positive *Mtb* culture results, or a positive AFB smear result confirmed by a positive *Mtb* culture on separate specimens. All untreated TB cases that were identified were referred to the local TB clinic for chemotherapy.

6.3.3 Data Analysis

Data were analyzed using STATA 10.0 (StataCorp, College Station, Texas). Bivariate analyses employed Student’s t-, Wilcoxon sum rank and chi^2 tests, as appropriate. A comparison of age and gender of randomly selected residents and enrolled participants to the population demographics for 2005 and 2008 were performed to confirm the representativeness of the randomly selected and enrolled groups.

TB prevalence was calculated as the proportion of TB cases identified in the total study sample, and as the proportion of TB cases identified in the HIV-infected and uninfected subsets. Multiple logistic regression models were developed to examine changes in overall TB prevalence, as well as treated and untreated TB prevalence between the two surveys, after adjusting for variation in individual participant characteristics. These models were weighted for the proportion of the population sampled in each survey. Median CD4 counts were calculated for the total HAART cohorts in 2005 and 2008, based on each HAART patients’ averaged CD4 count recorded in the survey year. Annual median CD4 counts were compared across the two years with Wilcoxon’s sum rank test. Case-finding proportion was calculated as the proportion of prevalent cases, overall and by HIV status, that were reported as treated TB cases.

Univariate logistic regression analyses were performed to determine the key risk factors for prevalent TB (overall, treated and untreated) in each year. Subsequently, a multivariate logistic regression model for TB was developed, adjusting for significant risk factors and the potential confounders of age and gender.

95% Confidence intervals (CI) were based on the Poisson distribution and all statistical tests were 2-sided at alpha=0.05.

6.4 STUDY FINDINGS

6.4.1 Demographic Characteristics

Of the 1,500 residents selected for participation in the 2008 survey, home visits confirmed that 1,383 of these individuals (92%) were still resident in the community, and eligible for the study. Of these 1,250 (90%) consented to enrol in the study, 121 (9%) refused participation and 12 (1%) were not contacted after 5 home visits. In the initial survey in 2005, 762 (78%) of 971 randomly selected, eligible residents were enrolled in the study, with a refusal rate of 15%[179]. Demographic characteristics of the two samples are shown in Table 6.1.

In 2005, residents selected for study participation did not differ significantly from the general population by age (median age 27 vs 28 years respectively; p=0.54) or by gender (48% vs 51% male in both groups; p=0.11). Similarly, participants enrolled in that study were representative of the general population in terms of age (median age 27 vs 28; p=0.31), and gender (51% males vs 48% respectively; p=0.11). In 2008, residents selected for study participation did not differ from the general population by age (median age 28 years for both groups; p=0.43) or by gender (54% males in both groups; p=0.81) and nor did participants who enrolled in the study (median age 28 years in both groups; p=0.41 and 54% males in both groups; p=0.84).

6.4.2 TB and HIV Testing

All participants were nebulised for sputum specimens, however, a small proportion of participants were unable to produce sputum. In 2005 sputum samples were obtained from 761 of the participants (99%), and in 2008, sputum specimens were obtained form 1,225 (98%) of the sample. The culture contamination rate of specimens was consistent across the two surveys (7% in 2005 vs 8% in 2008; p=0.41).

In 2005, 4 participants (1%) declined HIV testing; among those that were tested the HIV prevalence was 23% (95% CI: 20-26%)[179]. Of the 174 HIV-infected participants, 5% (95% CI: 2-10%) were receiving HAART. In 2008, 43 participants (3%) declined HIV testing; among those that were tested 25% (95% CI: 22-27%) were HIV-infected and of

these 20% (95% CI: 14-23%) were receiving HAART. There was no significant difference in HIV prevalence between the two surveys (p=0.23), although significantly more HIV-infected participants were receiving HAART in 2008 compared to 2005.

Table 6.1: Demographic, TB risk factors and clinical characteristics of two survey samples

	2005 Survey	2008 Survey	p-value
Community size	±10,500	±15,000	
Study sample	762 (15% refusal)	1,250 (9% refusal)	
Demographics			
Median age, yr (IQR)	27 (22-35)	27 (22-33)	0.23
Gender: male (%)	340 (45)	648 (52)	0.002
Median school grade completed (IQR)	10 (8-11)	10 (8-11)	0.001
Presently employed (%)	398 (52)	662 (53)	0.75
Median residence, yr (IQR)	5 (3-7)	5 (2-10)	0.52
Median residents in household (IQR)	3 (2-5)	3 (2-4)	0.01
Median persons sleeping in same Room (IQR)	2 (2-3)	2 (1-3)	0.09
Risk factors n(%)			
Ever had TB in past	58 (8)	101 (8)	0.71
Alcohol intake in past 6 mo	324 (43)	408 (33)	<0.001
Visited shebeen (bar) in past 6 mo	180 (24)	261 (21)	0.15
Smoked in past 6 mo	205 (27)	289 (23)	0.06
Recreational drugs in past 6 mo	29 (4)	54 (4)	0.57
Employment History			
Past mining	43 (6)	44 (4)	0.02
Health Care Worker	21 (3)	21 (2)	0.10
Prison	11 (1)	15 (1)	0.64
HIV-infected* [95% CI]	174 (23) [20-26%]	306 (25) [22-27%]	0.23
On HAART (% of HIV-infected) [95% CI]	9 (5) [2-10%]	60 (20) [15-25%]	<0.001

IQR = Interquartile range; CI = Confidence Interval
All values are n(%) unless otherwise specified.
* In 2005, 4 participants declined HIV testing, and in 2008, 43 people declined HIV testing.

6.4.3 Comparison of TB Prevalence in 2005 and 2008

Total Study Population TB prevalence

In 2008, 12 participants (1%) reported receiving TB treatment at the time of study participation, and a further 8 (0.6%) untreated cases were identified. The prevalence results of the 2005 and 2008 surveys, overall and by HIV status are reported in Tables 6.2A and 6.2B. The overall TB prevalence (treated and untreated cases) declined significantly from 3% in 2005[179] to 1.6% in 2008 (p=0.04). When adjusted for age, gender, HIV status, as well as demographic and risk profile characteristics that differed between the two surveys, this reduction in prevalence bordered on significant (p=0.05), as shown in Table 6.3. The reduction in prevalent treated TB (from 1.5 to 1.0%) was not significant (adjusted p=0.34); however the reduction in prevalent untreated TB from 1.6% to 0.6% was significant, and remained significant following adjustment as above (crude and adjusted p=0.05) (Table 6.3 and Figure 6.1).

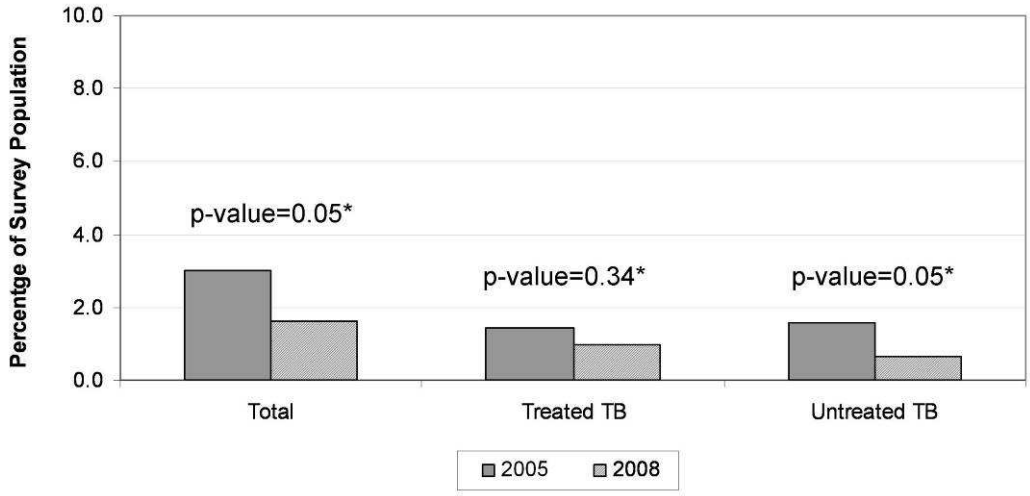


Figure 6.1: Prevalence of treated and untreated TB in the total study sample

*****adjusted for age, gender, HIV status, education level, mean residents in household, reported alcohol use, smoking and history of working in a mine

In 2005, 50% (n=6) of the untreated TB cases had smear-positive PTB[179], while in 2008, 13% (n=1) of the untreated cases had smear-positive PTB (p=0.09).

In 2008, untreated TB was not associated with reported symptoms, including cough (p=0.20), night sweats (p=0.35), loss of appetite (p=0.32) or loss of weight (p=0.22). This was in keeping with the 2005 survey findings[179].

HIV-uninfected participants

The total TB prevalence in HIV-uninfected participants remained unchanged between surveys (adjusted p=0.90), as did the prevalence of both treated TB and untreated TB in this group (adjusted p=0.90 and p=0.69 respectively) (Figure 6.2).

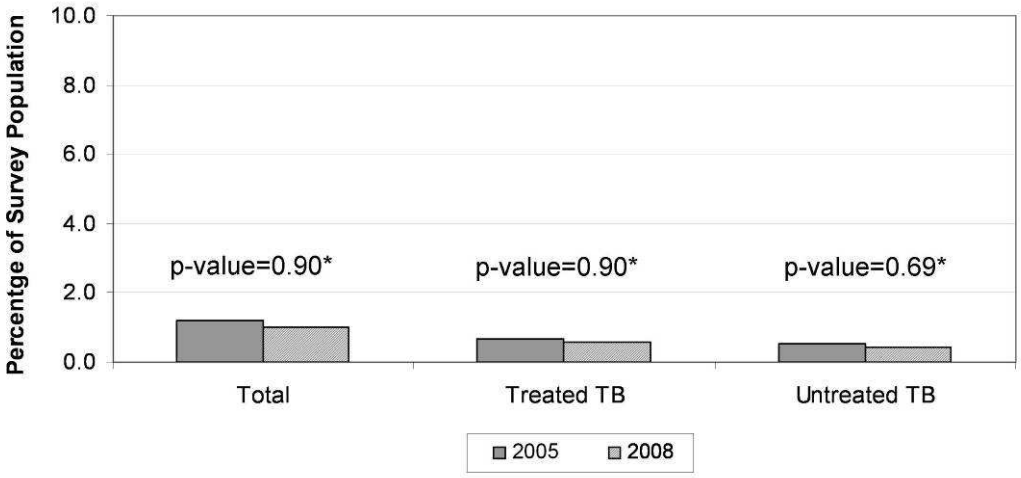


Figure 6.2: Prevalence of treated and untreated TB among HIV-uninfected participants

*****adjusted for age, gender, education level, mean residents in household, reported alcohol use, smoking and history of working in a mine

HIV-infected participants

The total TB prevalence dropped significantly in HIV-infected participants from 9.2% to 3.6% (adjusted p=0.01). While the decrease in treated TB prevalence was not significant in this group (4.0% in 2005 vs 2.3% in 2008; p=0.22 after adjustment for age, gender, HAART status, and demographic and risk profile characteristics as above), the prevalence of untreated TB cases declined significantly from 5.2% in 2005 to 1.3% in 2008 (adjusted p=0.02) (Figure 6.3). The multivariate logistic models for total TB prevalence and untreated TB prevalence in HIV-infected participants are reported in Table 6.4.

HAART

The distribution of treated and untreated TB cases by HAART status in HIV–infected patients is shown in Table 6.2A (2005) and Table 6.2B (2008). The proportion of both overall and untreated TB cases on HAART decreased significantly from 2005 to 2008 (p=0.001 and p<0.001 respectively). The median CD4 count for the total HAART cohort

in this community was 269 (interquartile range [IQR]: 177-350) in 2005, and 350 (IQR: 240-504) in 2008 (p<0.001).

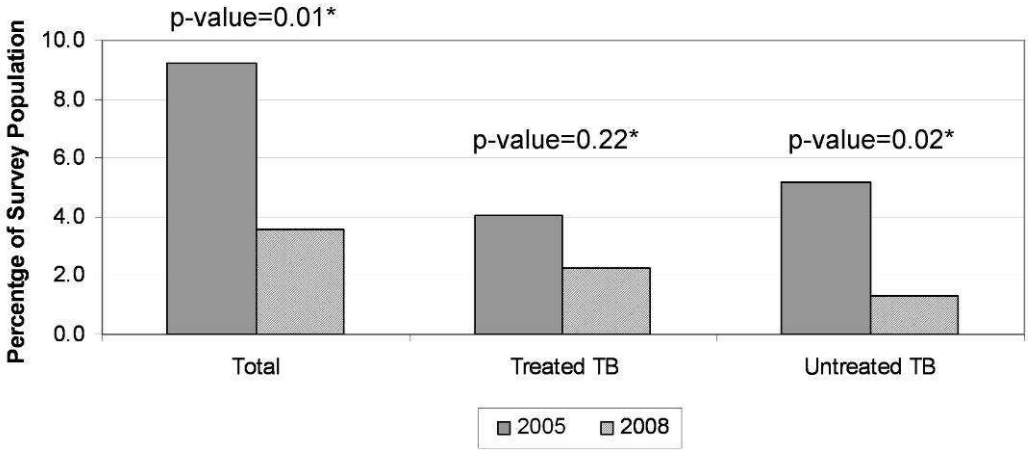


Figure 6.3: Prevalence of treated and untreated TB among HIV-infected participants

* adjusted for age, gender, education level, mean residents in household, reported alcohol use, smoking and history of working in a mine

Case Finding Proportions

As shown in Table 6.2A and 6.2B the case finding proportion by the TB clinic in this community increased from 48% in 2005 to 60% in 2008. Case-finding proportions did not change significantly in HIV-uninfected participants (57% vs 56%). However, in the HIV-infected population case-finding increased substantially from 44% to 64%. Case-finding increased for patients on HAART, from 50% in 2005, to 100% in 2008.

Table 6.2A: 2005 TB prevalence survey results, overall and by HIV and HAART status

2005 Tuberculosis Survey					
	Number at risk	Total prevalence % (n)	Treated prevalence % (n)	Untreated prevalence % (n)	Case finding proportion
Total TB cases	762	3.0 (23)	1.5 (11)	1.6 (12)	48%
HIV-uninfected cases	584	1.2 (7)	0.7 (4)	0.5 (3)	57%
HIV-infected cases	174	9.2 (16)	4.0 (7)	5.2 (9)	44%
HIV-infected not on HAART	165	7.3 (12)	3.0 (5)	4.2 (7)	42%
HIV-infected on HAART	9	44.4 (4)	22.2 (2)	22.2 (2)	50%

Table 6.2B: 2008 TB prevalence survey results, overall and by HIV and HAART status

2008 Tuberculosis Survey					
	Number at risk	Total prevalence % (n)	Treated prevalence % (n)	Untreated prevalence % (n)	Case finding proportion
Total TB cases	1250	1.6 (20)	1.0 (12)	0.6 (8)	60%
HIV-uninfected cases	901	1.0 (9)	0.6 (5)	0.4 (4)	56%
HIV-infected cases	306	3.6 (11)	2.3 (7)	1.3 (4)	64%
HIV-infected not on HAART	246	2.8 (7)	1.2 (3)	1.6 (4)	43%
HIV-infected on HAART	60	6.7 (4)	6.7 (4)	0.0 (0)	100%

6.4.4 Comparison of TB Risk Factors in 2005 and 2008

Risk factors of overall prevalent TB in 2005 and 2008 are reported in Appendix C. In summary, in 2005, in both univariate and multivariate analysis, the significant risk factors associated with prevalent TB were being in prison in the last 6 months (odds ratio [OR]: 21.5; 95% CI: 4.4-105.4; p<0.001), being HIV-infected (OR: 8.8; 95% CI: 3.1-24.8; p<0.001) and receiving HAART (OR: 111.2; 95% CI: 20.8-594.5; p<0.001).
In 2008, following adjustment for gender, age and relevant risk factors, only HIV-infected status (OR: 2.8; 95% CI: 0.9-7.7; p=0.05) and receiving HAART (OR: 7.9; 95% CI:2.3-27.8; p=0.001) remained significant risk factors for any TB, although a history of employment in the mines bordered on significant risk (OR: 4.4; 95% CI: 0.9-21.3; p=0.07). A history of imprisonment in the past 6 months was not significantly associated with risk of TB prevalence.

In 2005, the significant risk factors for treated prevalent TB in univariate analysis were previous history of imprisonment in the past 6 months (OR: 20.3; 95% CI: 3.8-109.3; p<0.001), HIV-infected status (OR: 3.2 compared to HIV-uninfected; 95% CI: 0.9-10.5; p=0.06) and HIV-infected persons receiving HAART (OR: 32.4 compared to HIV-uninfected; 95% CI: 5.5-189.8; p<0.001). These risk factors persisted in a multivariate model adjusting for age and gender (Table 6.5).

Table 6.3: Multivariate logistic model for TB prevalence (overall, treated and untreated) in the total study population

	Total TB prevalence		Treated TB prevalence		Untreated TB prevalence	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Survey year						
2005	1		1		1	
2008	0.53 (0.28 – 0.97)	0.05	0.66 (0.28 – 1.56)	0.34	0.39 (0.15 – 0.96)	0.05
Age (in years)	1.01 (0.97 – 1.05)	0.61	1.03 (0.97 – 1.07)	0.19	0.98 (0.91 – 1.04)	0.48
Gender						
Male	1		1		1	
Female	0.88 (0.40 – 1.96)	0.75	0.71 (0.25 – 2.09)	0.52	1.08 (0.31 – 3.84)	0.90
Education (years in school)	0.99 (0.89 – 1.11)	0.92	0.90 (0.79 – 1.02)	0.11	1.26 (0.99 – 1.60)	0.06
Number of residents in household	1.08 (0.94 – 1.25)	0.29	1.17 (0.98 – 1.40)	0.09	1.00 (0.78 – 1.27)	0.98
Alcohol intake in past 6 months	0.96 (0.46 – 1.97)	0.90	0.84 (0.31 – 2.31)	0.74	1.12 (0.40 – 3.12)	0.83
Smoked in past 6 months	2.14 (0.94 – 4.90)	0.07	2.27 (0.74 – 6.90)	0.15	1.77 (0.49 – 6.40)	0.38
Past employment in mines	1.97 (0.62 – 6.30)	0.25	0.34 (0.04 – 3.03)	0.34	12.19 (2.48 – 59.92)	0.002
HIV status						
HIV-uninfected	1		1		1	
HIV-infected	6.42 (3.34 – 12.32)	<0.001	6.14 (2.55 – 14.80)	<0.001	6.48 (2.43 -17.25)	<0.001

- p-values compare total TB, treated TB and untreated TB to participants with no TB, adjusted for the variables presented in the table
- OR = odds ratio

Table 6.4: Multivariate logistic model for TB prevalence (overall and untreated) in the HIV-infected study population

	Total TB prevalence		Untreated TB prevalence	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
Survey year				
2005	1		1	
2008	0.35 (0.15 – 0.80)	0.01	0.20 (0.07 – 0.74)	0.02
Age (in years)	1.03 (0.98 – 1.09)	0.22	1.04 (0.96 – 1.14)	0.29
Gender				
Male	1		1	
Female	0.93 (0.31 – 2.83)	0.90	1.40 (0.23 – 8.45)	0.72
Education (years in school)	1.00 (0.87 – 1.16)	0.95	1.24 (0.92 – 1.67)	0.15
Number of residents in household	0.98(0.79 – 1.23)	0.86	0.80 (0.55 – 1.17)	0.25
Alcohol intake in past 6 months	1.01 (0.41 – 2.50)	0.99	1.80 (0.50 – 6.43)	0.37
Smoked in past 6 months	1.26 (0.40 – 3.98)	0.70	1.57 (0.28 – 8.97)	0.61
Past employment in mines	2.81 (0.67 – 11.76)	0.16	7.44 (1.12 – 49.58)	0.04

- p-values compare total TB and untreated TB to HIV-infected participants with no TB, adjusted for the variables presented in the table
- OR = odds ratio

Participants with untreated prevalent TB in 2005 had similar risk factors as treated TB cases with a previous history of imprisonment in the past 6 months (OR: 13.5; 95% CI: 1.3-143.9; p=0.03), HIV-infected status (OR: 10.8 compared to HIV-uninfected; 95% CI: 2.4-47.8; p=0.002) and HIV-infected persons receiving HAART (OR: 133.6 compared to HIV-uninfected; 95% CI: 12.4-1438.9; p<0.001) persisting as significant risk factors for untreated TB after adjustment for age and gender (Table 6.6).

In 2008, the only significant risk factor for treated prevalent TB in univariate analysis was a history of receiving HAART (OR: 10.5 compared to HIV-uninfected participants; 95% CI: 3.1-35.9; p<0.001), and this persisted after adjusting for age and gender (Table 6.7).

In univariate analysis untreated prevalent TB in 2008 was associated with a history of employment in the mines (OR: 17.4; 95% CI: 4.0-75.2; p<0.001), and imprisonment in the past 6 months (OR: 12.4; 95% CI: 1.4-107.7; p=0.02) which carried twice the risk of those who had ever been in prison (excluding the last 6 months) (OR: 6.5; 95% CI: 1.5-27.6; p=0.01). HIV-infected participants off HAART bordered on statistical significance as a risk factor for untreated TB. In multivariate analysis, only a history of mining employment remained a significant risk factor (OR: 28.2; 95% CI: 2.6-308.2; p=0.01), although ever having been imprisoned bordered on significance (OR: 5.4; 95% CI: 0.9-33.8; p=0.07). HIV-infected status was not significantly associated with risk of untreated TB, and receiving HAART remained perfectly predictive of not having untreated TB (Table 6.8).

In these models, no prevalent TB was considered “failure”, and therefore variables that perfectly predicted failure had no prevalent TB identified in those groups.

Table 6.5: Risk factors for treated tuberculosis in 2005

Risk factor	Univariate analysis		Multivariate analysis	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age	1.03 (0.99-1.07)	0.17	1.04 (0.98-1.10)	0.19
Gender: Male	1		1	
Female	0.67 (0.20-2021)	0.51	0.50 (0.13-2.00)	0.33
Median residents in household	1.05 (0.81-1.37)	0.71		
Median persons sleeping in same room	0.97 (0.57-1.66)	0.92		
Ever had TB in the past	Perfectly predicts failure			
Alcohol intake in past 6 months	1.63 (0.49-5.40)	0.42		
Visited shebeen (bar) in past 6 months	1.40 (0.30-6.52)	0.67		
Smoked in past 6 months	2.31 (0.70-7.65)	0.17		
Recreational drugs in past 6 months	0.39 (0.05-3.18)	0.38		
Employment History				
Past mining	1.70 (0.21-13.62)	0.62		
Health Care Worker	0.28 (0.03-2.28)	0.23		
Prison in past 6 months	20.31 (3.77-109.29)	<0.001	27.14 (4.15-177.54)	0.001
HIV-uninfected	1		1	
HIV-infected	3.17 (0.95-10.53)	0.06	7.04 (1.65-30.05)	0.01
HIV-infected on HAART	32.44 (5.54-189.84)	<0.001	93.53 (11.18-782.30)	<0.001

- p-values compare treated TB to participants with no TB in 2005
- OR = odds ratio

Table 6.6: Risk factors for untreated tuberculosis in 2005

Risk factor	Univariate analysis		Multivariate analysis	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age	0.98 (0.92-1.04)	0.52	0.96 (0.88-1.05)	0.41
Gender: Male	1		1	
Female	1.12 (0.35-3.57)	0.84	0.59 (0.16-2.27)	0.45
Median residents in household	0.70 (0.48-1.03)	0.07	0.77 (0.52-1.15)	0.20
Median persons sleeping in same room	0.89 (0.51-1.54)	0.68		
Ever had TB in the past	Perfectly predicts failure			
Alcohol intake in past 6 months	0.97 (0.31-3.09)	0.96		
Visited shebeen (bar) in past 6 months	0.93 (0.25-3.48)	0.92		
Smoked in past 6 months	1.39 (0.41-4.65)	0.60		
Recreational drugs in past 6 months	Perfectly predicts failure			
Employment History				
Past mining	1.55 (0.20-12.28)	0.68		
Health Care Worker	Perfectly predicts failure			
Prison in past 6 months	8.31 (0.96-72.20)	0.06	13.49 (1.26-143.99)	0.03
HIV-uninfected	1		1	
HIV-infected	5.33 (1.67-17.01)	0.01	10.76 (2.42-47.84)	0.002
HIV-infected on HAART	29.2 (5.05-168.80)	<0.001	133.60 (12.40-1438.98)	<0.001

- p-values compare untreated TB to participants with no TB in 2005
- OR = odds ratio

Table 6.7: Risk factors for treated tuberculosis in 2008

Risk factor	Univariate analysis		Multivariate analysis	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age	1.04 (0.99-0.108)	0.11	1.03 (0.98 – 1.09)	0.24
Gender: Male	1		1	
Female	0.76 (0.24-2.42)	0.65	0.60 (0.18-1.97)	0.40
Median residents in household	1.15 (0.91-1.45)	0.26		
Median persons sleeping in same room	0.76 (0.42-1.37)	0.36		
Ever had TB in the past	Perfectly predicts failure			
Alcohol intake in past 6 months	1.04 (0.31-3.47)	0.95		
Visited shebeen (bar) in past 6 months	2.89 (0.37-22.52)	0.31		
Smoked in past 6 months	2.41 (0.76-7.66)	0.14		
Recreational drugs in past 6 months	Perfectly predicts failure			
Employment History				
Past mining	Perfectly predicts failure			
Health Care Worker	Perfectly predicts failure			
Prison in past 6 months	Perfectly predicts failure			
Ever spent time in prison (excl last 6 months)	0.98 (0.13-7.70)	0.99		
HIV-uninfected	1		1	
HIV-infected not on HAART	1.30 (0.35-4.84)	0.69	2.3 (0.55-10.15)	0.25
HIV-infected on HAART	10.49 (3.06-35.92)	<0.001	13.13 (3.34-51.56)	<0.001

- p-values compare treated TB to participants with no TB in 2008
- OR = odds ratio

Table 6.8: Risk factors for untreated tuberculosis in 2008

Risk factor	Univariate analysis		Multivariate analysis	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age	1.02 (0.96-1.09)	0.50	0.96 (0.87-1.06)	0.41
Gender: Male	1		1	
Female	0.64 (0.15-2.70)	0.55	3.30 (0.42-26.13)	0.26
Median residents in household	1.16 (0.88-1.55)	0.29		
Median persons sleeping in same room	0.82 (0.41-1.65)	0.59		
Ever had TB in the past	Perfectly predicts failure			
Alcohol intake in past 6 months	2.01 (0.52-8.34)	0.30		
Visited shebeen (bar) in past 6 months	0.26 (0.07-1.06)	0.06	0.37 (0.07-2.06)	0.25
Smoked in past 6 months	2.03 (0.48-8.53)	0.34		
Recreational drugs in past 6 months	Predicts failure perfectly			
Employment History				
Past mining	17.39 (4.02-75.22)	<0.001	28.24 (2.59-308.21)	0.01
Health Care Worker	Predicts failure perfectly			
Prison in past 6 months	12.41 (1.43-107.65)	0.02	10.12 (0.57-179.61)	0.12
Ever spent time in prison (excl last 6 months)	6.50 (1.53-27.56)	0.01	5.40 (0.86-33.75)	0.07
HIV-uninfected	1		1	
HIV-infected not on HAART	3.90 (0.97-15.73)	0.06	2.48 (0.54-11.43)	0.24
HIV-infected on HAART	Predicts failure perfectly		Predicts failure perfectly	

- p-values compare untreated TB to participants with no TB in 2008
- OR = odds ratio

6.5 DISCUSSION

The key finding of this study was that the prevalence of microbiologically-confirmed adult PTB declined significantly between 2005 and 2008, and this decline was due to a nearly three-fold decrease in TB prevalence in the HIV-infected population (from 9.2% to 3.6%). Among HIV-infected participants, the decrease in TB prevalence was predominantly due to a four-fold decline in untreated TB. This decrease was temporally associated with the scale-up of a HAART programme and the greatest reduction in prevalence was noted among HIV-infected patients on HAART. In this group TB prevalence dropped from 44% to 6.7%, and the largest decline was seen in untreated TB cases.

In keeping with the notification data for Site M, the prevalence of treated TB among HIV-infected patients decreased by nearly 50%, and this decrease was greatest among HIV-infected patients on HAART (although these reductions did not reach statistical significance). Similarly, this survey also reported a relatively steady state of TB among HIV-uninfected patients.

6.5.1 Understanding the Change in TB Prevalence

To explain these findings, we postulate that a wide-spread HAART programme can decrease prevalent TB in a community through two mechanisms: increased TB active case-finding, and immune recovery associated with HAART.

Increased Case-Finding in the HAART Program

When patients entered the HAART programme, they underwent active screening for TB, based on national and WHO policies[199;510]. Screening for TB was based on symptom review and, in patients with symptoms suggestive of TB sputum, staining for AFB and/or culture for *Mtb* growth was performed. The effective implementation of this policy is demonstrated by the increased proportion of case finding in the HIV-infected population, most notably among those on HAART, while the case finding proportion in HIV-uninfected remained unchanged. This has resulted in a significant decrease in the previously large burden of undiagnosed, untreated TB in HIV-infected patients reported in the first survey.

ACF may be removing patients from the infectious pool more quickly than would have occurred through the passive case-finding policy of the national TB control programme[195]. Furthermore, the substantial decrease in untreated smear-positive TB cases may suggest that active case-finding is removing the more infectious cases[55;56] from the community. As a result, it is possible that part of the decrease in TB prevalence may be due to a reduction in TB transmission. This hypothesis would also explain in part the decrease in notification rates observed in the community (Chapter 5). The effect of reduced transmission may be more evident among the HIV-infected patients due to the more rapid rate of progression to TB disease following recent infection in this group compared to HIV-uninfected individuals[102]. Subsequently, any benefit to the HIV-uninfected population accruing from decreased transmission may not be evident in the

short interval between these surveys. Indeed, the proportion of clustering of *Mtb* strains in this community has not changed over the past 4 years, suggesting persistent transmission. However, given the short post-HAART observation period, the decreasing notification rates and prevalence of TB overall and the variable incubation period for TB, there may be a time lag in observing the impact of decreased transmission. Increased follow-up time is needed to confirm or refute this hypothesis.

None-the-less, the reduction of TB prevalence noted in this study is in keeping with the results of mathematical models which have assessed the impact of intervention strategies such as active case-finding on population TB rates[189;511].

Risk of Disease: Immune Recovery on HAART

ACF would transfer untreated TB cases into the treated, notified group. However, rates of treated TB did not increase in this population; in contrast, treated TB in HIV-infected participants had declined between the two surveys. This finding may reflect changes in the immune status of the HIV-infected population related to high HAART coverage. It is well documented that HAART, and the subsequent CD4 count recovery, is associated with a substantial reduction in TB risk in HIV-infected patients[45;46;152;170]. The rapid scale-up of the HAART programme in this community (with 20% of HIV-infected population receiving HAART in 2008) resulted in a large treatment cohort, with an increasing mean CD4 count (as found in treatment cohorts in similar settings[35]) and, therefore, a decrease in risk of TB disease in HIV-infected patients. This is also reflected in the decreasing TB notification rates among HIV-infected patients, particularly those on HAART (Chapter 5). While the HAART cohort had a higher mean CD4 count in 2008 compared to 2005, the median CD4 count in 2008 is still relatively low. Therefore it is possible that the impact on reduction of TB prevalence may continue to increase with ongoing HAART initiation and accumulative immune recovery.

The revised HIV treatment guidelines in South Africa now allow for HAART initiation in TB patients with CD4 counts <350cell/μl[512]. As initiating HAART at higher CD4 counts has been shown to be associated with improved immune recovery[149;150], this policy may further enhance the benefits noted in this study.

6.5.2 Alternative Explanations for Study Findings

Over the study period there have been no significant changes in the TB control programme, other than the ACF in HAART patients over the study. Therefore, the decrease in TB prevalence in this community is unlikely to be due to changes in TB control policies. This is supported by the stable rates of treated and untreated TB in HIV-uninfected participants in this study. Similarly, TB-associated mortality rates have declined in this community (Chapter 5), particularly among HIV-infected patients, and as such an increase in mortality is not likely to be responsible for the decrease in TB prevalence. Emigration could result in a decreased prevalence of disease, but biennial censuses performed from 2002 to 2008, show that net immigration is greater than emigration in this growing community, with a 12% increase in population size between the two surveys (Appendix A). Furthermore, the same TB control programme laboratory services, with consistent specimen processing protocols, were used for the microscopy, culture and differentiation of sputum specimens in both surveys, and the same culture contamination rate was reported across both surveys.

6.5.3 Active Case-Finding in Different Populations

The recently published DETECTB study reported on the impact of population-based ACF strategies on TB prevalence[280]. As with our study, the DETECTB study was performed in a southern African community with high TB and HIV burdens and assessed the impact of ACF interventions by performing two community-based surveys on randomly selected samples of the adult residents, pre and post the implementation of the interventions. Similar to our study, participants, regardless of symptoms, provided sputum specimens for TB testing, including *Mycobacterium tuberculosis* culture, and also underwent HIV testing.

However, DETECTB assessed the impact of a different intervention strategy: employing regularly repeated ACF interventions in the general population. In addition, the researchers of the DETECTB study reported that there was little change in the coverage of the HAART programme in their study site over the study period, compared to the rapid scale-up of HAART coverage in Site M.

As with our study, the DETECTB study reported a substantial reduction in the population culture-positive TB prevalence over the study period. However, in contrast to our study

findings, the reduction in prevalence was predominantly due to a decrease in TB prevalence among HIV-uninfected participants, with a non-significant reduction among HIV-infected participants.

The difference in the study findings are most likely due to the targeting of different populations. The ACF interventions in DETECTB relied on symptom-based screening to identify TB suspects, and on sputum microscopy for TB diagnosis. Screening participants by the presence of a cough, as done in DETECTB, has a very low sensitivity for detecting TB among HIV-infected patients[513] and more recently a systematic review and meta-analysis of ACF studies has shown that testing HIV-infected patients for TB regardless of reported symptoms provides a significantly higher TB yield[507]. Similarly, sputum microscopy has been shown to have a low yield in HIV-infected patients[513], who have an increased prevalence of smear-negative culture-positive TB disease[18;118]. As a result, the DETECTB interventions predominantly targeted the detection of TB disease among the HIV-uninfected population, and consequently this is where the benefit was most noted. In contrast, our intervention was targeted at the HIV-infected population.

The TB epidemic in high TB and HIV settings comprises both HIV-associated TB and HIV-unassociated TB. These two studies show that community-based ACF strategies that utilize symptom-based screening will have a substantial impact on TB prevalence among the HIV-uninfected population, while the scale-up of HAART programmes, with associated ACF, have a marked impact on HIV-associated TB. These studies suggest that combining these two interventions, each targeting a different subset of the community population, may have an additive benefit for TB epidemics in high TB and HIV burdened settings.

6.5.4 Risk Factors Associated with Prevalent TB

In 2008, treated prevalent TB was associated with HIV-infected status, and in particular it was strongly associated with HIV-infected participants receiving HAART. In contrast HIV-infected patients on HAART did not have an increased risk of untreated TB. These findings most likely reflect the ACF among HIV-infected patients starting HAART.

In direct contrast to the findings in 2005, in 2008 HIV-infected patients not on HAART were not at significantly increased risk for untreated TB. This may reflect an increased

community awareness of TB in this population, due to increased screening among HIV-infected patients initiating HAART, as well as the community surveys. With the implementation of a high coverage HAART programme, the greatest risk for untreated TB was associated with more “traditional” risk factors such as past employment in the mines, or time spent in overcrowded prison conditions.

6.5.5 Strengths and Weaknesses

This is the first repeated cross-sectional TB prevalence survey following the large-scale availability of HAART in a sub-Saharan Africa community. The study was performed among randomly selected individuals in a well-defined community, with HIV and HAART data linked to TB results.

The sample of residents randomly selected to participate in both studies, as well as those residents that enrolled in the studies, did not differ demographically from the total study populations in each survey year. The 2008 survey participants did have a lower TB risk profile compared to the participants in the 2005 survey, including less crowding in the household, as well as lower rates of previous mining employment and alcohol use. However, following adjustment for these variables, the decrease in both overall TB prevalence as well as untreated TB prevalence in HIV-infected participants between the two surveys remained statistically significant.

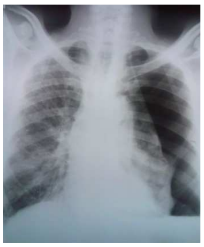
HIV testing was accepted by 98% of the participants (averaged across both surveys) and HIV status was available for all TB cases, both treated and untreated.

This relatively small community is typical of many recently urbanised populations in South Africa, but there is a need for further investigation to confirm our findings in other high HIV and TB prevalence settings. A third survey to confirm the downward trend in TB prevalence would also strengthen these findings.

6.5.6 Conclusion

Undiagnosed TB prevalence is an important driver of TB transmission[49;60;65;114]. This study showed a significant decline in adult TB prevalence, in particular undiagnosed TB prevalence in HIV-infected patients, associated with the introduction of a rapid and high coverage HAART programme in the community. These findings are in keeping with the

decrease in TB notifications reported in Chapter 5, and may be due to the increased case-finding in the HAART programme, and as well as the impact of HAART-associated immune recovery on TB risk. These findings suggest that high coverage HAART programmes may contribute to TB control in high TB and HIV prevalence settings. It is possible that HAART programmes combined with symptom-based ACF interventions among the general population may have an even greater impact on TB prevalence in high TB and HIV burdened settings.



Chapter 7
DISCUSSION

7.1 OVERVIEW

This thesis has described the epidemiology of tuberculosis (TB) in a high HIV and TB prevalent community, with a focus on the effect of HIV and a highly active antiretroviral therapy (HAART) programme on the TB epidemic in this setting.

Because of the extremely variable incubation period for TB (i.e. the period from infection to disease), it is helpful to consider TB pathogenesis, and associated risk factors, in two phases: the acquisition of infection (i.e. transmission) and the development of disease[71]. Below I discuss the impact of an HIV epidemic and a HAART programme on firstly, the transmission of TB and secondly, the burden of TB disease in the study community.

7.2 TB TRANSMISSION

This work has reported a substantial rate of ongoing TB transmission in this community, as shown by the high risk of acquiring TB infection in children, ranging from 4-7% per year. These high rates of transmission resulted in substantial prevalence of TB infection among children and adolescents. This prevalence increased with age, reaching over 50% among adolescents, and consequently, the majority of adults in this community have been infected with TB.

7.2.1 Impact of HIV on TB transmission

Tests for assessing TB infection are based on measuring, directly or indirectly, an immune response to TB antigens, and it is therefore difficult to assess the impact of HIV on the risk of TB infection, and transmission. However, this thesis provided some important insights into this complex relationship.

Not withstanding the caveats of immune-based testing, data from the secondary school tuberculin skin test survey showed that HIV-infected patients were not at an increased risk of acquiring TB infection. However, the role of HIV infection in the transmission of TB is more complicated. Analysis of molecular epidemiologically linked clusters has shown that the HIV-associated and HIV-unassociated TB epidemics are inter-related in

this community, with most clusters comprising of both HIV-infected and HIV-uninfected patients.

The contribution of HIV to TB transmission is postulated to depend on the prevalence of HIV-infected sputum smear-positive disease. Data presented here showed that while HIV-infected patients had lower odds of smear-positive pulmonary TB (PTB) disease compared to HIV-uninfected patients, the rates of smear-positive disease in this group were still higher than those in HIV-uninfected patients, and HIV-infected patients were responsible for at least 50% of the total burden of smear-positive PTB disease.

While HIV-infected and -uninfected adults contribute equally to the smear-positive PTB, molecular epidemiological data reported that HIV-infected patients were significantly less likely to be an index case for clusters in this community (while HIV-infected patients accounted for 61% of all sputum positive PTB cases, only 40% of index cases were HIV-infected), and the association between HIV status and index cases persisted even after adjusting for sputum smear-positive status. Therefore, it appears that HIV-infected sputum-positive cases may be less infectious than their HIV-uninfected counterparts, and this is in keeping with other reports[124;176;177]. A shorter infectious period in the community, due to rapid progression of TB disease and increased mortality[124;178] among HIV-infected patients may also explain the decreased contribution of HIV-infected patients to ongoing transmission.

Other evidence to support the argument that HIV-infected adults contribute proportionally less to transmission include the observation that TB notification rates among HIV-uninfected adults have remained stable over the time of escalating HIV prevalence and HIV-associated TB epidemics, and only declined modestly following the implementation of the HAART programme. This finding also suggests that while the HIV-associated and HIV-unassociated TB epidemics are inter-related, the HIV-associated epidemic has not had a substantial impact on transmission to the HIV-uninfected population. Even considering the longer incubation period of TB in HIV-uninfected patients, data spanning 12 years has not shown an increase in the HIV-uninfected TB epidemic.

The risk of transmission to children may be different to that of adults. While children were at increased risk of acquiring TB infection and disease from adult TB source cases on their residential plot, the HIV status of the adult source case was not a risk factor for this transmission. It is probable that the extended contact period between the sputum-positive adult case and the susceptible child may have overcome the less infectious nature of HIV-infected adult cases.

In conclusion, the HIV-associated TB epidemic has undoubtedly contributed to the transmission in this community by increasing the burden of sputum smear-positive TB cases. However, it appears that the HIV-associated epidemic contributed proportionally less to transmission, compared to HIV-uninfected patients.

7.2.2 Impact of a HAART Programme on TB transmission

Given the conclusion above, it is possible that a HAART programme would not have a dramatic impact on TB transmission in this setting. Indeed, the stable rates of TB disease in children despite the scale-up of the HAART programme would support this hypothesis, as does the consistent proportions of clustered TB cases noted in the molecular epidemiological data.

Of note was that the scale-up of the HAART programme was associated with a significant decline in adult PTB prevalence, including sputum smear-positive PTB, as well as an increase in TB case-finding proportions in the community. One would have anticipated that these findings would be associated with a decrease in TB transmission. The apparent lack of impact on transmission may be due to the fact that both the reduction in TB prevalence and the increase in case-finding were predominantly noted in the HIV-infected population and there was no difference in either measure among the HIV-uninfected population. The relatively less infectious nature of HIV-infected PTB may explain the modest impact noted.

However, there was some suggestion of a mild impact of the HAART programme on transmission, as seen in the modest decline noted in HIV-uninfected TB notification rates despite constant case-finding proportions. The effect of reduced transmission may be noted first among the HIV-infected patients due to the more rapid rate of progression to TB disease following recent infection in this group[102], and a significant reduction in TB

notifications among HIV-infected patients not receiving HAART was observed. The incubation period of TB is longer in HIV-uninfected patients, and as such, there may be a time lag to observing the impact of this intervention on transmission more generally. Therefore, the full effect of the reduced incident and prevalent disease among HIV-infected patients on transmission may not yet be evident and the four years of follow-up post-HAART implementation may be insufficient time to detect the overall impact on the reduction in TB transmission. The modest decline in sputum smear-positive PTB notification rates noted among both HIV-infected and HIV-uninfected patients suggest that longer term review of the potential impact of the intervention may be important.

7.2.3 Reducing TB Transmission

While HAART was associated with improved immune function in the HIV-infected cohort and the HAART programme was associated with increased TB case-finding, we have not reported a substantial impact on TB transmission.

Molecular epidemiological data reported that recent transmission was responsible for at least 55% of the TB disease burden in this community, and there was further evidence to suggest that this may be a conservative estimate. Therefore, specific steps to decrease TB transmission may be critical to control TB in this setting. Reducing TB transmission may be comprised of two components: firstly reducing the prevalence and infectious period of TB cases, and secondly, inplementing social and environmental changes that would decrease the effective contact number of infectious cases. Both these approaches require an understanding of the transmission dynamics in the community.

This thesis has reported that children have an extremely high annual exposure to adult TB disease within households and close geographic social networks, and child TB infection and disease rates were strongly correlated with this residential exposure to adult TB. Therefore, strategies for reducing the prevalence and infectious period of TB cases could include targeted active case-finding (ACF). Current ACF activities are restricted to patients initiating HAART and the screening of children in close contact with adult PTB cases[334]. However, the screening and management of child contacts is poorly implemented[319;335;336]. The data presented here suggest that improving the implementation of and expanding current contact tracing policies could assist in the earlier identification of infectious cases. One strategy could entail expanding ACF among

TB contacts to contacts of all ages, regardless of HIV status, and extending case-finding beyond the household to the close social network of the TB case. In this community, that would entail extending ACF to the patient's residential plot.

In contrast, the molecular epidemiological data indicated that adult to adult transmission did not occur primarily on residential plots. Of importance, it was noted that transmission did not appear to be occurring at the local HAART clinic. If high transmission locations for adults were identified, targeted ACF could be implemented in these locations. Further, environmental interventions, including improved ventilation and possible installment of ultra-violet lights, might impact transmission in these locations.

7.3 BURDEN OF TB DISEASE

The extra-ordinarily high transmission rates in this community are reflected in the substantial rates of TB disease in the community. TB notification rates reached over 2,000/100,000 in Site M, and TB prevalence was as high as 3% among adult residents in 2005.

While the HIV epidemic may not be primarily responsible for transmission, it was certainly contributing significantly to the burden of TB disease in this community. Consequently, it was in TB disease burden that the greatest benefit of a HAART programme was noted.

7.3.1 Impact of HIV on Burden of Disease

HIV-infected patients have a considerably increased risk of progression to TB disease, both from reactivation of latent infection and following recent infection[14;102]. Therefore, the substantial HIV prevalence among adults in Site M[179;197] together with the high prevalence and force of TB infection, account for the overwhelming burden of TB disease in this setting.

The HIV epidemic is driving the TB burden of disease, and this was reflected in the observation that the 25% of the community population infected with HIV were responsible for approximately 70% of the TB disease burden. As a result, overall community TB notification rates escalated from 1997, even while notification rates among HIV-uninfected patients remained relatively stable.

7.3.2 Impact of a HAART Programme on Burden of Disease

In terms of benefit at an individual level, the HAART programme was associated with a substantial reduction in TB mortality among HIV-infected patients. At the community level, the scale-up of the HAART programme was associated with a substantial decrease in TB disease burden. The trend of increasing TB notification rates was reversed, with a 20% reduction in adult TB incidence, as well as a 50% reduction in adult TB prevalence, from 2005 to 2008. In both incidence and prevalence, the decrease in TB rates was due predominantly to a decline among HIV-infected patients, particularly among patients receiving HAART.

While the greatest reduction in TB notification rates was noted among patients on HAART, a decrease in notification rates was also noted in HIV-infected patients not receiving HAART, as well as modest decline noted in HIV-uninfected patients. Similarly, while the greatest reduction in the prevalence of TB disease was noted among HIV-infected patients receiving HAART, a decline was also noted among HIV-infected patients not receiving HAART (although the latter was not statistically significant). However, prevalence among HIV-uninfected residents remained unchanged.

The reduction in TB notification rates and TB prevalence (especially previously unrecognized TB prevalence) among patients on HAART was most likely due to the combination of screening for TB disease among patients initiating HAART and the immune system recovery associated with antiretroviral therapy[45;46;152;170]. The risk of TB disease increases as the CD4 cell count decreases[45;75;113] and the reduction in TB rates in patients not receiving HAART was probably due to the removal of those with the highest TB risk (lowest CD4 counts) from this group into the group on HAART. There was an increasing overall average CD4 count in the HAART cohort, and it is possible that the ongoing identification and treatment of those patients with lowest CD4 counts may have resulted in an improvement in the average CD4 count in the overall HIV-infected population in this community, thus impacting TB rates among HIV-infected patients generally.

While there was a strong temporal association between the decline in both TB notification and prevalence rates and the implementation of a high coverage HAART programme, and there is substantive biological plausibility to support this association, I

have also discussed alternative explanations for the study findings. Changes in the TB control programme, in TB treatment outcomes as well as changes in the population structure were excluded as possible causes of the declining TB rates.

In summary, although the greatest benefit to TB burden was noted among HIV-infected patients receiving HAART, the net result was a decreased overall burden of TB disease, both incidence and prevalence, in the community. These studies, therefore, showed that a high coverage HAART programme did have an impact on TB burden at a community level.

7.4 Overall Impact of HAART Programme on TB Control

This thesis has shown that HAART can have a positive impact on a community TB epidemic. However, the extent and sustainability of that impact will depend on:

1) The impact of HAART on TB transmission in the community. The impact of HAART on TB transmission is dependent on the degree to which the HIV-associated epidemic is contributing to transmission in the community. In this study community, the contribution of HIV-infected TB patients to transmission was proportionally less than that of the HIV-uninfected patients. Consequently, the impact of HAART on transmission appeared to be relatively minor. However, longer term follow-up and analysis of TB rates in this community are required to assess this relationship more fully.

2) The point in the HIV disease process at which HAART is initiated. A substantial proportion of patients initiating HAART in South Africa have already had at least one episode of TB[35;174], and patients initiating HAART at low baseline CD4 counts have a high risk of TB in the first months of treatment[76;152;166]. A nearly 2-fold higher TB rate ratio in HIV-infected patients off HAART compared to those patients on HAART was reported by the end of this study period, and patients off HAART accounted for 64% of the total burden of TB disease in 2008. Starting patients on HAART earlier in the HIV disease process may prevent much of this pre- and early HAART disease. This strategy may also result in improved immune recovery[150], which may have long term benefits with regards to risk of TB disease.

3) The rate of HAART programme implementation and the coverage obtained among the HIV-infected population. In this study community, 22% of the HIV-infected population was receiving HAART and this high coverage was achieved over a short time period. Given that HIV-infected patients are responsible for 70% of TB disease and that patients receiving HAART have a 2-fold lower rate of disease, it is probably that the greater the proportion of HIV-infected patients receiving HAART, the greater the overall reduction in TB rates. The decline of notification rates among HIV-infected patients not receiving HAART indicates that the rate at which a HAART programme is implemented in a community might be an important variable determining the impact of this intervention on overall HIV-associated TB rates. However, it should be noted that as the coverage of a HAART programme increases, treatment adherence and the monitoring and management of patients with drug interactions and side effects become more challenging[514] and this may in turn adversely impact the sustainability of the benefits of a HAART programme.

7.5 FUTURE RESEARCH

A number of future research questions have been highlighted in this work. Firstly, it is clear that the issue of transmission has been neglected recently in TB research. While this thesis has provided important insights into transmission dynamics in high HV and TB prevalent settings, there are still unanswered questions that are critical for informing the development of effective intervention strategies. One such question involves the identification of key locations for adult TB transmission, and further molecular epidemiological studies could provide these data. In addition, a tuberculin skin test survey among young children (<5 years of age), would provide insights into possible changes in transmission rates over the period of HAART availability.

Secondly, these data have suggested that targeted ACF may assist in the earlier identification and treatment of TB cases. Studies are needed to assess the effectiveness of targeted ACF among TB contacts as an adjunctive intervention. Based on our findings, we would recommend using an expanded definition of contacts to include people of all ages and HIV status, as well as extending contact tracing beyond the household to include the TB patient's close social network.

Thirdly, the variability of the TB incubation period may mean that any indirect benefit of a HAART programme among HIV-uninfected patients may not have been observed over the time period of this study. In addition, due to the fact that patients on HAART remain at a higher risk for TB disease compared to HIV-uninfected patients[35;156;165;169], the benefits shown to TB burden may be off-set in the long term, to a greater or lesser extent, by the increased life expectancy of patients on HAART. Therefore, ongoing monitoring and analysis of trends in communities such as Site M are needed to assess the long term impact of this intervention.

Chapter 8

CONCLUSION

In 2008, there were over 9 million new cases of tuberculosis (TB) disease globally and TB remains a significant cause of morbidity and mortality in countries with high HIV prevalence, such as South Africa[7]. An improved understanding of the interaction between these epidemics, and the impact of interventions, in high TB and HIV settings is required in order to inform TB control strategies. The body of work outlined in this doctoral thesis has provided a detailed description of the epidemiology of TB in a well-defined community with a high burden of HIV and TB, and has focused on the effect of the HIV epidemic and a highly active antiretroviral therapy (HAART) programme on the TB epidemic in this setting.

A high rate of TB transmission was reported among both children and adults over the study period. This has resulted in a large pool of latently infected individuals in the study community and, given the increased risk of TB disease following recent infection[49,71-73], has also contributed to the community's burden of disease.

The risk factors identified for transmission to children and adults were different. Children were at increased risk of acquiring TB infection, and therefore disease, from adult TB source cases on their residential plot, and the HIV status of adult source cases was not a risk factor for this transmission. In contrast, the majority of adult to adult TB transmission occurred away from the individuals' residential plots. Of note is that there was no evidence of significant nosocomial transmission at the HAART clinic.

While the adult HIV-infected and HIV-uninfected TB epidemics were inter-related, HIV-infected TB patients contributed proportionally less to the transmission of TB than HIV-uninfected peers. Furthermore, HIV infection was not associated with an increased risk of acquiring TB infection.

The substantial HIV prevalence in the study site, combined with the high prevalence and incidence of TB infection, resulted in the HIV epidemic contributing significantly to the burden of TB disease in this community. The HAART programme was associated with active TB screening at HAART initiation, and improved immune function in patients receiving treatment. Consequently, the high coverage HAART programme resulted in a significant reduction in TB notification, TB-associated mortality and TB prevalence rates. Although the greatest benefit to TB burden was observed among HIV-infected patients, a net decrease in the overall burden of TB disease, both incidence and prevalence, was noted in the community.

This work has highlighted the urgent need for interventions that reduce TB transmission as an adjunct to improved case management. Despite the reduction in TB incidence and prevalence, the impact of HAART on TB transmission appeared to be relatively small. However, due to the prolonged incubation period for TB disease, longer term observation is required to confirm this finding.The findings reported here suggest that interventions aimed at reducing transmission will need to target the HIV-uninfected population.

In conclusion, in a high TB and HIV disease setting, the HIV epidemic contributed significantly to the burden of TB disease, but was responsible for proportionally less TB transmission compared to HIV-uninfected patients. A high coverage HAART programme was associated with a reduction in the overall burden of community TB disease, as well as a marked reduction in TB-associated mortality, reflecting a significant public health and individual benefit of this intervention. However, the impact of HAART on TB transmission was relatively minor, albeit over a short period of observation. Further studies on TB transmission are warranted, and interventions aimed at reducing transmission will be needed in order to gain control of this epidemic.

Appendix A

Population and HIV Model for Study Community

A.1 SITE M POPULATION

Population data for the population model were obtained from the 1996 South African national census, and from house-to-house census' performed by the Desmond Tutu HIV Centre (DTHC) in the study community in 2002, 2004, 2006, and 2008.

The 1996 South African national census provided age and gender composition of the community population for that year. The 2002 DTHC survey provided age composition of the community in 10-year age groups. The 2004, 2006 and 2008 DTHC census' collected the age (in years) and gender of each individual in the community.

For the population model 1996, 2004, 2006 and 2008 data were divided into 5-year age strata for the total population. The population was also divided into male and female categories, with 5-year age strata in each gender. The 10-year strata in 2002 were divided into 5-year age groups, by extrapolating the proportions of the 5-year age strata from 2004 and 2006. Similarly, the gender distribution for 2002 was extrapolated from the gender proportions in each age strata in 2004 and 2006.

Linear growth in each age stratum was extrapolated for the years between surveys, both for the total population and for gender categories.

The total population model is tabulated in Table A1.

A.2 HIV PREVALENCE

HIV prevalence for 1996 to 2004, by 5-year age strata and gender, were derived from the Actuarial Society of South Africa (ASSA) 2003 AIDS and Demographic model for the African population[198;465].

In 2005 and 2008, the DTHC performed community-based, random cross-sectional HIV prevalence surveys among adults ≥15 years of age in the study community[179;197]. HIV

status was available by age (years) and gender. These survey results were compared to the ASSA model predictions for the community.

The HIV prevalence results of the 2005 survey were not statistically different to that of the ASSA model predictions for the community (overall adult prevalence 23.2 vs 23.5% respectively; p=0.71). 95% confidence intervals were calculated for survey HIV prevalence in each age strata, assuming a poisson distribution. The ASSA model predictions for 5-year age strata fell within these confidence intervals (Figure A1).

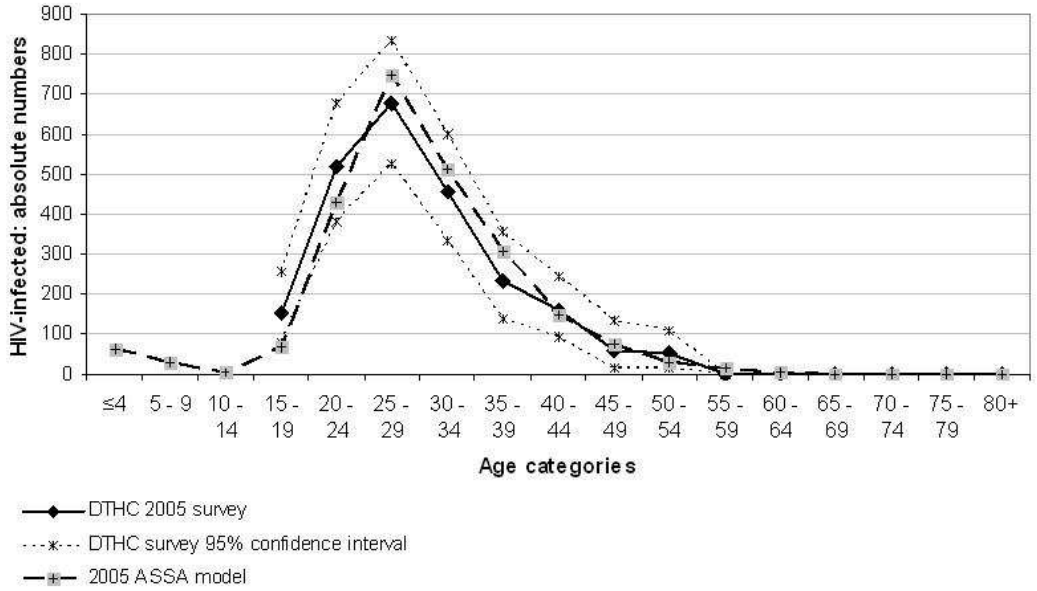


Figure A1: Comparison of HIV prevalence by 5-year age strata as reported by the 2005 DTHC HIV survey (with 95% confidence intervals) versus ASSA model predictions

The HIV prevalence reported in the 2008 survey was higher than that predicted by the ASSA model (overall adult prevalence 26.5% vs 24.1% respectively; p=0.001). 95% confidence intervals were calculated for HIV prevalence in each age strata, assuming a poisson distribution. On comparison of the survey data vs ASSA model predictions, the survey showed a shift towards older HIV-infected population (Figure A2). A comparison of HIV prevalence and gender between the survey and ASSA model, also showed shift towards higher HIV prevalence among older women in the survey (Figure A3(i) and A3(ii)). This shift in prevalence was less marked in men (Figure A4(i) and A4(ii)).

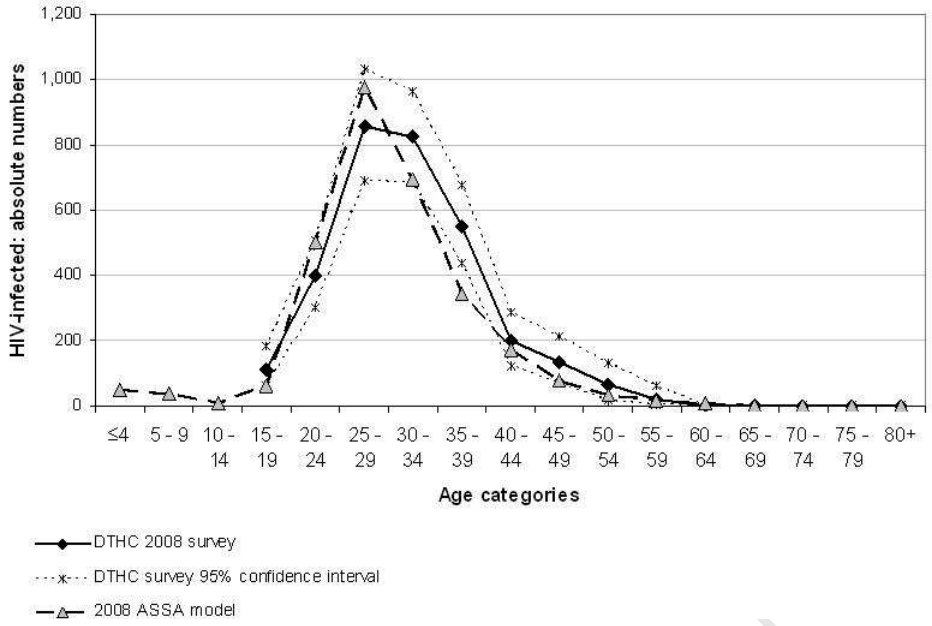
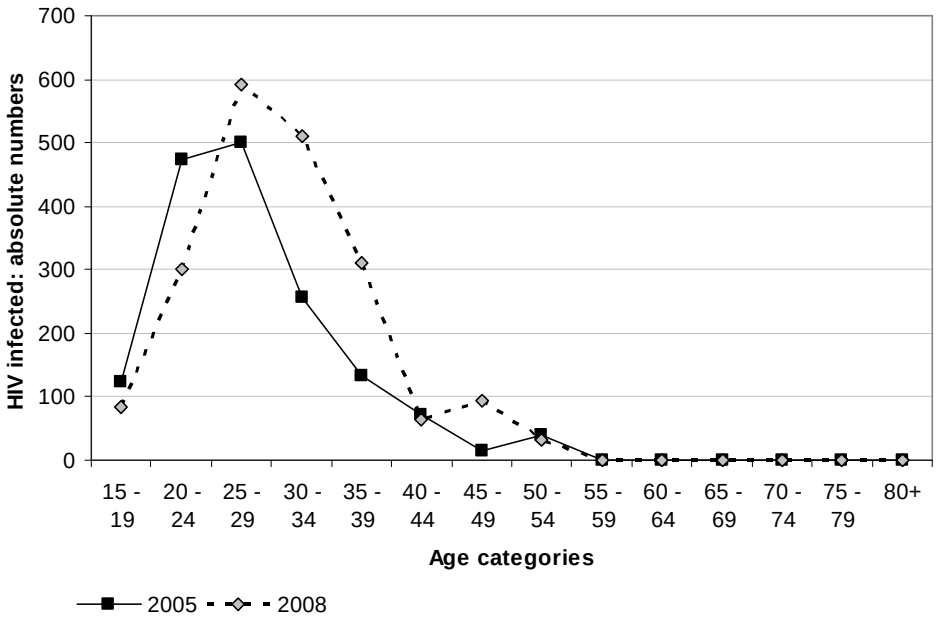


Figure A2: Comparison of HIV prevalence by 5-year age strata as reported by the 2008 DTHC HIV survey (with 95% confidence intervals) versus ASSA model predictions

The ASSA model did not adjust for the impact of an antiretroviral programme in the community. The differences noted in 2008 are in keeping with the expected impact of a highly active antiretroviral therapy (HAART) programme: with decreased mortality in the HIV-infected population on HAART[22-24], both a moderate increase in HIV prevalence as well as a shift in age distribution, towards an older HIV-infected population, is expected. Similarly, as the HAART programme enrolled a greater proportion of women, this change is expected to be most marked in this gender[468].

Based on these findings, the DTHC HIV survey data were used for the community HIV prevalence model for 2005 and 2008. HIV prevalence by 5-year age strata and gender were extrapolated between 2005 and 2008, assuming a linear trend.
As the DTHC surveys did not include 0-14 year olds, the HIV prevalence for these age groups were derived from the ASSA model, updated for revised paediatric survival assumptions[289].

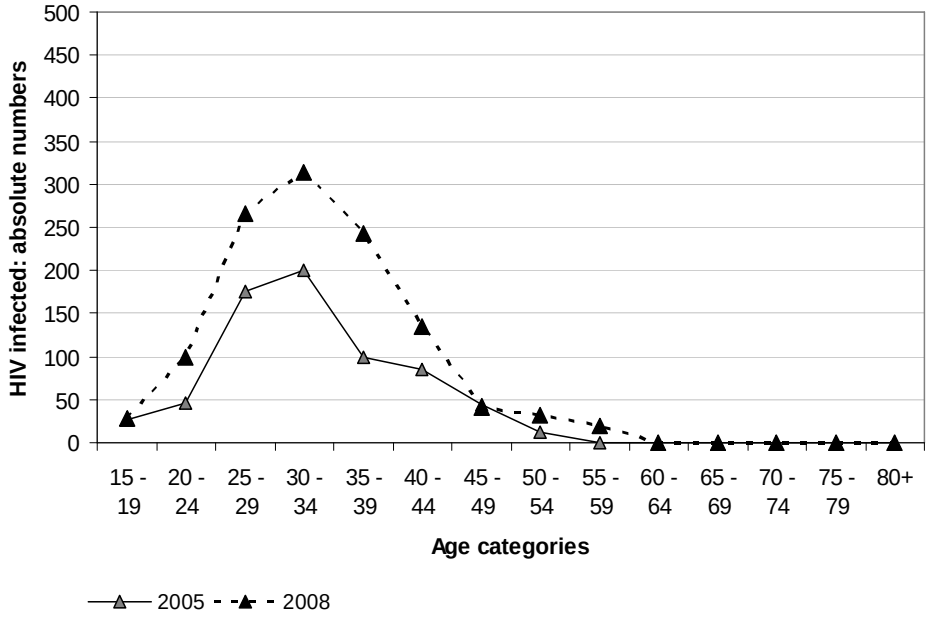
3i



3ii

Figure A3: Changes in HIV prevalence in 5-year age strata in females as reported by the 2005 and 2008 DTHC HIV survey (3i) and as predicted by the ASSA model for the same years (3ii)

4i



4ii

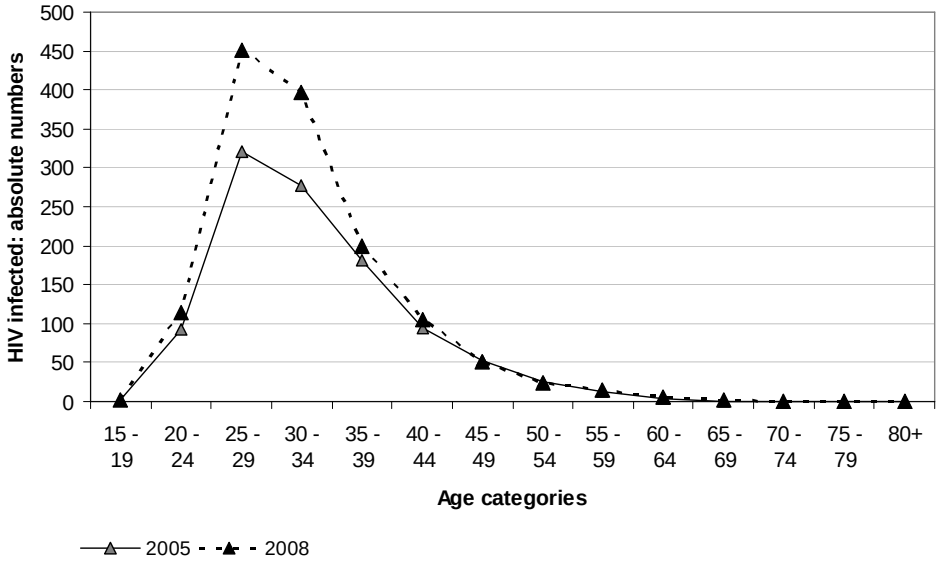


Figure A4: Changes in HIV prevalence in 5-year age strata in males as reported by the 2005 and 2008 DTHC HIV survey 4(i) and as predicted by the ASSA model for the same years (4ii)

The final HIV model is demonstrated in Figure A5 and Table A2.

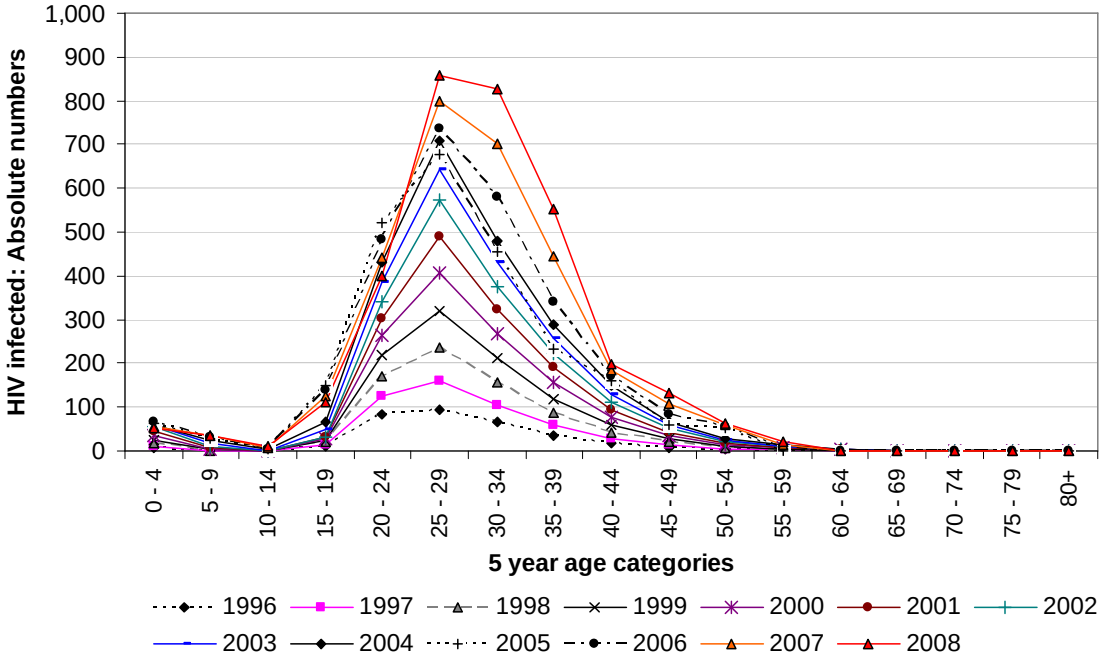


Figure A5: HIV prevalence in study community by year, in 5-year age strata

Table A1: Study Community Population: 1996 - 2008

	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
≤4	611	687	762	838	914	989	1,065	1,061	1,057	1,120	1,182	1,098	1,013
5 - 9	475	551	627	703	778	854	930	983	1,036	992	947	883	818
10 - 14	348	421	494	567	639	712	785	894	1,002	944	886	845	803
15 - 19	411	453	495	537	579	621	637	958	1,278	1,277	1,276	1,255	1,234
20 - 24	878	1,020	1,162	1,305	1,447	1,589	1,731	1,917	2,102	2,118	2,134	2,331	2,527
25 - 29	799	991	1,182	1,374	1,566	1,757	1,949	2,118	2,286	2,385	2,484	2,809	3,134
30 - 34	584	695	805	916	1,027	1,137	1,248	1,379	1,510	1,581	1,651	1,897	2,142
35 - 39	403	473	544	614	684	755	825	910	995	1,046	1,096	1,153	1,209
40 - 44	305	339	372	406	440	473	507	561	615	607	599	665	730
45 - 49	232	247	262	277	292	307	322	344	366	386	406	403	400
50 - 54	139	149	160	170	180	191	201	203	205	217	228	226	223
55 - 59	108	111	115	118	121	125	128	137	145	138	131	145	159
60 - 64	87	87	87	87	86	86	86	87	88	78	67	80	93
65 - 69	84	80	75	71	66	62	57	55	53	52	51	54	57
70 - 74	26	24	23	21	19	18	16	27	37	29	20	23	26
75 - 79	2	3	4	5	6	7	8	10	12	13	14	14	14
80+	26	23	20	17	13	10	7	10	13	11	8	9	10
Total	*5,518*	*6,353*	*7,188*	*8,023*	*8,858*	*9,693*	*10,502*	*11,651*	*12,801*	*12,990*	*13,180*	*13,886*	*14,592*

Table A2: Study Commmunity HIV-infected Population: 1997 - 2008

	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
≤ 4	10	17	25	34	45	55	59	61	63	64	57	51
5 - 9	0	1	2	3	6	11	16	23	28	32	35	35
10 - 14	0	0	0	0	0	0	1	2	3	5	7	10
15 - 19	15	19	24	28	31	32	49	66	151	138	124	111
20 - 24	125	172	219	263	303	339	385	430	520	481	441	401
25 - 29	158	235	320	406	491	572	642	707	676	737	798	859
30 - 34	106	155	211	267	322	374	429	481	456	579	701	825
35 - 39	57	86	120	155	190	223	258	289	232	339	446	553
40 - 44	28	42	58	75	93	110	130	148	158	171	185	198
45 - 49	13	19	27	35	43	51	59	67	59	84	109	133
50 - 54	5	8	11	14	18	23	26	28	51	55	60	64
55 - 59	2	3	4	6	7	9	11	13	0	7	13	20
60 - 64	1	1	1	2	2	3	4	4	0	0	0	0
65 - 69	0	0	0	0	0	0	0	0	0	0	0	0
70 - 74	0	0	0	0	0	0	0	0	0	0	0	0
75 - 79	0	0	0	0	0	0	0	0	0	0	0	0
80+	0	0	0	0	0	0	0	0	0	0	0	0
Total	521	758	1,020	1,288	1,552	1,803	2,069	2,319	2,398	2,693	2,976	3,259

Appendix B

Sensitivity Analyses for TB Notification Rates

TB notification rates were calculated based on population and HIV models that were generated based on assumptions of the trends of population growth and best fit HIV prevalence rates. Therefore, to determine the robustness of the notification analyses, sensitivity studies were performed with models assuming different trends of population growth and different assumptions for HIV prevalence from the final study population model. Analysis of the adult notification rates, overall and by HIV status were performed for these sensitivity analyses. Analyses for HIV-infected patients on ART were not included in the sensitivity studies, as the denominators for these rates were not modeled, but obtained from ART databases.

B.1 SITE M POPULATION MODEL

As described in Appendix A, the community population model that supplied denominators for rates calculations was based on repeated community censuses and assumed linear growth of the population between census years. Although other population growth trends were assessed, the linear trend was the best fit, based on the R^2 Goodness of Fit test (r^2=0.98 for total population, and r^2=0.99 for adult population).

However, TB notification rates were re-calculated based on population models assuming different population tends.

The different population growth trends assessed were:

- exponential growth between census years
  (r^2=0.95 for total population, and r^2=0.97 for adult population)
- logarithmic growth between census years
  (r^2=0.96 for total population, and r^2=0.91 for adult population)
- power growth between census years
  (r^2=0.99 for total population, and r^2=0.97 for adult population)

The proportion of population HIV-infected was obtained from the HIV model used throughout the main analysis, and was therefore constant across the 4 population models.

As shown in Figures B1, B2 and B3, the different models yielded no substantive changes in adult TB notification trends, overall or by HIV status. In overall adult TB rates all models showed a significant increase from 1997 to 2004, and a significant decline from 2005.

With regards to adult HIV-uninfected TB notification trends, all models showed a stable trend in TB rates from 2002 to 2004, and all models reporting a decrease in rates from 2005, although only the linear and exponential models reported the decrease to be statistically significant.

With regards to adult HIV-infected TB notification trends, all models showed an increase in TB rates from 2002 to 2004 (with only the power trend population model not reporting a statistically significant increase), and all models reporting a significant decrease in rates from 2005.

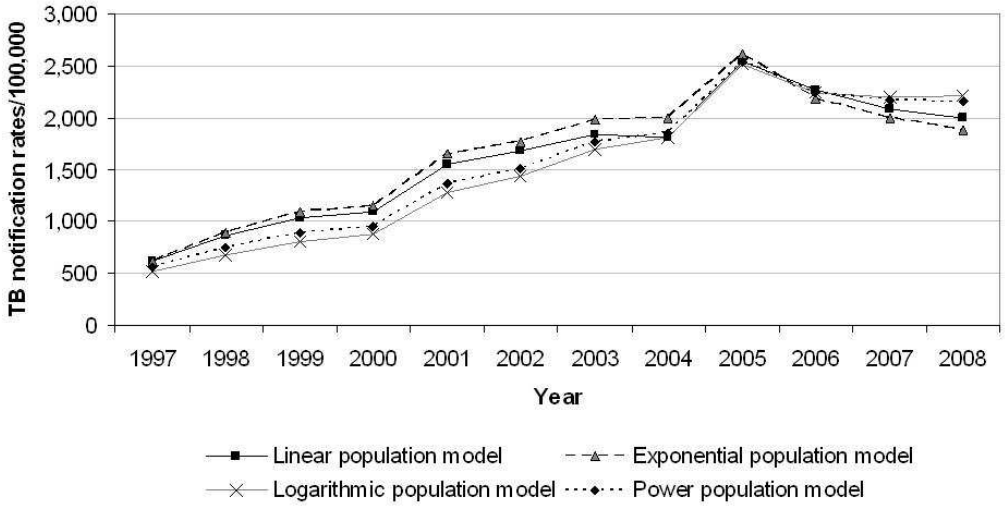


Figure B1: Total adult TB notification rates as calculated using the linear, exponential, logarithmic and power trend based population models

TB notification rates escalated from 1997 to 2004 in the linear population model (ie the model used in the main analysis; p=0.004), as well as in the exponential population model (p<0.001), the logarithmic population model (p<0.001) and the power population model (p<0.001). TB notification rates decreased from 2005 to 2008 in the linear population model (p=0.02), as well as in the exponential population model (p<0.001), the logarithmic population model (p=0.01) and the power population model (p<0.001).

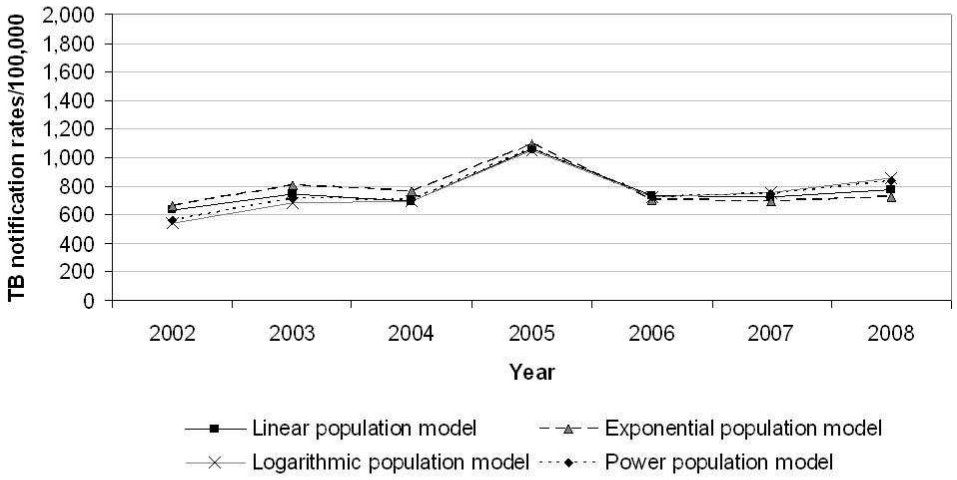


Figure B2: HIV-uninfected adult TB notification rates as calculated using the linear, exponential, logarithmic and power trend based population models

Among HIV-uninfected patients TB notification rates remained stable from 2002 to 2004 in the linear population model (ie the model used in the main analysis; p=0.59), as well as in the exponential population model (p=0.46), the logarithmic population model (p=0.45) and the power population model (p=0.46). TB notification rates decreased among HIV-uninfected patients from 2005 to 2008 in the linear population model (p=0.01), as well as in the exponential population model (p=0.002), the logarithmic population model (p=0.11) and the power population model (p=0.06).

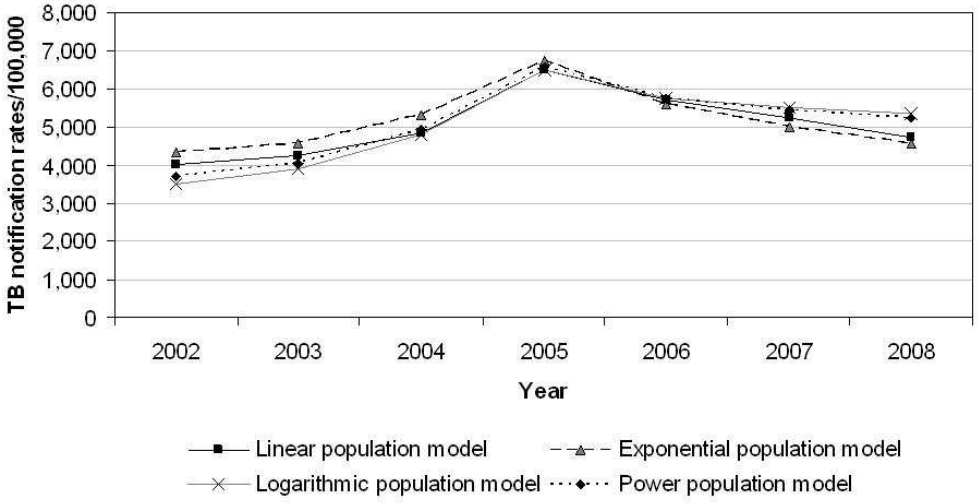


Figure B3: HIV-infected adult TB notification rates as calculated using the linear, exponential, logarithmic and power trend based population models

Among HIV-infected patients TB notification rates escalated from 2002 to 2004 in the linear population model (ie the model used in the main analysis; p=0.01), as well as in the exponential population model (p<0.001), the logarithmic population model (p=0.05) and the power population model (p=0.09). TB notification rates decreased among HIV-infected patients from 2005 to 2008 in the linear population model (p<0.001), as well as in the exponential population model (p<0.001), the logarithmic population model (p=0.01) and the power population model (p<0.001).

In summary, for overall TB notification rates, as well as rates in HIV-uninfected and HIV-infected adults, the exponential trend model showed a marginally higher rate of TB notification increase from 1997 to 2004, and a slightly sharper decline following 2005. The logarithmic trend model conversely showed a marginally slower escalation of all TB notification rates, followed by a less marked decline. The linear model therefore presents a "middle road" denominator for the data analysis.

B.2 HIV PREVALENCE MODEL

As described in Appendix A, the community HIV prevalence model used to determine denominators for TB rates calculations was obtained from the Actuarial Society of South Africa (ASSA) 2003 AIDS and Demographic model[465] for the African population, and adjusted from 2005 onwards based on the results of the 2005 and 2008 cross-sectional HIV prevalence surveys[179;197] performed in the study community.

The analysis for adult TB rates in HIV-uninfected and HIV-infected patients, as well as HIV-infected patients not receiving ART status, were repeated using the ASSA 2003 model for the African population for the full duration of the study (ie, unadjusted for community prevalence survey results), the ASSA 2003 model for the Western Cape (province in which study community is based) and the Eastern Cape. The Eastern Cape was chosen as the study population is predominantly Xhosa speaking individuals who have immigrated from that province. The study population size was obtained from the population model used throughout the main analysis, and was therefore constant across the 3 models.

Figures B4 and B5 illustrate the estimated HIV prevalence for Site M population, as well as the HAART coverage, based on each model.

Due to the fact that the ASSA 2003 AIDS and Demographic model did not account for the impact of ART programmes, the final population model has a slightly high HIV prevalence estimate compared to the different provincial models generated by the ASSA model. Consequently, the HAART coverage for the Site M HIV-infected population is lower in the final Site M model compared to each of the ASSA models.

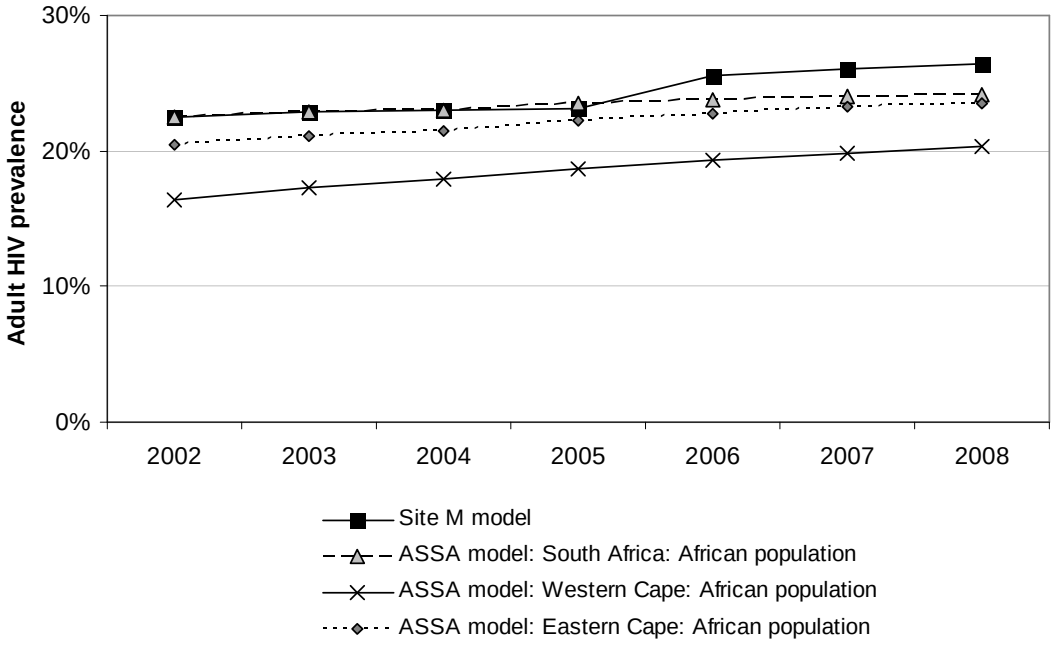


Figure B4: The population HIV prevalence estimated by each of the models

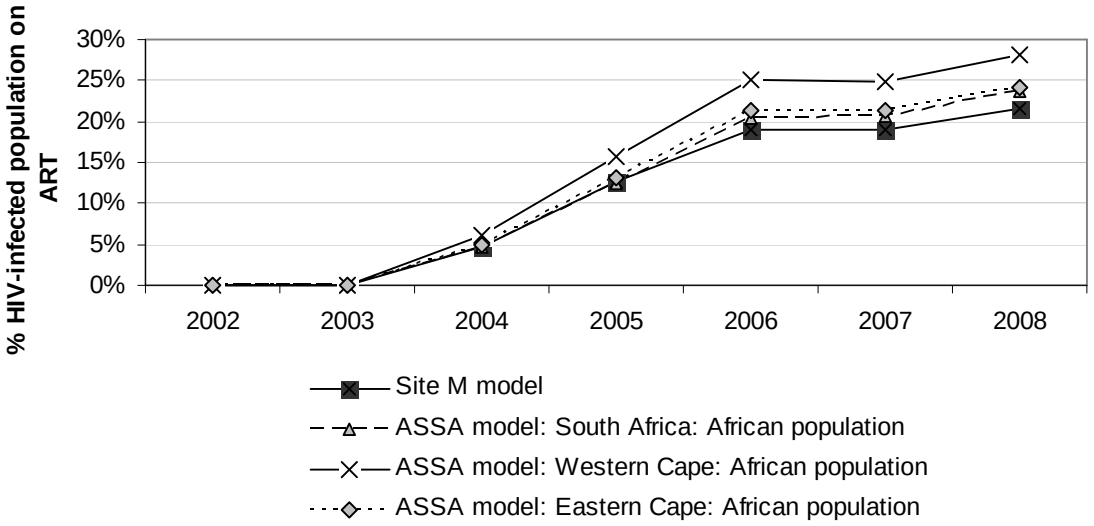


Figure B5: The HAART coverage among HIV-infected population as estimated by each model

The HAART coverage by end 2008 was 21% according to the final study model, 24% based on the ASSA national model for Africans and the ASSA Eastern Cape model for Africans, and 28% based on the ASSA Western Cape model for Africans.

Below are the TB rates among the HIV-uninfected, HIV-infected and HIV-infected not receiving HAART populations, as determined by each model (Figures B6-8).

Among HIV-uninfected patients (Figure B6), all four models showed stable TB rates from 2002 to end 2004, and all the models reported a modest but significant decline in HV-uninfected TB rates from 2005.

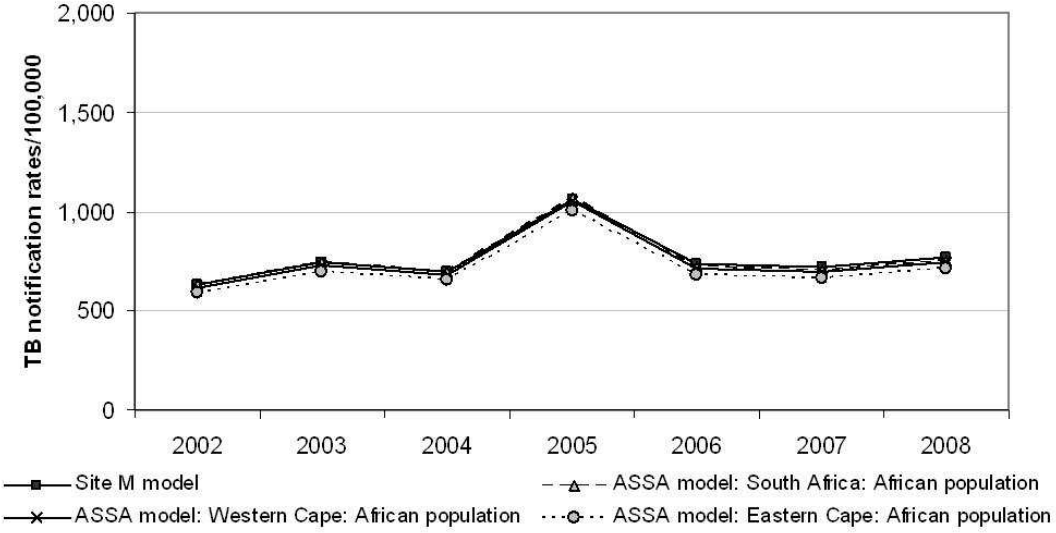


Figure B6: The TB rates among HIV-uninfected population as estimated by each model

Among HIV-uninfected patients TB notification rates remained stable from 2002 to 2004 in the final Site M model (p=0.59), as well as in the ASSA models for African populations in South Africa (p=0.61), the Western Cape (p=0.60) and the Eastern Cape (p=0.59). TB notification rates decreased among HIV-uninfected patients from 2005 to 2008 in the final Site M model (p=0.01), as well as in the ASSA models for African populations in South Africa (p=0.002), the Western Cape (p=0.002) and the Eastern Cape (p=0.003).

Among HIV-infected patients (Figure B7), all four models showed a significant increase in TB rates from 2002 to end 2004, and all the models reported a significant decline in TB rates in this group from 2005.

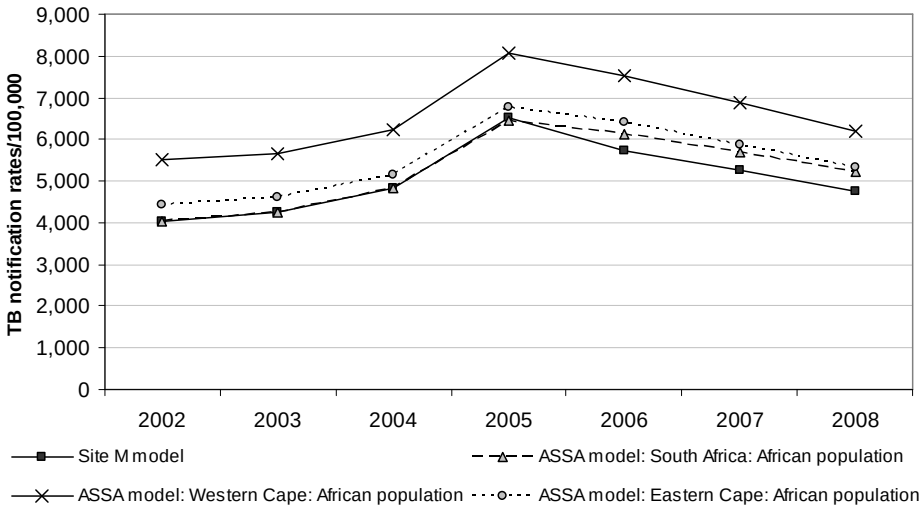


Figure B7: The TB rates among HIV-infected population, as estimated by each model

Among HIV-infected patients TB notification rates increased significantly from 2002 to 2004 in the final Site M model (p=0.01), as well as in the ASSA models for African populations in South Africa (p<0.001), the Western Cape (p<0.001) and the Eastern Cape (p<0.001). TB notification rates decreased significantly among HIV-infected patients from 2005 to 2008 in the final Site M model (p<0.001), as well as in the ASSA models for African populations in South Africa (p<0.001), the Western Cape (p<0.001) and the Eastern Cape (p<0.001).

Among HIV-infected patients not receiving HAART (Figure B8), all four models showed an increase in TB rates from 2002 to end 2004, although the increases were only significant in the ASSA South African and Western Cape models. All the models reported a significant decline in TB rates in this group from 2005.

The final population model provided conservative estimates of HIV-infected TB rates, compared to the other models. However, as these figures illustrate, using the different models to determine community HIV prevalence does not result in any substantive changes in study inferences.

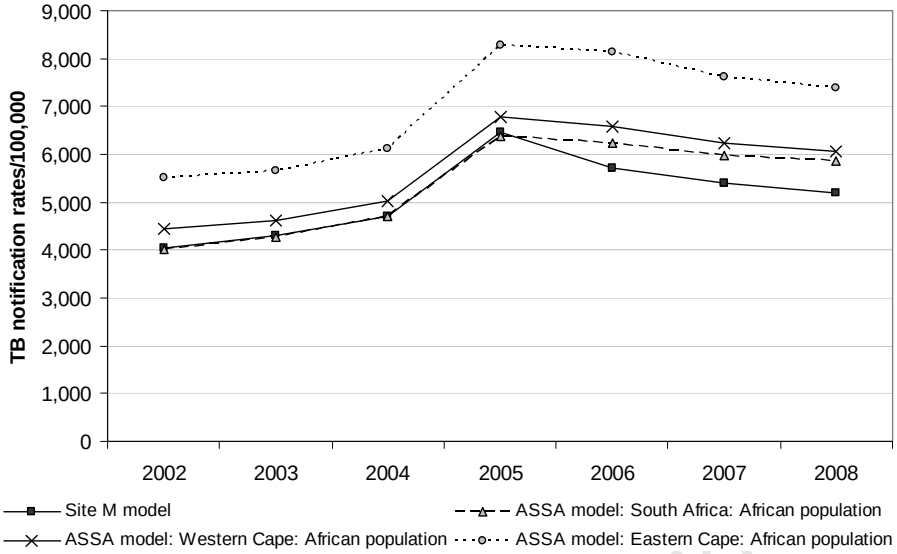


Figure B8: The TB rates among HIV-infected patients not receiving HAART, as estimated by each model

Among HIV-infected patients not receiving HAART TB notification rates increased, but not significantly from 2002 to 2004 in the final Site M model (p=0.34), as well as in the ASSA models for the Eastern Cape (p=0.21). The increases noted in the ASSA South African (p<0.001) and the Western Cape (p<0.001) models were significant. TB notification rates decreased significantly among HIV-infected patients not on HAART from 2005 to 2008 in the final Site M model (p<0.001), as well as in the ASSA models for African populations in South Africa (p<0.001), the Western Cape (p<0.001) and the Eastern Cape (p=0.2).

B.3 FURTHER SENSITIVITY ANALYSES

Pre and post HAART cut-off

The use of 2005 as the pre and post HAART cut-off was an a priori decision, based on the scale-up of the ART programme in this year: HAART coverage increased from 5% in 2004 to 13% in 2005.

However a sensitivity analysis was performed using 2004 as the HAART cut-off. In this revised analysis the post HAART trends remained in the same direction as the main analysis (ie showing a decrease), although these tends no longer reached statistical significance. Overall TB rates declined by an annual average of 23 cases/100,000 following 2003 (p=0.66), HIV-uninfected TB rates declined by an average of 52 cases per year (p=0.47) and TB rates in HIV-infected patients declined by an annual average of 238 cases (p=0.35).

Definition of TB patients "on HAART"

In the main analysis all patients who were receiving HAART at the time of TB diagnosis were classified as "on HAART", regardless of duration of HAART treatment. Given the possibility that TB disease diagnosed in the first 3 months of HAART treatment represents TB disease that was present, although unrecognised, at the time of HAART initiation[76;152;166], these patients could also conceivably be considered to belong in the TB patients "off HAART" group. However, in the study clinic all HIV-infected patients were actively screened for TB disease prior to starting HAART. Given that no evidence of clinical disease was evident at the time of HAART initiation, it was decided to classify all TB patients receiving HAART as "on HAART", regardless of HAART treatment duration. However, a sensitivity analysis was performed, re-classifying all TB patients who had received HAART for less than 85 days (ie 3 months) in the "off HAART" group.

Overall 31(30% of the original "receiving HAART" group) TB patients were re-classified from the "HIV-infected patients receiving HAART" group to the "HIV-infected patients not receiving HAART" group. The sensitivity anlaysis findings are presented in Figure B9. The TB notification rates of patients on HAART were lower than those presented in the main analysis, and the decreasing tend in these rates was not significant (p=0.84). However, if year 2004 was excluded from the analysis (only two TB patients were receiving HAART in that year), the decreasing trend in this group reached statistical significance (p=0.002). Overall, this sensitivity analysis confirmed the trends reported in Chapter 5.

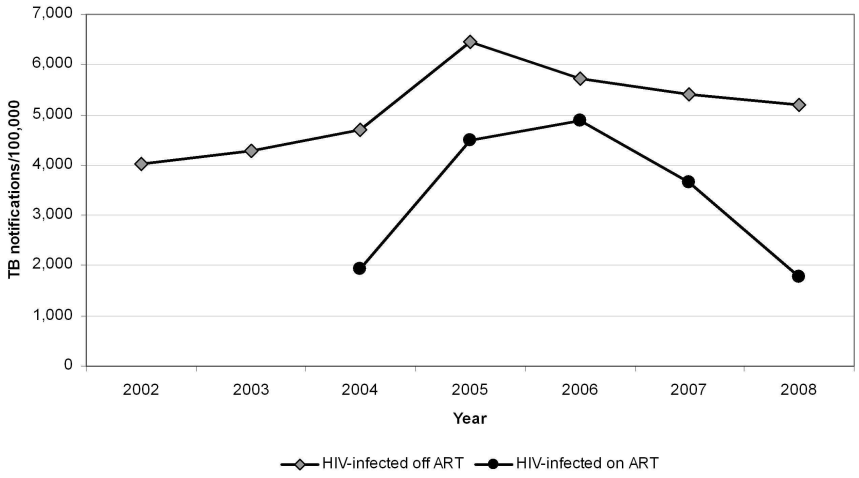


Figure B9: Adult TB notification rates by HAART status, with TB patients in the first 3 months of HAART treatment classified as "HIV-infected off HAART".

This figure shows the increase in TB notification rates from 2002 to 2004 among HIV-infected patients not receiving HAART (p=0.16), followed by a decrease in notification rates from 2005 to 2008 (p<0.001). This figure also shows the decreasing TB notification rates from 2004 to 2008 among HIV-infected patients receiving HAART (p=0.84).

Impact of TB Prevalence Survey

In 2005 a cross-sectional community TB survey was performed in Site M (Chapter 6). This survey was performed on a randomly selected 10% of the adult population. Based on the findings of the survey, 12 additional patients were started on TB treatment in 2005 and 2006, 6 of whom were HIV-infected.

To determine if the survey had a direct impact on TB notifications, analyses were re-run excluding the patients identified by the survey. The analyses are shown in Figures B10 and B11. These analyses showed no significant difference in the main findings of the study.

Overall TB rates still showed a significant increase from 1997 to 2004, followed by a significant decline from 2005. Similarly, HIV-uninfected TB rates were stable from 2002 to end 2004, followed by a moderate decline (as noted in the main analysis), and HIV-infected TB rates increased significantly from 2002 to 2004, after which they decreased significantly (as in the main analysis).

Of note is that the peak in TB notifications observed in 2005 was unaffected in this analysis. This supports our theory that the increase in 2005 is predominantly due to the increased screening of patients initiating ART and increased TB awareness in the community.

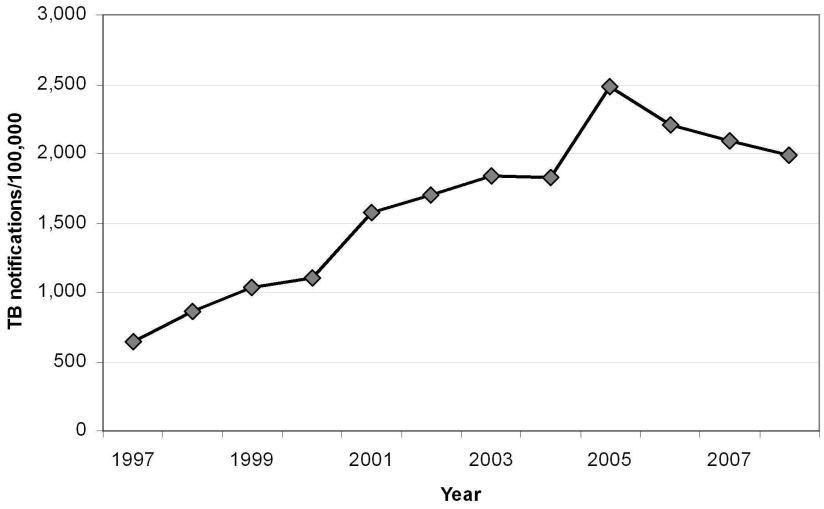


Figure B10: Overall adult TB notification rates in Site M, excluding patients identified in the 2005 survey

TB notification rates increased significantly from1997 to 2004 (p<0.001), followed by a significant decrease in TB notification rates from 2005 to 2008 (p=0.01).

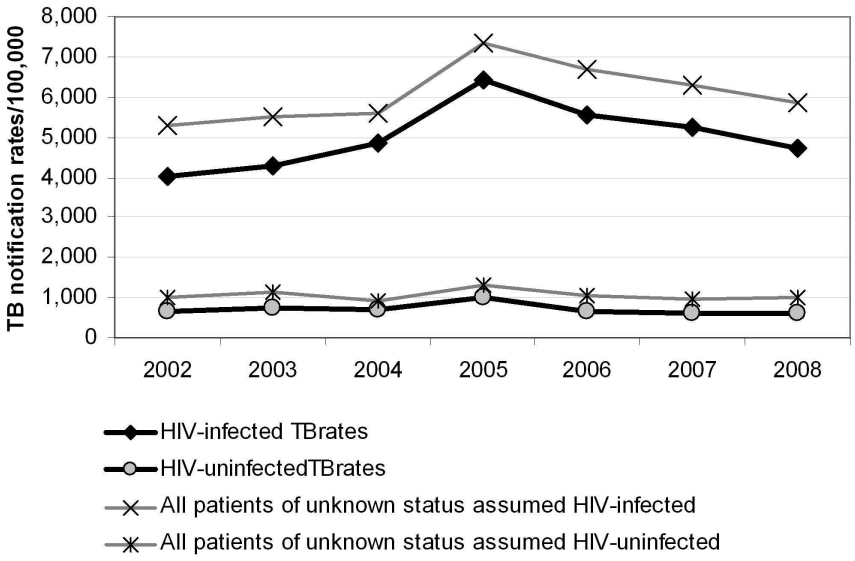


Figure B11: Adult TB notification rates by HIV status, excluding patients identified in the 2005 survey

This figure shows the stable TB notification rates among HIV-uninfected patients from 2002 to 2004 (p=0.61), followed by a moderate decline from 2005 (p<0.001). This figure also shows increasing TB notification rates among HIV-infected patients from 2002 to 2004 (p=0.01), followed by a decrease in notification rates from 2005 to 2008 (p<0.001).

B.4 ATERNATIVE ANALYSIS

Logistic Regression Models

Logistic regression provides an alternative method for modeling the population TB rates. This approach is based on the absolute count data (TB cases and population size), rather than converting to TB cases/100,000. Therefore a logistic regression analysis was performed as a sensitivity analysis. Figure B12 illustrates the odds ratios of overall adult TB disease in the population, using 1997 as the reference year. Figure B13 reports the odds ratios of adult TB overall and in HIV-infected and uninfected populations using 2002 as the reference year. These analyses confirm the overall trends reported in Chapter 5.

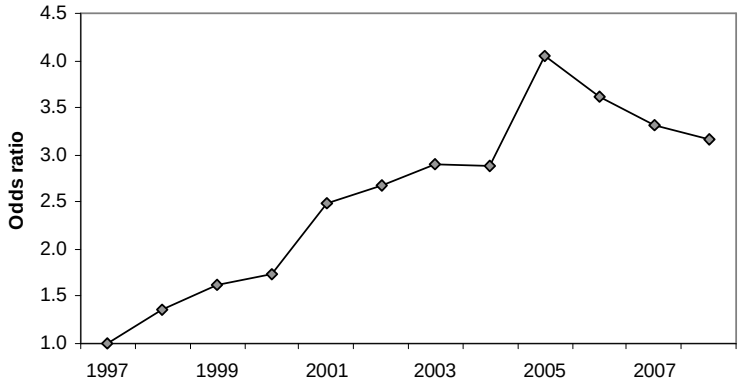


Figure B12: The odds ratios of overall adult TB disease in the population, using 1997 as the reference year

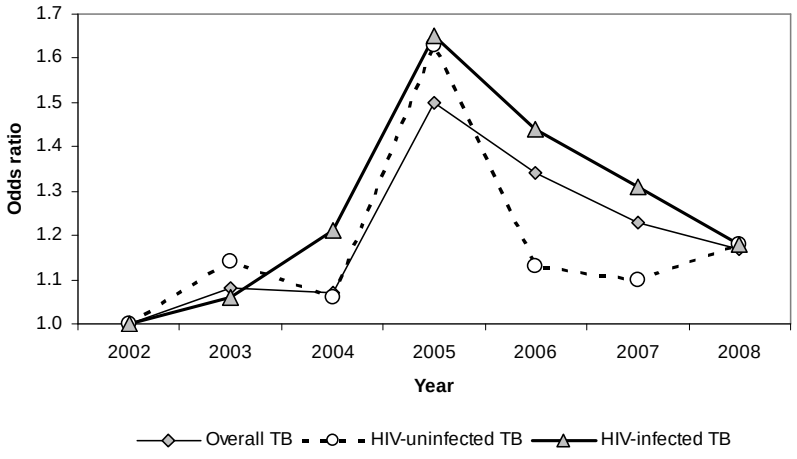


Figure B13: The odds ratios of adult TB overall and in HIV-infected and uninfected populations using 2002 as the reference year

B.5 CONCLUSION

In conclusion, exhaustive sensitivity analyses were performed for this study, including differing population and HIV prevalence models and considering an alternative HAART cut-off date, the possible impact of other studies in the community on TB notifications, as well as alternative analyses techniques. All analyses supported the main findings of the study and did not substantively alter the study results.

Appendix C

Risk Factor Models for TB Prevalence in 2005 and 2008

Table C1: Total Prevalent Tuberculosis in 2005

Risk factor	Univariate analysis		Multivariate analysis	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age	1.01 (0.97-1.04)	0.61	1.01 (0.96-1.06)	0.78
Gender: Male	1		1	
Female	0.88 (0.38-2.01)	0.75	0.54 (0.20-1.44)	0.22
Median residents in household	0.89 (0.71-1.11)	0.30		
Median persons sleeping in same room	0.93 (0.63-1.37)	0.72		
Ever had TB in the past	Perfectly predicts failure			
Alcohol intake in past 6 months	0.25 (0.54-2.86)	0.60		
Visited shebeen (bar) in past 6 months	1.12 (0.41-3.05)	0.83		
Smoked in past 6 months	1.78 (0.76-4.18)	0.19		
Recreational drugs in past 6 months	0.87 (0.11-6.66)	0.89		
Employment History				
Past mining	1.62 (0.67-7.15)	0.52		
Health Care Worker	0.61 (0.08-4.77)	0.64		
Prison in past 6 months	13.71 (3.38-55.54)	<0.001	21.47 (4.37-105.43)	<0.001
HIV-uninfected	1		1	
HIV-infected	4.15 (1.80-9.59)	0.001	8.79 (3.12-24.76)	<0.001
HIV-infected on HAART	30.74 (7.64-123.58)	<0.001	111.22 (20.81-594.54)	<0.001

Table C2: Total Prevalent Tuberculosis in 2008

Risk factor	Univariate analysis		Multivariate analysis	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Age	1.03 (0.99-1.07)	0.10	1.01 (0.96-1.06)	0.83
Gender: Male	1		1	
Female	0.71 (0.29-1.76)	0.46	1.06 (0.33-3.42)	0.92
Median residents in household	1.15 (-.96-1.39)	0.13		
Median persons sleeping in same room	0.78 (0.50-1.23)	0.29		
Ever had TB in the past	Perfectly predicts failure			
Alcohol intake in past 6 months	1.38 (0.56-3.41)	0.48		
Visited shebeen (bar) in past 6 months	0.79 (0.28-2.19)	0.65		
Smoked in past 6 months	2.25 (0.91-5.56)	0.08	2.10 (0.71-6.25)	0.18
Recreational drugs in past 6 months	Perfectly predicts failure			
Employment History				
Past mining	5.11 (1.44-8.14)	0.01	4.39 (0.91-21.30)	0.07
Health Care Worker	Perfectly predicts failure			
Prison in past 6 months	4.57 (0.57-36.55)	0.15		
Ever spent time in prison (excl last 6 months)	2.71 (0.89-8.25)	0.08	1.57 (0.48-5.83)	0.47
HIV-uninfected	1		1	
HIV-infected	2.10 (0.83-5.33)	0.12	2.77 (0.99-7.73)	0.05
HIV-infected on HAART	5.25 (1.70-16.22)	0.004	7.91 (2.25-27.81)	0.001

Reference List

1. Sutherland I. Recent studies in the epidemiology of tuberculosis, based on the risk of being infected with tubercle bacilli. *Adv Tuberc Res* 1976; 19:1-63.

2. Cauthen GM, Pio A, ten Dam HG. Annual risk of tuberculous infection. 1988. *Bull World Health Organ* 2002; 80(6):503-511.

3. The Oxford Medical Companion. Edited by: Walton, J, Baronders, JA, and Lock, S. Oxford University Press, Oxford. 1994.

4. Morse D, Brothwell DR, Ucko PJ. Tuberculosis in Ancient Egypt. *Am Rev Respir Dis* 1964; 90:524-541.

5. Salo WL, Aufderheide AC, Buikstra J, Holcomb TA. Identification of Mycobacterium tuberculosis DNA in a pre-Columbian Peruvian mummy. *Proc Natl Acad Sci U S A* 1994; 91(6):2091-2094.

6. World Health Organization. The Global Burden of Disease. 2004 Update. 2004; http://www.who.int/healthinfo/global_burden_disease/GBD_report_2004update_part2.pdf

7. World Health Organization. Global tuberculosis control: surveillance, planning, financing. 2009; http://www.who.int/tb/publications/global_report/2009/en/index.html

8. Raviglione MC, Snider DE, Jr., Kochi A. Global epidemiology of tuberculosis. Morbidity and mortality of a worldwide epidemic. *JAMA* 1995; 273(3):220-226.

9. Corbett EL, Watt CJ, Walker N, Maher D, Williams BG, Raviglione MC, Dye C. The growing burden of tuberculosis: global trends and interactions with the HIV epidemic. *Arch Intern Med* 2003; 163(9):1009-1021.

10. World Health Organization. Factsheet: Tuberculosis. 2010; http://www.who.int/mediacentre/factsheets/fs104/en/

11. World Health Organization. Global Tuberculosis Control. A short update to the 2009 report. 2009; http://www.who.int/tb/publications/global_report/2009/update/tbu_9.pdf

12. UNAIDS. Global Report. UNAIDS report on the global AIDS epidemic. 2010; http://www.unaids.org/documents/20101123_GlobalReport_em.pdf

13. UNAIDS and World Health Organization. AIDS Epidemic Update. 2009; http://data.unaids.org/pub/Report/2009/JC1700_Epi_Update_2009_en.pdf

14. Selwyn PA, Hartel D, Lewis VA, Schoenbaum EE, Vermund SH, Klein RS, Walker AT, Friedland GH. A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. *N Engl J Med* 1989; 320(9):545-550.

15. Braun MM, Badi N, Ryder RW, Baende E, Mukadi Y, Nsuami M, Matela B, Willame JC, Kaboto M, Heyward W. A retrospective cohort study of the risk of tuberculosis among women of childbearing age with HIV infection in Zaire. *Am Rev Respir Dis* 1991; 143(3):501-504.

16. Sonnenberg P, Glynn JR, Fielding K, Murray J, Godfrey-Faussett P, Shearer S. How soon after infection with HIV does the risk of tuberculosis start to increase? A retrospective cohort study in South African gold miners. *J Infect Dis* 2005; 191(2):150-158.

17. De Cock KM, Soro B, Coulibaly IM, Lucas SB. Tuberculosis and HIV infection in sub-Saharan Africa. *JAMA* 1992; 268(12):1581-1587.

18. Harries AD. Tuberculosis and human immunodeficiency virus infection in developing countries. *Lancet* 1990; 335(8686):387-390.

19. Styblo K. Impact of HIV infection on the tuberculosis problem worldwide. *Kekkaku* 1990; 65(6):429-438.

20. Control of tuberculosis in the United States. American Thoracic Society. *Am Rev Respir Dis* 1992; 146(6):1623-1633.

21. Nunn P, Williams B, Floyd K, Dye C, Elzinga G, Raviglione M. Tuberculosis control in the era of HIV. *Nat Rev Immunol* 2005; 5(10):819-826.

22. Mocroft A, Ledergerber B, Katlama C, Kirk O, Reiss P, d'Arminio MA, Knysz B, Dietrich M, Phillips AN, Lundgren JD. Decline in the AIDS and death rates in the EuroSIDA study: an observational study. *Lancet* 2003; 362(9377):22-29.

23. Egger M, May M, Chene G, Phillips AN, Ledergerber B, Dabis F, Costagliola D, d'Arminio MA, de Wolf F, Reiss P, Lundgren JD, Justice AC, Staszewski S, Leport C, Hogg RS, Sabin CA, Gill MJ, Salzberger B, Sterne JA. Prognosis of HIV-1-infected patients starting highly active antiretroviral therapy: a collaborative analysis of prospective studies. *Lancet* 2002; 360(9327):119-129.

24. Palella FJ, Jr., Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* 1998; 338(13):853-860.

25. Enger C, Graham N, Peng Y, Chmiel JS, Kingsley LA, Detels R, Munoz A. Survival from early, intermediate, and late stages of HIV infection. *JAMA* 1996; 275(17):1329-1334.

26. World Health Organization. Treating 3 million people by 2005: Making it Happen. The WHO Strategy. 2003; http://www.who.int/3by5/publications/documents/en/3by5StrategyMakingItHappen.pdf

27. The United States President's Emergency Plan For AIDS Relief: Fact Sheet. 2009; http://www.pepfar.gov/documents/organization/115411.pdf

28. The Global Fund to Fight AIDS. Annual Report 2008. 2008; http://www.theglobalfund.org/documents/publications/annualreports/2008/AnnualReport2008.pdf

29. World Health Organization, UNAIDS, UNICEF. Towards universal access: scaling up priority HIV/AIDS interventions in the health sector. 2008; http://www.who.int/hiv/pub/towards_universal_access_report_2008.pdf

30. World Health Organization, *Addressing Poverty in TB Control: options for national TB control programmes 2005.* WHO, Geneva, Switzerland. 2005; http://whqlibdoc.who.int/hq/2005/who_htm_tb_2005.352.pdfhttp://whqlibdoc.who.int/hq/2005/who_htm_tb_2005.352.pdf

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

32. Elender F, Bentham G, Langford I. Tuberculosis mortality in England and Wales during 1982-1992: its association with poverty, ethnicity and AIDS. *Soc Sci Med* 1998; 46(6):673-681.

33. Rana FS, Hawken MP, Mwachari C, Bhatt SM, Abdullah F, Ng'ang'a LW, Power C, Githui WA, Porter JD, Lucas SB. Autopsy study of HIV-1-positive and HIV-1-negative adult medical patients in Nairobi, Kenya. *J Acquir Immune Defic Syndr* 2000; 24(1):23-29.

34. Grant AD, Djomand G, De Cock KM. Natural history and spectrum of disease in adults with HIV/AIDS in Africa. *AIDS* 1997; 11 Suppl B:S43-S54.

35. Lawn SD, Myer L, Bekker LG, Wood R. Burden of tuberculosis in an antiretroviral treatment programme in sub-Saharan Africa: impact on treatment outcomes and implications for tuberculosis control. *AIDS* 2006; 20(12):1605-1612.

36. Lawn SD, Myer L, Orrell C, Bekker LG, Wood R. Early mortality among adults accessing a community-based antiretroviral service in South Africa: implications for programme design. *AIDS* 2005; 19(18):2141-2148.

37. Kok P and Collinson M. 2006: Migration and urbanisation in South Africa. Report 03-04-02. 2006.

38. State of the World Population 2007. United Nations Population Fund, United Nations, Geneva, Switzerland. 2007; http://www.unfpa.org/swp/2007/english/introduction.htmlhttp://www.unfpa.org/swp/2007/english/introduction.html

39. Lawn SD, Bekker LG, Middelkoop K, Myer L, Wood R. Impact of HIV infection on the epidemiology of tuberculosis in a peri-urban community in South Africa: the need for age-specific interventions. *Clin Infect Dis* 2006; 42(7):1040-1047.

40. UNAIDS, World Health Organization, UNICEF. Epidemiological Fact Sheet on HIV and AIDS: Core data on epidemiology and response. South Africa. 2008; http://apps.who.int/globalatlas/predefinedReports/EFS2008/full/EFS2008_ZA.pdf

41. De Cock KM and Chaisson RE. Will DOTS do it? A reappraisal of tuberculosis control in countries with high rates of HIV infection. *Int J Tuberc Lung Dis* 1999; 3(6):457-465.

42. Whalen CC. Failure of directly observed treatment for tuberculosis in Africa: a call for new approaches. *Clin Infect Dis* 2006; 42(7):1048-1050.

43. World Health Organization. WHO declares TB an emergency in Africa. Call for "urgent and extraordinary actions" to halt a worsening epidemic. 2005; http://www.who.int/mediacentre/news/2005/africa_emergency/en/

44. World Health Organization HIV/AIDS and TB Department. WHO Three I's Meeting: Intensified Case Finding (ICF), Isoniazid Preventive Theraoy (IPT) and TB Infection Control (IC) for people living with HIV. 2008; http://www.who.int/hiv/pub/meetingreports/WHO_3Is_meeting_report.pdf

45. Badri M, Wilson D, Wood R. Effect of highly active antiretroviral therapy on incidence of tuberculosis in South Africa: a cohort study. *Lancet* 2002; 359(9323):2059-2064.

46. Lawn SD, Badri M, Wood R. Tuberculosis among HIV-infected patients receiving HAART: long term incidence and risk factors in a South African cohort. *AIDS* 2005; 19(18):2109-2116.

47. World Health Organization: *The Global Plan to Stop TB, 2006-2015 Stop TB Partnership,* WHO Geneva, Switzerland. 2006; http://www.stoptb.org/globalplan/plan_p2main.asp?p=2http://www.stoptb.org/globalplan/plan_p2main.asp?p=2

48. Koch. The aetiology of tuberculosis: A translation by Berna Pinner and Max Pinner. *Am Rev Tuberc* 1932; 25:284-323.

49. Rieder H. Epidemiologic Basis of Tuberculosis Control. First Edition. International Union Against Tuberculosis and Lung Disease, Paris. 1999.

50. Vynnycky E and Fine PE. The natural history of tuberculosis: the implications of age-dependent risks of disease and the role of reinfection. *Epidemiol Infect* 1997; 119(2):183-201.

51. Richardson M, Carroll NM, Engelke E, van der Spuy GD, Salker F, Munch Z, Gie RP, Warren RM, Beyers N, van Helden PD. Multiple Mycobacterium tuberculosis strains in early cultures from patients in a high-incidence community setting. *J Clin Microbiol* 2002; 40(8):2750-2754.

52. Ashwin S Dharmadhikari and Edward A Nardell. Transmission of *Mycobacterium tuberculosis*. *Tuberculosis. A Comprehensive Clinical Reference.* Edited by: Zumla & Schaaf. Elsevier Health Sciences Saunders, 2009;8-16.

53. Riley RL, Mills CC, O'Grady F, Sultan LU, Wittstadt F, Shivpuri DN. Infectiousness of air from a tuberculosis ward. Ultraviolet irradiation of infected air: comparative infectiousness of different patients. *Am Rev Respir Dis* 1962; 85:511-525.

54. An investigation of household contacts of open cases of pulmonary tuberculosis amongst the Kikuyu in Kiambu, Kenya. *Bull World Health Organ* 1961; 25(6):831-850.

55. Shaw JB and Wynn-Williams N. Infectivity of pulmonary tuberculosis in relation to sputum status. *Am Rev Tuberc* 1954; 69(5):724-732.

56. Zeidberg LD, Gass RS, Dillon A, Hutcheson RH. The Williamson County Tuberculosis Study. A twenty-four-year epidemiologic study. *Am Rev Respir Dis* 1963; 87(3) Pt 2:1-88.

57. Grzybowski S, Barnett GD, Styblo K. Contacts of cases of active pulmonary tuberculosis. *Bull Int Union Tuberc* 1975; 50(1):90-106.

58. Grzybowski S and Allen EA. The Challenge of Tuberculosis in Decline. A Study based on the epidemiology of Tuberculosis in Ontario, Canada. *Am Rev Respir Dis* 1964; 90:707-720.

59. Chapman JS and Dyerly MD. Social and other factors in intrafamilial transmission of tuberculosis. *Am Rev Respir Dis* 1964; 90:48-60.

60. Rouillon A, Perdrizet S, Parrot R. Transmission of tubercle bacilli: The effects of chemotherapy. *Tubercle* 1976; 57(4):275-299.

61. van Geuns HA, Meijer J, Styblo K. Results of contact examination in Rotterdam, 1967-1969. *Bull Int Union Tuberc* 1975; 50(1):107-121.

62. Elwood RK, Cook VJ, Hernandez-Garduno E. Risk of tuberculosis in children from smear-negative source cases. *Int J Tuberc Lung Dis* 2005; 9(1):49-55.

63. Loudon RG and Roberts RM. Droplet expulsion from the respiratory tract. *Am Rev Respir Dis* 1966; 95:435-442.

64. Wells FW. Airborne Contagion and Air Hygiene. *Edition:1.* Harvard University Press, Cambridge. 1955.

65. Iseman MD. Treatment of multidrug-resistant tuberculosis. *N Engl J Med* 1993; 329(11):784-791.

66. DiStasio AJ and Trump DH. The investigation of a tuberculosis outbreak in the closed environment of a U.S. Navy ship, 1987. *Mil Med* 1990; 155(8):347-351.

67. Aparicio JP, Capurro AF, Castillo-Chavez C. Transmission and dynamics of tuberculosis on generalized households. *J Theor Biol* 2000; 206(3):327-341.

68. Kenyon TA, Valway SE, Ihle WW, Onorato IM, Castro KG. Transmission of multidrug-resistant Mycobacterium tuberculosis during a long airplane flight. *N Engl J Med* 1996; 334(15):933-938.

69. Comstock GW, Livesay VT, Woolpert SF. The prognosis of a positive tuberculin reaction in childhood and adolescence. *Am J Epidemiol* 1974; 99(2):131-138.

70. Vynnycky E and Fine PE. Lifetime risks, incubation period, and serial interval of tuberculosis. *Am J Epidemiol* 2000; 152(3):247-263.

71. Comstock GW. Epidemiology of tuberculosis. *Am Rev Respir Dis* 1982; 125(3 Pt 2):8-15.

72. Sutherland I. The Evolution of Clinical Tuberculosis in Adolescents. *Tubercle* 1966; 47:308.

73. Moran-Mendoza O, Marion SA, Elwood K, Patrick D, FitzGerald JM. Risk factors for developing tuberculosis: a 12-year follow-up of contacts of tuberculosis cases. *Int J Tuberc Lung Dis* 2010; 14(9):1112-1119.

74. Rieder HL, Cauthen GM, Comstock GW, Snider DE, Jr. Epidemiology of tuberculosis in the United States. *Epidemiol Rev* 1989; 11:79-98.

75. Holmes CB, Wood R, Badri M, Zilber S, Wang B, Maartens G, Zheng H, Lu Z, Freedberg KA, Losina E. CD4 decline and incidence of opportunistic infections in Cape Town, South Africa: implications for prophylaxis and treatment. *J Acquir Immune Defic Syndr* 2006; 42(4):464-469.

76. Badri M, Lawn SD, Wood R. Short-term risk of AIDS or death in people infected with HIV-1 before antiretroviral therapy in South Africa: a longitudinal study. *Lancet* 2006; 368(9543):1254-1259.

77. Silwer H and Oscarsson PN. Incidence and coincidence of diabetes mellitus and pulmonary tuberculosis in a Swedish county. *Acta Med Scand Suppl* 1958; 335:1-48.

78. Webb EA, Hesseling AC, Schaaf HS, Gie RP, Lombard CJ, Spitaels A, Delport S, Marais BJ, Donald K, Hindmarsh P, Beyers N. High prevalence of Mycobacterium tuberculosis infection and disease in children and adolescents with type 1 diabetes mellitus. *Int J Tuberc Lung Dis* 2009; 13(7):868-874.

79. Snider DE, Jr. The relationship between tuberculosis and silicosis. *Am Rev Respir Dis* 1978; 118(3):455-460.

80. Cowie RL. The epidemiology of tuberculosis in gold miners with silicosis. *Am J Respir Crit Care Med* 1994; 150(5 Pt 1):1460-1462.

81. Parker F Jr, Jackson H Jr, Bethea JM, Otis F. The coexistence of tuberculosis with Hodgkin's Disease and other forms of malignant lymphoma. *The American Journal of the Medical Sciences* 1932; 184:694-699.

82. Kaplan MH, Armstrong D, Rosen P. Tuberculosis complicating neoplastic disease. A review of 201 cases. *Cancer* 1974; 33(3):850-858.

83. Millar JW and Horne NW. Tuberculosis in immunosuppressed patients. *Lancet* 1979; 1(8127):1176-1178.

84. Johnson JR and Davey WN. Cortisone, corticotropin, and antimicrobial therapy in tuberculosis in animals and man; a review. *Am Rev Tuberc* 1954; 70(4):623-636.

85. Lurie MB. Role of adrenal cortex in native resistance. *Resistance to Tuberculosis: Experimental Studies in Native and Acquired Defensive Mechanisms. Edition:Edition 1.* Harvard University Press, Cambridge, Massachusetts, 1964;244-264.

86. Reed MB, Domenech P, Manca C, Su H, Barczak AK, Kreiswirth BN, Kaplan G, Barry CE, III. A glycolipid of hypervirulent tuberculosis strains that inhibits the innate immune response. *Nature* 2004; 431(7004):84-87.

87. Valway SE, Sanchez MP, Shinnick TF, Orme I, Agerton T, Hoy D, Jones JS, Westmoreland H, Onorato IM. An outbreak involving extensive transmission of a virulent strain of Mycobacterium tuberculosis. *N Engl J Med* 1998; 338(10):633-639.

88. Yu GP, Hsieh CC, Peng J. Risk factors associated with the prevalence of pulmonary tuberculosis among sanitary workers in Shanghai. *Tubercle* 1988; 69(2):105-112.

89. Lowe CR. An association between smoking and respiratory tuberculosis. *Br Med J* 1956; 2(5001):1081-1086.

90. Jacobson JM. Alcoholism and tuberculosis. *Alcohol Health & Research World* 21992; 16:39-45.

91. Szabo G. Alcohol and susceptibility to tuberculosis. *Alcohol Health & Research World* 1997; 21:39-41.

92. Rehm J, Samokhvalov AV, Neuman MG, Room R, Parry C, Lonnroth K, Patra J, Poznyak V, Popova S. The association between alcohol use, alcohol use disorders and tuberculosis (TB). A systematic review. *BMC Public Health* 2009; 9:450.

93. Tverdal A. Body mass index and incidence of tuberculosis. *Eur J Respir Dis* 1986; 69(5):355-362.

94. Gupta KB, Gupta R, Atreja A, Verma M, Vishvkarma S. Tuberculosis and nutrition. *Lung India* 2009; 26(1):9-16.

95. Floyd K and Wilkinson D. Tuberculosis in the HIV / AIDS era: interactions, impacts and solutions. *AIDS Anal Afr* 1997; 7(5):5-7.

96. Toossi Z. Virological and immunological impact of tuberculosis on human immunodeficiency virus type 1 disease. *J Infect Dis* 2003; 188(8):1146-1155.

97. Sheriff FG, Manji KP, Manji MP, Chagani MM, Mpembeni RM, Jusabani AM, Alwani ZR, Karimjee TS. Latent tuberculosis among pregnant mothers in a resource poor setting in Northern Tanzania: a cross-sectional study. *BMC Infect Dis* 2010; 10:52.

98. Suwanagool S, Chuenarom V, Pechthanon L, Sonjai A, Leelarasamee A, Pathrakom C. A comparative study of tuberculin skin test reactivity between asymptomatic HIV-1 seropositive subjects and healthy volunteers. *Asian Pac J Allergy Immunol* 1995; 13(2):139-144.

99. Cobelens FG, Egwaga SM, van Ginkel T, Muwinge H, Matee MI, Borgdorff MW. Tuberculin skin testing in patients with HIV infection: limited benefit of reduced cutoff values. *Clin Infect Dis* 2006; 43(5):634-639.

100. Markowitz N, Hansen NI, Wilcosky TC, Hopewell PC, Glassroth J, Kvale PA, Mangura BT, Osmond D, Wallace JM, Rosen MJ, Reichman LB. Tuberculin and anergy testing in HIV-seropositive and HIV-seronegative persons. Pulmonary Complications of HIV Infection Study Group. *Ann Intern Med* 1993; 119(3):185-193.

101. Huebner RE, Schein MF, Hall CA, Barnes SA. Delayed-type hypersensitivity anergy in human immunodeficiency virus-infected persons screened for infection with Mycobacterium tuberculosis. *Clin Infect Dis* 1994; 19(1):26-32.

102. Daley CL, Small PM, Schecter GF, Schoolnik GK, McAdam RA, Jacobs WR, Jr., Hopewell PC. An outbreak of tuberculosis with accelerated progression among persons infected with the human immunodeficiency virus. An analysis using restriction-fragment-length polymorphisms. *N Engl J Med* 1992; 326(4):231-235.

103. Schluger NW and Rom WN. The host immune response to tuberculosis. *Am J Respir Crit Care Med* 1998; 157(3 Pt 1):679-691.

104. Lawn SD, Butera ST, Shinnick TM. Tuberculosis unleashed: the impact of human immunodeficiency virus infection on the host granulomatous response to Mycobacterium tuberculosis. *Microbes Infect* 2002; 4(6):635-646.

105. Orme IM and Cooper AM. Cytokine/chemokine cascades in immunity to tuberculosis. *Immunol Today* 1999; 20(7):307-312.

106. Lawn SD, Bekker LG, Miller RF. Immune reconstitution disease associated with mycobacterial infections in HIV-infected individuals receiving antiretrovirals. *Lancet Infect Dis* 2005; 5(6):361-373.

107. Saunders BM and Cooper AM. Restraining mycobacteria: role of granulomas in mycobacterial infections. *Immunol Cell Biol* 2000; 78(4):334-341.

108. May M, Wood R, Myer L, Taffe P, Rauch A, Battegay M, Egger M. CD4(+) T cell count decreases by ethnicity among untreated patients with HIV infection in South Africa and Switzerland. *J Infect Dis* 2009; 200(11):1729-1735.

109. Clerici M, Stocks NI, Zajac RA, Boswell RN, Lucey DR, Via CS, Shearer GM. Detection of three distinct patterns of T helper cell dysfunction in asymptomatic, human immunodeficiency virus-seropositive patients. Independence of CD4+ cell numbers and clinical staging. *J Clin Invest* 1989; 84(6):1892-1899.

110. Lawn SD and Bekker LG. Co-pathogenesis of tuberculoss and HIV. *Tuberculosis. A Comprehensive Clinical Reference.* Edited by: Zumla & Schaaf. Elsevier Health Sciences Saunders, 2009;96.

111. Dooley SW, Villarino ME, Lawrence M, Salinas L, Amil S, Rullan JV, Jarvis WR, Bloch AB, Cauthen GM. Nosocomial transmission of tuberculosis in a hospital unit for HIV-infected patients. *JAMA* 1992; 267(19):2632-2634.

112. Badri M, Ehrlich R, Wood R, Pulerwitz T, Maartens G. Association between tuberculosis and HIV disease progression in a high tuberculosis prevalence area. *Int J Tuberc Lung Dis* 2001; 5(3):225-232.

113. Wood R, Maartens G, Lombard CJ. Risk factors for developing tuberculosis in HIV-1-infected adults from communities with a low or very high incidence of tuberculosis. *J Acquir Immune Defic Syndr* 2000; 23(1):75-80.

114. Styblo K, Dankova D, Drapela J, Galliova J, Jezek Z, Krivanek J, Kubik A, Langerova M, Radkovsky J. Epidemiological and Clinical Study of Tuberculosis in the District of Kolin, Czechoslovakia: report for the first 4 years of the study, 1961–64. *Bulletin of World Health Organization* 1967; 37:819-874.

115. Krivinka R, Drapela J, Kubik A, Dankova D, Krivanek J, Ruzha J, Mikova Z, Hejdova E. Epidemiological and Clinical Study of Tuberculosis in the District of Kolin, Czechoslovakia: second report (1965–72). *Bulletin of World Health Organization* 1974; 51:59-69.

116. Cauthen GM, Pio A, ten Dam HG. Annual risk of tuberculous infection.1988. *Bull World Health Organ* 2002; 80(6):503-511.

117. Murray CJ, Styblo K, Rouillon A. Tuberculosis in developing countries: burden, intervention and cost. *Bull Int Union Tuberc Lung Dis* 1990; 65(1):6-24.

118. Chum HJ, O'Brien RJ, Chonde TM, Graf P, Rieder HL. An epidemiological study of tuberculosis and HIV infection in Tanzania, 1991-1993. *AIDS* 1996; 10(3):299-309.

119. Havlir DV and Barnes PF. Tuberculosis in patients with human immunodeficiency virus infection. *N Engl J Med* 1999; 340(5):367-373.

120. Lee MP, Chan JW, Ng KK, Li PC. Clinical manifestations of tuberculosis in HIV-infected patients. *Respirology* 2000; 5(4):423-426.

121. Jones BE, Young SM, Antoniskis D, Davidson PT, Kramer F, Barnes PF. Relationship of the manifestations of tuberculosis to CD4 cell counts in patients with human immunodeficiency virus infection. *Am Rev Respir Dis* 1993; 148(5):1292-1297.

122. Brindle RJ, Nunn PP, Githui W, Allen BW, Gathua S, Waiyaki P. Quantitative bacillary response to treatment in HIV-associated pulmonary tuberculosis. *Am Rev Respir Dis* 1993; 147(4):958-961.

123. Mwandumba HC, Bertel SS, White SA, Nyirenda MH, Kampondeni SD, Rhoades ER, Zijlstra EE, Molyneux ME, Russell DG. Association between sputum smear status and local immune responses at the site of disease in HIV-infected patients with pulmonary tuberculosis. *Tuberculosis (Edinb)* 2008; 88(1):58-63.

124. Cauthen GM, Dooley SW, Onorato IM, Ihle WW, Burr JM, Bigler WJ, Witte J, Castro KG. Transmission of Mycobacterium tuberculosis from tuberculosis patients with HIV infection or AIDS. *Am J Epidemiol* 1996; 144(1):69-77.

125. Korenromp EL, Scano F, Williams BG, Dye C, Nunn P. Effects of human immunodeficiency virus infection on recurrence of tuberculosis after rifampin-based treatment: an analytical review. *Clin Infect Dis* 2003; 37(1):101-112.

126. Sonnenberg P, Murray J, Glynn JR, Shearer S, Kambashi B, Godfrey-Faussett P. HIV-1 and recurrence, relapse, and reinfection of tuberculosis after cure: a cohort study in South African mineworkers. *Lancet* 2001; 358(9294):1687-1693.

127. van Rie A, Warren R, Richardson M, Victor TC, Gie RP, Enarson DA, Beyers N, van Helden PD. Exogenous reinfection as a cause of recurrent tuberculosis after curative treatment. *N Engl J Med* 1999; 341(16):1174-1179.

128. Small PM, Shafer RW, Hopewell PC, Singh SP, Murphy MJ, Desmond E, Sierra MF, Schoolnik GK. Exogenous reinfection with multidrug-resistant Mycobacterium tuberculosis in patients with advanced HIV infection. *N Engl J Med* 1993; 328(16):1137-1144.

129. Fine PE and Small PM. Exogenous reinfection in tuberculosis. *N Engl J Med* 1999; 341(16):1226-1227.

130. Ansari NA, Kombe AH, Kenyon TA, Hone NM, Tappero JW, Nyirenda ST, Binkin NJ, Lucas SB. Pathology and causes of death in a group of 128 predominantly HIV-positive patients in Botswana, 1997-1998. *Int J Tuberc Lung Dis* 2002; 6(1):55-63.

131. Abouya YL, Beaumel A, Lucas S, Dago-Akribi A, Coulibaly G, N'Dhatz M, Konan JB, Yapi A, De Cock KM. Pneumocystis carinii pneumonia. An uncommon cause of death in African patients with acquired immunodeficiency syndrome. *Am Rev Respir Dis* 1992; 145(3):617-620.

132. Stoneburner R, Laroche E, Prevots R, Singh T, Blum S, Terry P, Reatrice S, Adler J. Survival in a cohort of human immunodeficiency virus-infected tuberculosis patients in New York City. Implications for the expansion of the AIDS case definition. *Arch Intern Med* 1992; 152(10):2033-2037.

133. Nunn P, Brindle R, Carpenter L, Odhiambo J, Wasunna K, Newnham R, Githui W, Gathua S, Omwega M, McAdam K. Cohort study of human immunodeficiency virus infection in patients with tuberculosis in Nairobi, Kenya. Analysis of early (6-month) mortality. *Am Rev Respir Dis* 1992; 146(4):849-854.

134. Mukadi YD, Maher D, Harries A. Tuberculosis case fatality rates in high HIV prevalence populations in sub-Saharan Africa. *AIDS* 2001; 15(2):143-152.

135. Whalen C, Horsburgh CR, Hom D, Lahart C, Simberkoff M, Ellner J. Accelerated course of human immunodeficiency virus infection after tuberculosis. *Am J Respir Crit Care Med* 1995; 151(1):129-135.

136. Toossi Z, Mayanja-Kizza H, Hirsch CS, Edmonds KL, Spahlinger T, Hom DL, Aung H, Mugyenyi P, Ellner JJ, Whalen CW. Impact of tuberculosis (TB) on HIV-1 activity in dually infected patients. *Clin Exp Immunol* 2001; 123(2):233-238.

137. Goletti D, Weissman D, Jackson RW, Graham NM, Vlahov D, Klein RS, Munsiff SS, Ortona L, Cauda R, Fauci AS. Effect of Mycobacterium tuberculosis on HIV replication. Role of immune activation. *J Immunol* 1996; 157(3):1271-1278.

138. Donovan RM, Bush CE, Markowitz NP, Baxa DM, Saravolatz LD. Changes in virus load markers during AIDS-associated opportunistic diseases in human immunodeficiency virus-infected persons. *J Infect Dis* 1996; 174(2):401-403.

139. del Amo J, Malin AS, Pozniak A, De Cock KM. Does tuberculosis accelerate the progression of HIV disease? Evidence from basic science and epidemiology. *AIDS* 1999; 13(10):1151-1158.

140. Day JH, Grant AD, Fielding KL, Morris L, Moloi V, Charalambous S, Puren AJ, Chaisson RE, De Cock KM, Hayes RJ, Churchyard GJ. Does tuberculosis increase HIV load? *J Infect Dis* 2004; 190(9):1677-1684.

141. Lawn SD, Shattock RJ, Acheampong JW, Lal RB, Folks TM, Griffin GE, Butera ST. Sustained plasma TNF-alpha and HIV-1 load despite resolution of other parameters of immune activation during treatment of tuberculosis in Africans. *AIDS* 1999; 13(16):2231-2237.

142. Bekker LG, Maartens G, Steyn L, Kaplan G. Selective increase in plasma tumor necrosis factor-alpha and concomitant clinical deterioration after initiating therapy in patients with severe tuberculosis. *J Infect Dis* 1998; 178(2):580-584.

143. Munsiff SS, Alpert PL, Gourevitch MN, Chang CJ, Klein RS. A prospective study of tuberculosis and HIV disease progression. *J Acquir Immune Defic Syndr Hum Retrovirol* 1998; 19(4):361-366.

144. Leroy V, Salmi LR, Dupon M, Sentilhes A, Texier-Maugein J, Dequae L, Dabis F, Salamon R. Progression of human immunodeficiency virus infection in patients with tuberculosis disease. A cohort study in Bordeaux, France, 1988-1994. The Groupe d'Epidemiologie Clinique du Sida en Aquitaine (GECSA). *Am J Epidemiol* 1997; 145(4):293-300.

145. Perneger TV, Sudre P, Lundgren JD, Hirschel B. Does the onset of tuberculosis in AIDS predict shorter survival? Results of a cohort study in 17 European countries over 13 years. AIDS in Europe Study Group. *BMJ* 1995; 311(7018):1468-1471.

146. Lederman MM. Immune restoration and CD4+ T-cell function with antiretroviral therapies. *AIDS* 2001; 15 Suppl 2:S11-S15.

147. Autran B, Carcelain G, Li TS, Blanc C, Mathez D, Tubiana R, Katlama C, Debre P, Leibowitch J. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. *Science* 1997; 277(5322):112-116.

148. Gandhi RT, Spritzler J, Chan E, Asmuth DM, Rodriguez B, Merigan TC, Hirsch MS, Shafer RW, Robbins GK, Pollard RB. Effect of baseline- and treatment-related factors on immunologic recovery after initiation of antiretroviral therapy in HIV-1-positive subjects: results from ACTG 384. *J Acquir Immune Defic Syndr* 2006; 42(4):426-434.

149. Lange CG, Valdez H, Medvik K, Asaad R, Lederman MM. CD4+ T-lymphocyte nadir and the effect of highly active antiretroviral therapy on phenotypic and functional immune restoration in HIV-1 infection. *Clin Immunol* 2002; 102(2):154-161.

150. Lange CG and Lederman MM. Immune reconstitution with antiretroviral therapies in chronic HIV-1 infection. *J Antimicrob Chemother* 2003; 51(1):1-4.

151. Mocroft A, Vella S, Benfield TL, Chiesi A, Miller V, Gargalianos P, d'Arminio MA, Yust I, Bruun JN, Phillips AN, Lundgren JD. Changing patterns of mortality across Europe in patients infected with HIV-1. EuroSIDA Study Group. *Lancet* 1998; 352(9142):1725-1730.

152. Lawn SD, Myer L, Edwards D, Bekker LG, Wood R. Short-term and long-term risk of tuberculosis associated with CD4 cell recovery during antiretroviral therapy in South Africa. *AIDS* 2009; 23(13):1717-1725.

153. Narain JP, Raviglione MC, Kochi A. HIV-associated tuberculosis in developing countries: epidemiology and strategies for prevention. *Tuber Lung Dis* 1992; 73(6):311-321.

154. Girardi E, Antonucci G, Vanacore P, Libanore M, Errante I, Matteelli A, Ippolito G. Impact of combination antiretroviral therapy on the risk of tuberculosis among persons with HIV infection. *AIDS* 2000; 14(13):1985-1991.

155. Jones JL, Hanson DL, Dworkin MS, DeCock KM. HIV-associated tuberculosis in the era of highly active antiretroviral therapy. The Adult/Adolescent Spectrum of HIV Disease Group. *Int J Tuberc Lung Dis* 2000; 4(11):1026-1031.

156. Golub JE, Pronyk P, Mohapi L, Thsabangu N, Moshabela M, Struthers H, Gray GE, McIntyre JA, Chaisson RE, Martinson NA. Isoniazid preventive therapy, HAART and tuberculosis risk in HIV-infected adults in South Africa: a prospective cohort. *AIDS* 2009; 23(5):631-636.

157. Moore D, Liechty C, Ekwaru P, Were W, Mwima G, Solberg P, Rutherford G, Mermin J. Prevalence, incidence and mortality associated with tuberculosis in HIV-infected patients initiating antiretroviral therapy in rural Uganda. *AIDS* 2007; 21(6):713-719.

158. Lawn SD, Bekker LG, Wood R. How effectively does HAART restore immune responses to Mycobacterium tuberculosis? Implications for tuberculosis control. *AIDS* 2005; 19(11):1113-1124.

159. Bonnet MM, Pinoges LL, Varaine FF, Oberhauser BB, O'Brien DD, Kebede YY, Hewison CC, Zachariah RR, Ferradini LL. Tuberculosis after HAART initiation in HIV-positive patients from five countries with a high tuberculosis burden. *AIDS* 2006; 20(9):1275-1279.

160. Dembele M, Saleri N, Carvalho AC, Saouadogo T, Hien AD, Zabsonre I, Koala ST, Simpore J, Matteelli A. Incidence of tuberculosis after HAART initiation in a

cohort of HIV-positive patients in Burkina Faso. *Int J Tuberc Lung Dis* 2010; 14(3):318-323.

161. Breen RA, Smith CJ, Cropley I, Johnson MA, Lipman MC. Does immune reconstitution syndrome promote active tuberculosis in patients receiving highly active antiretroviral therapy? *AIDS* 2005; 19(11):1201-1206.

162. Seyler C, Toure S, Messou E, Bonard D, Gabillard D, Anglaret X. Risk factors for active tuberculosis after antiretroviral treatment initiation in Abidjan. *Am J Respir Crit Care Med* 2005; 172(1):123-127.

163. Lawn SD, Harries AD, Wood R. Strategies to reduce early morbidity and mortality in adults receiving antiretroviral therapy in resource-limited settings. *Curr Opin HIV AIDS* 2010; 5(1):18-26.

164. Lawn SD and Wood R. Incidence of tuberculosis during highly active antiretroviral therapy in high-income and low-income countries. *Clin Infect Dis* 2005; 41(12):1783-1786.

165. Girardi E, Sabin CA, d'Arminio MA, Hogg B, Phillips AN, Gill MJ, Dabis F, Reiss P, Kirk O, Bernasconi E, Grabar S, Justice A, Staszewski S, Fatkenheuer G, Sterne JA. Incidence of Tuberculosis among HIV-infected patients receiving highly active antiretroviral therapy in Europe and North America. *Clin Infect Dis* 2005; 41(12):1772-1782.

166. Lawn SD, Wilkinson RJ, Lipman MC, Wood R. Immune reconstitution and "unmasking" of tuberculosis during antiretroviral therapy. *Am J Respir Crit Care Med* 2008; 177(7):680-685.

167. Park WB, Choe PG, Jo JH, Kim SH, Bang JH, Kim HB, Kim NJ, Oh MD, Choe KW. Tuberculosis manifested by immune reconstitution inflammatory syndrome during HAART. *AIDS* 2007; 21(7):875-877.

168. Meintjes G, Rabie H, Wilkinson RJ, Cotton MF. Tuberculosis-associated immune reconstitution inflammatory syndrome and unmasking of tuberculosis by antiretroviral therapy. *Clin Chest Med* 2009; 30(4):797-810.

169. Lawn SD, Kranzer K, Wood R. Antiretroviral therapy for control of the HIV-associated tuberculosis epidemic in resource-limited settings. *Clin Chest Med* 2009; 30(4):685-99, viii.

170. Golub JE, Durovni B, King BS, Cavalacante SC, Pacheco AG, Moulton LH, Moore RD, Chaisson RE, Saraceni V. Recurrent tuberculosis in HIV-infected patients in Rio de Janeiro, Brazil. *AIDS* 2008; 22(18):2527-2533.

171. Girardi E, Palmieri F, Cingolani A, Ammassari A, Petrosillo N, Gillini L, Zinzi D, De Luca A, Antinori A, Ippolito G. Changing clinical presentation and survival in HIV-associated tuberculosis after highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2001; 26(4):326-331.

172. Velasco M, Castilla V, Sanz J, Gaspar G, Condes E, Barros C, Cervero M, Torres R, Guijarro C. Effect of simultaneous use of highly active antiretroviral therapy on survival of HIV patients with tuberculosis. *J Acquir Immune Defic Syndr* 2009; 50(2):148-152.

173. Dheda K, Lampe FC, Johnson MA, Lipman MC. Outcome of HIV-associated tuberculosis in the era of highly active antiretroviral therapy. *J Infect Dis* 2004; 190(9):1670-1676.

174. Pacheco AG, Durovni B, Cavalcante SC, Lauria LM, Moore RD, Moulton LH, Chaisson RE, Golub JE. AIDS-related tuberculosis in Rio de Janeiro, Brazil. *PLoS One* 2008; 3(9):e3132.

175. Elliott AM, Hayes RJ, Halwiindi B, Luo N, Tembo G, Pobee JO, Nunn PP, McAdam KP. The impact of HIV on infectiousness of pulmonary tuberculosis: a community study in Zambia. *AIDS* 1993; 7(7):981-987.

176. Mohammad Z, Naing NN, Salleh R, Ahmad N, Hamzah WM, Mahmud R. A preliminary study of the influence of HIV infection in the transmission of tuberculosis. *Southeast Asian J Trop Med Public Health* 2002; 33(1):92-98.

177. Espinal MA, Perez EN, Baez J, Henriquez L, Fernandez K, Lopez M, Olivo P, Reingold AL. Infectiousness of Mycobacterium tuberculosis in HIV-1-infected patients with tuberculosis: a prospective study. *Lancet* 2000; 355(9200):275-280.

178. Corbett EL, Charalambous S, Moloi VM, Fielding K, Grant AD, Dye C, De Cock KM, Hayes RJ, Williams BG, Churchyard GJ. Human immunodeficiency virus and the prevalence of undiagnosed tuberculosis in African gold miners. *Am J Respir Crit Care Med* 2004; 170(6):673-679.

179. Wood R, Middelkoop K, Myer L, Grant AD, Whitelaw A, Lawn SD, Kaplan G, Huebner R, McIntyre J, Bekker LG. Undiagnosed tuberculosis in a community with high HIV prevalence: implications for tuberculosis control. *Am J Respir Crit Care Med* 2007; 175(1):87-93.

180. Cayla JA, Garcia dO, Galdos-Tanguis H, Vidal R, Lopez-Colomes JL, Gatell JM, Jansa JM. The influence of intravenous drug use and HIV infection in the transmission of tuberculosis. *AIDS* 1996; 10(1):95-100.

181. Carvalho AC, DeRiemer K, Nunes ZB, Martins M, Comelli M, Marinoni A, Kritski AL. Transmission of Mycobacterium tuberculosis to contacts of HIV-infected tuberculosis patients. *Am J Respir Crit Care Med* 2001; 164(12):2166-2171.

182. Middelkoop K, Bekker LG, Morrow C, Zwane E, Wood R. Childhood tuberculosis infection and disease: a spatial and temporal transmission analysis in a South African township. *S Afr Med J* 2009; 99(10):738-743.

183. Topley JM, Maher D, Mbewe LN. Transmission of tuberculosis to contacts of sputum positive adults in Malawi. *Arch Dis Child* 1996; 74(2):140-143.

184. Nunn P, Mungai M, Nyamwaya J, Gicheha C, Brindle RJ, Dunn DT, Githui W, Were JO, McAdam KP. The effect of human immunodeficiency virus type-1 on the infectiousness of tuberculosis. *Tuber Lung Dis* 1994; 75(1):25-32.

185. Klausner JD, Ryder RW, Baende E, Lelo U, Williame JC, Ngamboli K, Perriens JH, Kaboto M, Prignot J. Mycobacterium tuberculosis in household contacts of human immunodeficiency virus type 1-seropositive patients with active pulmonary tuberculosis in Kinshasa, Zaire. *J Infect Dis* 1993; 168(1):106-111.

186. Zahnow K, Matts JP, Hillman D, Finley E, Brown LS, Jr., Torres RA, Ernst J, El Sadr W, Perez G, Webster C, Barber B, Gordin FM. Rates of tuberculosis infection in healthcare workers providing services to HIV-infected populations. Terry Beirn Community Programs for Clinical Research on AIDS. *Infect Control Hosp Epidemiol* 1998; 19(11):829-835.

187. Cruciani M, Malena M, Bosco O, Gatti G, Serpelloni G. The impact of human immunodeficiency virus type 1 on infectiousness of tuberculosis: a meta-analysis. *Clin Infect Dis* 2001; 33(11):1922-1930.

188. Currie CS, Floyd K, Williams BG, Dye C. Cost, affordability and cost-effectiveness of strategies to control tuberculosis in countries with high HIV prevalence. *BMC Public Health* 2005; 5:130.

189. Currie CS, Williams BG, Cheng RC, Dye C. Tuberculosis epidemics driven by HIV: is prevention better than cure? *AIDS* 2003; 17(17):2501-2508.

190. Williams BG and Dye C. Antiretroviral drugs for tuberculosis control in the era of HIV/AIDS. *Science* 2003; 301(5639):1535-1537.

191. Williams BG, Granich R, Chauhan LS, Dharmshaktu NS, Dye C. The impact of HIV/AIDS on the control of tuberculosis in India. *Proc Natl Acad Sci U S A* 2005; 102(27):9619-9624.

192. Bacaer N, Ouifki R, Pretorius C, Wood R, Williams B. Modeling the joint epidemics of TB and HIV in a South African township. *J Math Biol* 2008.

193. Sanchez MS, Lloyd-Smith JO, Porco TC, Williams BG, Borgdorff MW, Mansoer J, Salomon JA, Getz WM. Impact of HIV on novel therapies for tuberculosis control. *AIDS* 2008; 22(8):963-972.

194. Chaisson RE and Martinson NA. Tuberculosis in Africa--combating an HIV-driven crisis. *N Engl J Med* 2008; 358(11):1089-1092.

195. The South African Tuberculosis Control Programme. Practical Guidelines.Pretoria, South Africa: South African Department of Health. 2004; http://www.capegateway.gov.za/Text/2003/tb_guidelines2000.pdf

196. Department of Health RoSA. National Tuberculosis Management Guidelines. 2009; http://familymedicine.ukzn.ac.za/Uploads/1d49d8e8-6720-4566-b292-9e2f6a212c06/TB%20Guidelines%202009.pdf

197. Middelkoop K, Bekker LG, Myer L, Whitelaw A, Grant A, Kaplan G, McIntyre J, Wood R. Antiretroviral program associated with reduction in untreated prevalent tuberculosis in a South african township. *Am J Respir Crit Care Med* 2010; 182(8):1080-1085.

198. Johnson L.F. and Dorrington R.E. Modelling the demographic impact of HIV/AIDS in South Africa and the likely impact of interventions. *Demographic Research* 2006; 14:541-574.

199. South African Department of Health. National Antiretroviral Treatment Guidelines. 2004; http://www.hst.org.za/uploads/files/sa_ART_Guidelines1.pdf

200. 1993 Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults. *MMWR Recomm Rep* 1992; 41(RR17):1-19; http://www.cdc.gov/mmwr/preview/mmwrhtml/00018871.htm

201. Sanne I, Orrell C, Fox MP, Conradie F, Ive P, Zeinecker J, Cornell M, Heiberg C, Ingram C, Panchia R, Rassool M, Gonin R, Stevens W, Truter H, Dehlinger M, van der HC, McIntyre J, Wood R. Nurse versus doctor management of HIV-infected patients receiving antiretroviral therapy (CIPRA-SA): a randomised non-inferiority trial. *Lancet* 2010; 376(9734):33-40.

202. Lawn SD, Myer L, Bekker LG, Wood R. CD4 cell count recovery among HIV-infected patients with very advanced immunodeficiency commencing antiretroviral treatment in sub-Saharan Africa. *BMC Infect Dis* 2006; 6:59.

203. World Health Organization and UNAIDS. Progress on Global Access to HIV Antiretroviral Therapy. An update on "3 by 5". 2005; http://www.who.int/hiv/pub/progressreports/3by5%20Progress%20Report_E_light.pdf

204. Bloch AB and Snider DE, Jr. How much tuberculosis in children must we accept? *Am J Public Health* 1986; 76(1):14-15.

205. Middelkoop K, Bekker LG, Myer L, Dawson R, Wood R. Rates of tuberculosis transmission to children and adolescents in a community with a high prevalence of HIV infection among adults. *Clin Infect Dis* 2008; 47(3):349-355.

206. Koch R. An Address on Bacteriological Research. *British Medical Journal* 1890; 2:380-383.

207. Von Pirquet C. Die Allergie probe zur Diagnose der Tuberkulose in Kindesalter. *Wien Med Wochenschr* 1907; 57:1369-1374.

208. Von Pirquet C. Frequency of tuberculosis in childhood. *JAMA* 1909; 52:675-678.

209. Menzies D, Schwartzman K, Pai M. Immune-based tests for tuberculosis. *Tuberculosis. A Comprehensive Clinical Reference.* Edited by: Zumla & Schaaf. Elsevier Health Sciences Saunders, 2009;179-196.

210. Huebner RE, Schein MF, Bass JB, Jr. The tuberculin skin test. *Clin Infect Dis* 1993; 17(6):968-975.

211. Menzies D. Interpretation of repeated tuberculin tests. Boosting, conversion, and reversion. *Am J Respir Crit Care Med* 1999; 159(1):15-21.

212. Fine PE, Bruce J, Ponnighaus JM, Nkhosa P, Harawa A, Vynnycky E. Tuberculin sensitivity: conversions and reversions in a rural African population. *Int J Tuberc Lung Dis* 1999; 3(11):962-975.

213. Beck JS. Skin changes in the tuberculin test. *Tubercle* 1991; 72(2):81-87.

214. Spiteri MA, Bowman A, Assefi AR, Clarke SW. Life threatening reaction to tuberculin testing. *Br Med J (Clin Res Ed)* 1986; 293(6541):243-244.

215. Tarlo SM, Day JH, Mann P, Day MP. Immediate hypersensitivity to tuberculin. In vivo and in vitro studies. *Chest* 1977; 71(1):33-37.

216. Rieder HL. Methodological issues in the estimation of the tuberculosis problem from tuberculin surveys. *Tuber Lung Dis* 1995; 76(2):114-121.

217. Further studies of geographic variation in naturally acquired tuberculin sensitivity. *Bull World Health Organ* 1955; 12(1-2):63-83.

218. Roelsgaard E, Iversen E, Blocher C. Tuberculosis in Tropical Africa. An Epidemiological Study. *Bull World Health Organ* 1964; 30:459-518.

219. Woodruff CE and Chapman PT. Tuberculin sensitivity in elderly patients. *Am Rev Respir Dis* 1971; 104(2):261-263.

220. Lordi GM and Reichman LB. Tuberculin Skin Testing. *Tuberculosis and Nontuberculosis Mycobacterial Infections.* Edited by: Schlossberg, David. W.B. Saunders Company, Philadelphia. 1999;65-70.

221. Lordi GM and Reichman LB. Tuberculin Skin Testing. *Tuberculosis and Nontuberculosis Mycobacterial Infections.* Edited by: Schlossberg, David. W.B. Saunders Company, Philadelphia. 1999;65-70.

222. Harrison BD, Tugwell P, Fawcett IW. Tuberculin reaction in adult Nigerians with sputum-positive pulmonary tuberculosis. *Lancet* 1975; 1(7904):421-424.

223. Arnadottir T, Rieder HL, Trebucq A, Waaler HT. Guidelines for conducting tuberculin skin test surveys in high prevalence countries. *Tuber Lung Dis* 1996; 77 Suppl 1:1-19.

224. Edwards PQ. Tuberculin negative? *N Engl J Med* 1972; 286(7):373-374.

225. Perez-Stable EJ and Slutkin G. A demonstration of lack of variability among six tuberculin skin test readers. *Am J Public Health* 1985; 75(11):1341-1343.

226. Nyboe J. The efficacy of the tuberculin test: an analysis based on results from 33 countries. *Bull World Health Organ* 1960; 22:5-37.

227. Edwards LB and Palmer CE. Geographic variation in naturally acquired tuberculin sensitivity. *Lancet* 1953; 1(6750):54-57.

228. Abrahams EW. Tuberculin hypersensitivity following BCG vaccination in Brisbane school children. *Tubercle* 1979; 60(2):109-113.

229. Menzies R and Vissandjee B. Effect of bacille Calmette-Guerin vaccination on tuberculin reactivity. *Am Rev Respir Dis* 1992; 145(3):621-625.

230. Further studies of geographic variation in naturally acquired tuberculin sensitivity. *Bull World Health Organ* 1955; 12(1-2):63-83.

231. Chaparas SD, Vandiviere HM, Melvin I, Koch G, Becker C. Tuberculin test. Variability with the Mantoux procedure. *Am Rev Respir Dis* 1985; 132(1):175-177.

232. Farhat M, Greenaway C, Pai M, Menzies D. False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? *Int J Tuberc Lung Dis* 2006; 10(11):1192-1204.

233. Epidemiology. A Research Manual for South Africa. *Edition:2nd.* Edited by: Joubert, G and Ehrlich R. Oxford University Press, Southern Africa, 2007.

234. Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. *Ann Intern Med* 2007; 146(5):340-354.

235. Miranda C, Tomford JW, Gordon SM. Interferon-gamma-release assays: Better than tuberculin skin testing? *Cleve Clin J Med* 2010; 77(9):606-611.

236. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med* 2008; 149(3):177-184.

237. Kobashi Y, Shimizu H, Ohue Y, Mouri K, Obase Y, Miyashita N, Oka M. Comparison of T-cell interferon-gamma release assays for Mycobacterium tuberculosis-specific antigens in patients with active and latent tuberculosis. *Lung* 2010; 188(4):283-287.

238. Thompson NJ, Glassroth JL, Snider DE, Jr., Farer LS. The booster phenomenon in serial tuberculin testing. *Am Rev Respir Dis* 1979; 119(4):587-597.

239. Bleiker MA and Styblo K. [Annual rate of tuberculosis infection and its evolution in developing countries]. *Bull Int Union Tuberc* 1978; 53(4):313-316.

240. Styblo K. The First Round of the National Tuberculin Survey in Tanzania, 1983-1987. Progress report 1992 of the Tuberculosis Surveillance Research Unit. 1992; Vol. Volume 1. The Hague: KNCV.

241. Styblo K. The relationship between the risk of tuberculosis infection and the risk of developing infectious tuberculosis. *Bulletin of the International Union Against Tuberculosis* 1985; 60:117-119.

242. Rieder H. Annual risk of infection with Mycobacterium tuberculosis. *Eur Respir J* 2005; 25(1):181-185.

243. Cauthen GM, Rieder HL, Geiter LJ. A model of the relation between age-specific prevalence of tuberculosis infection and incidence of infectious tuberculosis: implications for screening policies. *Tuberculosis Surveillance Research Unit of the IUATLD Progress Report 1991, Volume 1 The Hague: Royal Netherlands Tuberculosis Association* 1991; 1-20.

244. Borgdorff MW. Annual risk of infection – time for an update? *Bulletin of World Health Organization* 2002; 80:501-502.

245. Podgor MJ and Leske MC. Estimating incidence from age-specific prevalence for irreversible diseases with differential mortality. *Stat Med* 1986; 5(6):573-578.

246. Leske MC, Ederer F, Podgor M. Estimating incidence from age-specific prevalence in glaucoma. *Am J Epidemiol* 1981; 113(5):606-613.

247. Nagelkerke N, Heisterkamp S, Borgdorff M, Broekmans J, Van Houwelingen H. Semi-parametric estimation of age-time specific infection incidence from serial prevalence data. *Stat Med* 1999; 18(3):307-320.

248. Williams B, Gouws E, Wilkinson D, Karim SA. Estimating HIV incidence rates from age prevalence data in epidemic situations. *Stat Med* 2001; 20(13):2003-2016.

249. Hallett TB, Zaba B, Todd J, Lopman B, Mwita W, Biraro S, Gregson S, Boerma JT. Estimating incidence from prevalence in generalised HIV epidemics: methods and validation. *PLoS Med* 2008; 5(4):e80.

250. Freeman J and Hutchison GB. Prevalence, incidence and duration. *Am J Epidemiol* 1980; 112(5):707-723.

251. Menzies D. Tuberculin surveys--why? *Int J Tuberc Lung Dis* 1998; 2(4):263-264.

252. Sutherland I. On the risk of infection. *Bull Int Union Tuberc Lung Dis* 1991; 66(4):189-191.

253. Egwaga SM, Cobelens FG, Muwinge H, Verhage C, Kalisvaart N, Borgdorff MW. The impact of the HIV epidemic on tuberculosis transmission in Tanzania. *AIDS* 2006; 20(6):915-921.

254. Migliori GB, Borghesi A, Spanevello A, Eriki P, Raviglione M, Maciocco G, Morandi A, Ballardini L, Neri M. Risk of infection and estimated incidence of tuberculosis in northern Uganda. *Eur Respir J* 1994; 7(5):946-953.

255. Odhiambo JA, Borgdorff MW, Kiambih FM, Kibuga DK, Kwamanga DO, Ng'ang'a L, Agwanda R, Kalisvaart NA, Misljenovic O, Nagelkerke NJ, Bosman M. Tuberculosis and the HIV epidemic: increasing annual risk of tuberculous infection in Kenya, 1986-1996. *Am J Public Health* 1999; 89(7):1078-1082.

256. Arabin G, Gartig D, Kleeberg HH. First tuberculosis prevalence survey in KwaZulu. *S Afr Med J* 1979; 56(11):434-438.

257. Fourie PB. The prevalence and annual rate of tuberculous infection in South Africa. *Tubercle* 1983; 64(3):181-192.

258. Glatthaar E, Arabin G, Kleeberg HH. The significance of the tuberculosis infection risk and its application in Pretoria. *S Afr Med J* 1978; 53(16):615-619.

259. UNAIDS report: AIDS Epidemic Update December. 2005; www.unaids.org/epi/2005/doc/report_pdf.asp

260. UNAIDS, World Health Organization, UNICEF. Epidemiological Fact Sheet on HIV and AIDS: Core data on epidemiology and response. Uganda. 2008; http://apps.who.int/globalatlas/predefinedReports/EFS2008/short/EFSCountryProfiles2008_UG.pdf

261. Trebucq A, Guerin N, Ali IH, Bernatas JJ, Sevre JP, Rieder HL. Prevalence and trends of infection with Mycobacterium tuberculosis in Djibouti, testing an alternative method. *Int J Tuberc Lung Dis* 2005; 9(10):1097-1104.

262. Salaniponi FM, Kwanjana J, Veen J, Misljenovic O, Borgdorff MW. Risk of infection with Mycobacterium tuberculosis in Malawi: national tuberculin survey 1994. *Int J Tuberc Lung Dis* 2004; 8(6):718-723.

263. Shanaube K, Sismanidis C, Ayles H, Beyers N, Schaap A, Lawrence KA, Barker A, Godfrey-Faussett P. Annual risk of tuberculous infection using different methods in communities with a high prevalence of TB and HIV in Zambia and South Africa. *PLoS One* 2009; 4(11):e7749.

264. Kritzinger FE, den Boon S, Verver S, Enarson DA, Lombard CJ, Borgdorff MW, Gie RP, Beyers N. No decrease in annual risk of tuberculosis infection in endemic area in Cape Town, South Africa. *Trop Med Int Health* 2009; 14(2):136-142.

265. Wood R, Liang H, Wu H, Middelkoop K, Oni T, Rangaka MX, Wilkinson RJ, Bekker LG, Lawn SD. Changing prevalence of tuberculosis infection with increasing age in high-burden townships in South Africa. *Int J Tuberc Lung Dis* 2010; 14(4):406-412.

266. Guidance for National Tuberculosis Programmes on the management of tuberculosis in children. Chapter 1: introduction and diagnosis of tuberculosis in children. *Int J Tuberc Lung Dis* 2006; 10(10):1091-1097.

267. Cox DR and Stuart A. Some quick sign tests for trend in location and dispersion. *Biometrika* 1955; 42(1-2):80-95.

268. Conover WJ. Practical Nonparametric Statistics. *Edition:3rd.* John Wiley & Sons, Inc., 1999;169-176.

269. Narain R. Interpretation of the repeat tuberculin test. *Tubercle* 1968; 49(1):92-103.

270. Bass JA, Jr. and Serio RA. The use of repeat skin tests to eliminate the booster phenomenon in serial tuberculin testing. *Am Rev Respir Dis* 1981; 123(4 Pt 1):394-396.

271. Center for Disease Control and ATS/CDC Statement Committee on Latent Tuberculosis Infection Membership List. Targeted Tuberculin Testing and Treatment of Latent Tuberculosis Infection. 2000; 49(RR106): 1-54. MMWR 2000; http://www.cdc.gov/mmwr/preview/mmwrhtml/rr4906a1.htm

272. Rangaka MX, Wilkinson KA, Seldon R, van Cutsem G, Meintjes GA, Morroni C, Mouton P, Diwakar L, Connell TG, Maartens G, Wilkinson RJ. Effect of HIV-1 infection on T-Cell-based and skin test detection of tuberculosis infection. *Am J Respir Crit Care Med* 2007; 175(5):514-520.

273. Lawn SD, Bangani N, Vogt M, Bekker LG, Badri M, Ntobongwana M, Dockrell HM, Wilkinson RJ, Wood R. Utility of interferon-gamma ELISPOT assay responses in highly tuberculosis-exposed patients with advanced HIV infection in South Africa. *BMC Infect Dis* 2007; 7:99.

274. Pretorius C, Bacaer N, Williams B, Wood R, Ouifki R. On the relationship between age, annual rate of infection, and prevalence of mycobacterium tuberculosis in a South African township. *Clin Infect Dis* 2009; 48(7):994-996.

275. Rieder HL. Editorial commentary: on the risk of being and becoming infected with Mycobacterium tuberculosis. *Clin Infect Dis* 2008; 47(3):356-357.

276. Sun SJ, Bennett DE, Flood J, Loeffler AM, Kammerer S, Ellis BA. Identifying the sources of tuberculosis in young children: a multistate investigation. *Emerg Infect Dis* 2002; 8(11):1216-1223.

277. Mossong J, Hens N, Jit M, Beutels P, Auranen K, Mikolajczyk R, Massari M, Salmaso S, Tomba GS, Wallinga J, Heijne J, Sadkowska-Todys M, Rosinska

M, Edmunds WJ. Social contacts and mixing patterns relevant to the spread of infectious diseases. *PLoS Med* 2008; 5(3):e74.

278. Borgdorff MW, Nagelkerke NJ, van Soolingen D, Broekmans JF. Transmission of tuberculosis between people of different ages in The Netherlands: an analysis using DNA fingerprinting. *Int J Tuberc Lung Dis* 1999; 3(3):202-206.

279. Jaspan HB, Berwick JR, Myer L, Mathews C, Flisher AJ, Wood R, Bekker LG. Adolescent HIV prevalence, sexual risk, and willingness to participate in HIV vaccine trials. *J Adolesc Health* 2006; 39(5):642-648.

280. Corbett EL, Bandason T, Duong T, Dauya E, Makamure B, Churchyard GJ, Williams BG, Munyati SS, Butterworth AE, Mason PR, Mungofa S, Hayes RJ. Comparison of two active case-finding strategies for community-based diagnosis of symptomatic smear-positive tuberculosis and control of infectious tuberculosis in Harare, Zimbabwe (DETECTB): a cluster-randomised trial. *Lancet* 2010; 376(9748):1244-1253.

281. Snider DE, Jr. and Cauthen GM. Tuberculin skin testing of hospital employees: infection, "boosting," and two-step testing. *Am J Infect Control* 1984; 12(6):305-311.

282. Al Mazrou AM. Booster effect of two-step tuberculin skin testing among hospital employees from areas with a high prevalence of tuberculosis. *Infect Control Hosp Epidemiol* 2004; 25(12):1117-1120.

283. Menzies R, Vissandjee B, Amyot D. Factors associated with tuberculin reactivity among the foreign-born in Montreal. *Am Rev Respir Dis* 1992; 146(3):752-756.

284. Menzies R, Vissandjee B, Rocher I, St Germain Y. The booster effect in two-step tuberculin testing among young adults in Montreal. *Ann Intern Med* 1994; 120(3):190-198.

285. Friedland IR. The booster effect with repeat tuberculin testing in children and its relationship to BCG vaccination. *S Afr Med J* 1990; 77(8):387-389.

286. Narain R, Nair SS, Rao GR, Chandrasekhar P, Lal P. Enhancing of tuberculin allergy by previous tuberculin testing. *Bull World Health Organ* 1966; 34(4):623-638.

287. Perez-Stable EJ, Flaherty D, Schecter G, Slutkin G, Hopewell PC. Conversion and reversion of tuberculin reactions in nursing home residents. *Am Rev Respir Dis* 1988; 137(4):801-804.

288. Riley RL. Transmission and Environmental Control of Tuberculosis. *Tuberculosis. A Comprehensive International Approach.* Edited by: Reichman, L. B. and Hershfield, E. S. Marcel Dekker, Inc, New York. 1993;123-136.

289. Ferrand RA, Corbett EL, Wood R, Hargrove J, Ndhlovu CE, Cowan FM, Gouws E, Williams BG. AIDS among older children and adolescents in Southern Africa: projecting the time course and magnitude of the epidemic. *AIDS* 2009; 23(15):2039-2046.

290. Rehle TM, Hallett TB, Shisana O, Pillay-van Wyk V, Zuma K, Carrara H, Jooste S. A decline in new HIV infections in South Africa: estimating HIV incidence from three national HIV surveys in 2002, 2005 and 2008. *PLoS One* 2010; 5(6):e11094.

291. Pettifor AE, Levandowski BA, MacPhail C, Padian NS, Cohen MS, Rees HV. Keep them in school: the importance of education as a protective factor against HIV infection among young South African women. *Int J Epidemiol* 2008; 37(6):1266-1273.

292. Pettifor AE, Rees HV, Kleinschmidt I, Steffenson AE, MacPhail C, Hlongwa-Madikizela L, Vermaak K, Padian NS. Young people's sexual health in South Africa: HIV prevalence and sexual behaviors from a nationally representative household survey. *AIDS* 2005; 19(14):1525-1534.

293. Great Britain Medical Research Council. BCG and vole bacillus vaccines in the prevention of tuberculosis in adolescence and early life. *British Medical Journal* 1963; 1:973-978.

294. Comstock GW and Palmer CE. Long-term results of BCG vaccination in the southern United States. *Am Rev Respir Dis* 1966; 93(2):171-183.

295. Narain R, Geser A, Jambunathan MV, Subramanian M. Some aspects of a tuberculosis prevalence survey in a South Indian district. *Bull World Health Organ* 1963; 29:641-664.

296. Chan PC, Chang LY, Wu YC, Lu CY, Kuo HS, Lee CY, Huang LM, Chen CJ. Age-specific cut-offs for the tuberculin skin test to detect latent tuberculosis in BCG-vaccinated children. *Int J Tuberc Lung Dis* 2008; 12(12):1401-1406.

297. Buchanan M and Fransman D. BCG Vaccination change over in South Africa. Child Health Unit. 2008; http://web.uct.ac.za/depts/chu/mch16f.rtf

298. Grindulis H, Baynham MI, Scott PH, Thompson RA, Wharton BA. Tuberculin response two years after BCG vaccination at birth. *Arch Dis Child* 1984; 59(7):614-619.

299. Menzies D. What does tuberculin reactivity after bacille Calmette-Guerin vaccination tell us? *Clin Infect Dis* 2000; 31 Suppl 3:S71-S74.

300. Lockman S, Tappero JW, Kenyon TA, Rumisha D, Huebner RE, Binkin NJ. Tuberculin reactivity in a pediatric population with high BCG vaccination coverage. *Int J Tuberc Lung Dis* 1999; 3(1):23-30.

301. National HIV and Syphilis Antenatal Sero-Prevalence Survey in South Africa 2005, Department of Health, Republic of South Africa. 2008.

302. Newell ML, Coovadia H, Cortina-Borja M, Rollins N, Gaillard P, Dabis F. Mortality of infected and uninfected infants born to HIV-infected mothers in Africa: a pooled analysis. *Lancet* 2004; 364(9441):1236-1243.

303. Loeffler AM. Pediatric tuberculosis. *Semin Respir Infect* 2003; 18(4):272-291.

304. Starke JR, Jacobs RF, Jereb J. Resurgence of tuberculosis in children. *J Pediatr* 1992; 120(6):839-855.

305. World Health Organization. Global tuberculosis control: surveillance, planning, financing. 2008; http://www.who.int/tb/publications/global_report/2008/download_centre/en/index.html

306. Feja K and Saiman L. Tuberculosis in children. *Clin Chest Med* 2005; 26(2):295-312, vii.

307. Eamranond P and Jaramillo E. Tuberculosis in children: reassessing the need for improved diagnosis in global control strategies. *Int J Tuberc Lung Dis* 2001; 5(7):594-603.

308. Saunders LD, Irwig LM, Wilson TD, Kahn A, Groeneveld H. Tuberculosis management in Soweto. *S Afr Med J* 1984; 66(9):330-333.

309. Marais BJ, Hesseling AC, Gie RP, Schaaf HS, Beyers N. The burden of childhood tuberculosis and the accuracy of community-based surveillance data. *Int J Tuberc Lung Dis* 2006; 10(3):259-263.

310. van Rie A, Beyers N, Gie RP, Kunneke M, Zietsman L, Donald PR. Childhood tuberculosis in an urban population in South Africa: burden and risk factor. *Arch Dis Child* 1999; 80(5):433-437.

311. Chintu C, Mudenda V, Lucas S, Nunn A, Lishimpi K, Maswahu D, Kasolo F, Mwaba P, Bhat G, Terunuma H, Zumla A. Lung diseases at necropsy in African children dying from respiratory illnesses: a descriptive necropsy study. *Lancet* 2002; 360(9338):985-990.

312. Jeena PM, Pillay P, Pillay T, Coovadia HM. Impact of HIV-1 co-infection on presentation and hospital-related mortality in children with culture proven pulmonary tuberculosis in Durban, South Africa. *Int J Tuberc Lung Dis* 2002; 6(8):672-678.

313. Lienhardt C, Sillah J, Fielding K, Donkor S, Manneh K, Warndorff D, Bennett S, McAdam K. Risk factors for tuberculosis infection in children in contact with infectious tuberculosis cases in the Gambia, West Africa. *Pediatrics* 2003; 111(5 Pt 1):e608-e614.

314. Marks SM, Taylor Z, Qualls NL, Shrestha-Kuwahara RJ, Wilce MA, Nguyen CH. Outcomes of contact investigations of infectious tuberculosis patients. *Am J Respir Crit Care Med* 2000; 162(6):2033-2038.

315. Nolan RJ, Jr. Childhood tuberculosis in North Carolina: a study of the opportunities for intervention in the transmission of tuberculosis to children. *Am J Public Health* 1986; 76(1):26-30.

316. Andersen S and Geser A. The distribution of tuberculous infection among households in African communities. *Bull World Health Organ* 1960; 22:39-60.

317. Marais BJ, Hesseling AC, Schaaf HS, Gie RP, van Helden PD, Warren RM. Mycobacterium tuberculosis transmission is not related to household genotype in a setting of high endemicity. *J Clin Microbiol* 2009; 47(5):1338-1343.

318. Schaaf HS, Michaelis IA, Richardson M, Booysen CN, Gie RP, Warren R, van Helden PD, Beyers N. Adult-to-child transmission of tuberculosis: household or community contact? *Int J Tuberc Lung Dis* 2003; 7(5):426-431.

319. Sinfield R, Nyirenda M, Haves S, Molyneux EM, Graham SM. Risk factors for TB infection and disease in young childhood contacts in Malawi. *Ann Trop Paediatr* 2006; 26(3):205-213.

320. Nguyen TH, Odermatt P, Slesak G, Barennes H. Risk of latent tuberculosis infection in children living in households with tuberculosis patients: a cross sectional survey in remote northern Lao People's Democratic Republic. *BMC Infect Dis* 2009; 9:96.

321. Lutong L and Bei Z. Association of prevalence of tuberculin reactions with closeness of contact among household contacts of new smear-positive pulmonary tuberculosis patients. *Int J Tuberc Lung Dis* 2000; 4(3):275-277.

322. Aissa K, Madhi F, Ronsin N, Delarocque F, Lecuyer A, Decludt B, Remus N, Abel L, Poirier C, Delacourt C. Evaluation of a model for efficient screening of tuberculosis contact subjects. *Am J Respir Crit Care Med* 2008; 177(9):1041-1047.

323. Beyers N, Gie RP, Schaaf HS, Van Zyl S, Talent JM, Nel ED, Donald PR. A prospective evaluation of children under the age of 5 years living in the same household as adults with recently diagnosed pulmonary tuberculosis. *Int J Tuberc Lung Dis* 1997; 1(1):38-43.

324. Saloojee H, De Maayer T, Garenne ML, Kahn K. What's new? Investigating risk factors for severe childhood malnutrition in a high HIV prevalence South African setting. *Scand J Public Health Suppl* 2007; 69:96-106.

325. Singh M, Mynak ML, Kumar L, Mathew JL, Jindal SK. Prevalence and risk factors for transmission of infection among children in household contact with adults having pulmonary tuberculosis. *Arch Dis Child* 2005; 90(6):624-628.

326. Guwatudde D, Nakakeeto M, Jones-Lopez EC, Maganda A, Chiunda A, Mugerwa RD, Ellner JJ, Bukenya G, Whalen CC. Tuberculosis in household contacts of infectious cases in Kampala, Uganda. *Am J Epidemiol* 2003; 158(9):887-898.

327. Egsmose T, Ang'awa JO, Poti SJ. The use of isoniazid among household contacts of open cases of pulmonary tuberculosis. *Bull World Health Organ* 1965; 33(3):419-433.

328. Zachariah R, Spielmann MP, Harries AD, Gomani P, Graham SM, Bakali E, Humblet P. Passive versus active tuberculosis case finding and isoniazid preventive therapy among household contacts in a rural district of Malawi. *Int J Tuberc Lung Dis* 2003; 7(11):1033-1039.

329. Gilpin TP and Hammond M. Active case-finding - for the whole community or for tuberculosis contacts only? *S Afr Med J* 1987; 72(4):260-262.

330. UNAIDS, World Health Organization, UNICEF. Epidemiological Fact Sheet on HIV and AIDS: Core data on epidemiology and response. Gambia. 2008; http://apps.who.int/globalatlas/predefinedReports/EFS2008/short/EFSCountryProfiles2008_GM.pdf

331. UNAIDS, World Health Organization, UNICEF. Epidemiological Fact Sheet on HIV and AIDS: Core data on epidemiology and response. Kenya. 2008; http://apps.who.int/globalatlas/predefinedReports/EFS2008/short/EFSCountryProfiles2008_KE.pdf

332. UNAIDS, World Health Organization, UNICEF. Epidemiological Fact Sheet on HIV and AIDS: Core data on epidemiology and response. Congo. 2008; http://apps.who.int/globalatlas/predefinedReports/EFS2008/short/EFSCountryProfiles2008_CG.pdf

333. UNAIDS, World Health Organization, UNICEF. Epidemiological Fact Sheet on HIV and AIDS: Core data on epidemiology and response. Zambia. 2008; http://apps.who.int/globalatlas/predefinedReports/EFS2008/short/EFSCountryProfiles2008_ZM.pdf

334. World Health Organization. Guidance for national tuberculosis programmes on the management of tuberculosis in children. 2006; http://whqlibdoc.who.int/hq/2006/WHO_HTM_TB_2006.371_eng.pdf

335. Claessens NJ, Gausi FF, Meijnen S, Weismuller MM, Salaniponi FM, Harries AD. Screening childhood contacts of patients with smear-positive pulmonary tuberculosis in Malawi. *Int J Tuberc Lung Dis* 2002; 6(4):362-364.

336. Schaaf HS, Marais BJ, Whitelaw A, Hesseling AC, Eley B, Hussey GD, Donald PR. Culture-confirmed childhood tuberculosis in Cape Town, South Africa: a review of 596 cases. *BMC Infect Dis* 2007; 7:140.

337. Verver S, Warren RM, Munch Z, Richardson M, van der Spuy GD, Borgdorff MW, Behr MA, Beyers N, van Helden PD. Proportion of tuberculosis transmission that takes place in households in a high-incidence area. *Lancet* 2004; 363(9404):212-214.

338. Wood R, Johnstone-Robertson S, Uys P, Hargrove J, Middelkoop K, Lawn SD, Bekker LG. Tuberculosis transmission to young children in a South African community: modeling household and community infection risks. *Clin Infect Dis* 2010; 51(4):401-408.

339. Storla DG, Yimer S, Bjune GA. A systematic review of delay in the diagnosis and treatment of tuberculosis. *BMC Public Health* 2008; 8:15.

340. Sreeramareddy CT, Panduru KV, Menten J, Van den EJ. Time delays in diagnosis of pulmonary tuberculosis: a systematic review of literature. *BMC Infect Dis* 2009; 9:91.

341. Pronyk RM, Makhubele MB, Hargreaves JR, Tollman SM, Hausler HP. Assessing health seeking behaviour among tuberculosis patients in rural South Africa. *Int J Tuberc Lung Dis* 2001; 5(7):619-627.

342. Meintjes G, Schoeman H, Morroni C, Wilson D, Maartens G. Patient and provider delay in tuberculosis suspects from communities with a high HIV prevalence in South Africa: a cross-sectional study. *BMC Infect Dis* 2008; 8:72.

343. Narang P, Tyagi NK, Mendiratta DK, Jajoo UN, Bharambhe MS, Nayar S. Prevalence of sputum-positive pulmonary tuberculosis in tribal and non-tribal populations of the Ashti and Karanja tahsils in Wardha district, Maharashtra State, India. *Int J Tuberc Lung Dis* 1999; 3(6):478-482.

344. Demissie M, Zenebere B, Berhane Y, Lindtjorn B. A rapid survey to determine the prevalence of smear-positive tuberculosis in Addis Ababa. *Int J Tuberc Lung Dis* 2002; 6(7):580-584.

345. Sullam PM, Slutkin G, Hopewell PC. The benefits of evaluating close associates of child tuberculin reactors from a high prevalence group. *Am J Public Health* 1986; 76(9):1109-1111.

346. Lobato MN, Royce SE, Mohle-Boetani JC. Yield of source-case and contact investigations in identifying previously undiagnosed childhood tuberculosis. *Int J Tuberc Lung Dis* 2003; 7(12 Suppl 3):S391-S396.

347. Claessens NJ, Gausi FF, Meijnen S, Weismuller MM, Salaniponi FM, Harries AD. High frequency of tuberculosis in households of index TB patients. *Int J Tuberc Lung Dis* 2002; 6(3):266-269.

348. Becerra MC, Pachao-Torreblanca IF, Bayona J, Celi R, Shin SS, Kim JY, Farmer PE, Murray M. Expanding tuberculosis case detection by screening household contacts. *Public Health Rep* 2005; 120(3):271-277.

349. Morrison J, Pai M, Hopewell PC. Tuberculosis and latent tuberculosis infection in close contacts of people with pulmonary tuberculosis in low-income and middle-income countries: a systematic review and meta-analysis. *Lancet Infect Dis* 2008; 8(6):359-368.

350. Alland D, Kalkut GE, Moss AR, McAdam RA, Hahn JA, Bosworth W, Drucker E, Bloom BR. Transmission of tuberculosis in New York City. An analysis by DNA fingerprinting and conventional epidemiologic methods. *N Engl J Med* 1994; 330(24):1710-1716.

351. Small PM, Hopewell PC, Singh SP, Paz A, Parsonnet J, Ruston DC, Schecter GF, Daley CL, Schoolnik GK. The epidemiology of tuberculosis in San Francisco. A population-based study using conventional and molecular methods. *N Engl J Med* 1994; 330(24):1703-1709.

352. Bauer J, Yang Z, Poulsen S, Andersen AB. Results from 5 years of nationwide DNA fingerprinting of Mycobacterium tuberculosis complex isolates in a country with a low incidence of M. tuberculosis infection. *J Clin Microbiol* 1998; 36(1):305-308.

353. van Soolingen D, Borgdorff MW, de Haas PE, Sebek MM, Veen J, Dessens M, Kremer K, van Embden JD. Molecular epidemiology of tuberculosis in the Netherlands: a nationwide study from 1993 through 1997. *J Infect Dis* 1999; 180(3):726-736.

354. Borgdorff MW, Nagelkerke N, van Soolingen D, de Haas PE, Veen J, van Embden JD. Analysis of tuberculosis transmission between nationalities in the Netherlands in the period 1993-1995 using DNA fingerprinting. *Am J Epidemiol* 1998; 147(2):187-195.

355. Caminero JA, Pena MJ, Campos-Herrero MI, Rodriguez JC, Afonso O, Martin C, Pavon JM, Torres MJ, Burgos M, Cabrera P, Small PM, Enarson DA. Exogenous reinfection with tuberculosis on a European island with a moderate incidence of disease. *Am J Respir Crit Care Med* 2001; 163(3 Pt 1):717-720.

356. Glynn JR, Crampin AC, Yates MD, Traore H, Mwaungulu FD, Ngwira BM, Ndlovu R, Drobniewski F, Fine PE. The importance of recent infection with Mycobacterium tuberculosis in an area with high HIV prevalence: a long-term molecular epidemiological study in Northern Malawi. *J Infect Dis* 2005; 192(3):480-487.

357. Lockman S, Sheppard JD, Braden CR, Mwasekaga MJ, Woodley CL, Kenyon TA, Binkin NJ, Steinman M, Montsho F, Kesupile-Reed M, Hirschfeldt C, Notha M, Moeti T, Tappero JW. Molecular and conventional epidemiology of Mycobacterium tuberculosis in Botswana: a population-based prospective study of 301 pulmonary tuberculosis patients. *J Clin Microbiol* 2001; 39(3):1042-1047.

358. Wilkinson D, Pillay M, Crump J, Lombard C, Davies GR, Sturm AW. Molecular epidemiology and transmission dynamics of Mycobacterium tuberculosis in rural Africa. *Trop Med Int Health* 1997; 2(8):747-753.

359. Verver S, Warren RM, Munch Z, Vynnycky E, van Helden PD, Richardson M, van der Spuy GD, Enarson DA, Borgdorff MW, Behr MA, Beyers N. Transmission of tuberculosis in a high incidence urban community in South Africa. *Int J Epidemiol* 2004; 33(2):351-357.

360. Warren R, Richardson M, van der SG, Victor T, Sampson S, Beyers N, van Helden P. DNA fingerprinting and molecular epidemiology of tuberculosis: use and interpretation in an epidemic setting. *Electrophoresis* 1999; 20(8):1807-1812.

361. Middelkoop K, Bekker LG, Mathema B, Shashkina E, Kurepina N, Whitelaw A, Fallows D, Morrow C, Kreiswirth B, Kaplan G, Wood R. Molecular epidemiology of Mycobacterium tuberculosis in a South African community with high HIV prevalence. *J Infect Dis* 2009; 200(8):1207-1211.

362. Hall A. What is molecular epidemiology? *Trop Med Int Health* 1996; 1(4):407-408.

363. Mathema B, Kurepina NE, Bifani PJ, Kreiswirth BN. Molecular epidemiology of tuberculosis: current insights. *Clin Microbiol Rev* 2006; 19(4):658-685.

364. Niobe-Eyangoh SN, Kuaban C, Sorlin P, Cunin P, Thonnon J, Sola C, Rastogi N, Vincent V, Gutierrez MC. Genetic biodiversity of Mycobacterium tuberculosis complex strains from patients with pulmonary tuberculosis in Cameroon. *J Clin Microbiol* 2003; 41(6):2547-2553.

365. Warren R, Hauman J, Beyers N, Richardson M, Schaaf HS, Donald P, van Helden P. Unexpectedly high strain diversity of Mycobacterium tuberculosis in a high-incidence community. *S Afr Med J* 1996; 86(1):45-49.

366. Marais BJ, Victor TC, Hesseling AC, Barnard M, Jordaan A, Brittle W, Reuter H, Beyers N, van Helden PD, Warren RM, Schaaf HS. Beijing and Haarlem genotypes are overrepresented among children with drug-resistant tuberculosis in the Western Cape Province of South Africa. *J Clin Microbiol* 2006; 44(10):3539-3543.

367. Johnson R, Warren R, Strauss OJ, Jordaan AM, Falmer AA, Beyers N, Schaaf HS, Murray M, Cloete K, van Helden PD, Victor TC. An outbreak of drug-resistant tuberculosis caused by a Beijing strain in the Western Cape, South Africa. *Int J Tuberc Lung Dis* 2006; 10(12):1412-1414.

368. Rhee JT, Piatek AS, Small PM, Harris LM, Chaparro SV, Kramer FR, Alland D. Molecular epidemiologic evaluation of transmissibility and virulence of Mycobacterium tuberculosis. *J Clin Microbiol* 1999; 37(6):1764-1770.

369. Kong Y, Cave MD, Zhang L, Foxman B, Marrs CF, Bates JH, Yang ZH. Association between Mycobacterium tuberculosis Beijing/W lineage strain infection and extrathoracic tuberculosis: Insights from epidemiologic and clinical characterization of the three principal genetic groups of M. tuberculosis clinical isolates. *J Clin Microbiol* 2007; 45(2):409-414.

370. Theus SA, Cave MD, Eisenach K, Walrath J, Lee H, Mackay W, Whalen C, Silver RF. Differences in the growth of paired Ugandan isolates of Mycobacterium tuberculosis within human mononuclear phagocytes correlate with epidemiological evidence of strain virulence. *Infect Immun* 2006; 74(12):6865-6876.

371. van Crevel R, Nelwan RH, de Lenne W, Veeraragu Y, van der Zanden AG, Amin Z, van der Meer JW, van Soolingen D. Mycobacterium tuberculosis Beijing genotype strains associated with febrile response to treatment. *Emerg Infect Dis* 2001; 7(5):880-883.

372. Mathema B, Bifani PJ, Driscoll J, Steinlein L, Kurepina N, Moghazeh SL, Shashkina E, Marras SA, Campbell S, Mangura B, Shilkret K, Crawford JT, Frothingham R, Kreiswirth BN. Identification and evolution of an IS6110 low-copy-number Mycobacterium tuberculosis cluster. *J Infect Dis* 2002; 185(5):641-649.

373. Barnes PF and Cave MD. Molecular epidemiology of tuberculosis. *N Engl J Med* 2003; 349(12):1149-1156.

374. Gandhi NR, Moll A, Sturm AW, Pawinski R, Govender T, Lalloo U, Zeller K, Andrews J, Friedland G. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet* 2006; 368(9547):1575-1580.

375. Tsenova L, Ellison E, Harbacheuski R, Moreira AL, Kurepina N, Reed MB, Mathema B, Barry CE, III, Kaplan G. Virulence of selected Mycobacterium tuberculosis clinical isolates in the rabbit model of meningitis is dependent on phenolic glycolipid produced by the bacilli. *J Infect Dis* 2005; 192(1):98-106.

376. Manca C, Tsenova L, Bergtold A, Freeman S, Tovey M, Musser JM, Barry CE, III, Freedman VH, Kaplan G. Virulence of a Mycobacterium tuberculosis clinical isolate in mice is determined by failure to induce Th1 type immunity and is associated with induction of IFN-alpha /beta. *Proc Natl Acad Sci U S A* 2001; 98(10):5752-5757.

377. Manca C, Tsenova L, Barry CE, III, Bergtold A, Freeman S, Haslett PA, Musser JM, Freedman VH, Kaplan G. Mycobacterium tuberculosis CDC1551 induces a more vigorous host response in vivo and in vitro, but is not more virulent than other clinical isolates. *J Immunol* 1999; 162(11):6740-6746.

378. Newton SM, Smith RJ, Wilkinson KA, Nicol MP, Garton NJ, Staples KJ, Stewart GR, Wain JR, Martineau AR, Fandrich S, Smallie T, Foxwell B, Al Obaidi A, Shafi J, Rajakumar K, Kampmann B, Andrew PW, Ziegler-Heitbrock L, Barer MR, Wilkinson RJ. A deletion defining a common Asian lineage of Mycobacterium tuberculosis associates with immune subversion. *Proc Natl Acad Sci U S A* 2006; 103(42):15594-15598.

379. Lopez B, Aguilar D, Orozco H, Burger M, Espitia C, Ritacco V, Barrera L, Kremer K, Hernandez-Pando R, Huygen K, van Soolingen D. A marked difference in pathogenesis and immune response induced by different Mycobacterium tuberculosis genotypes. *Clin Exp Immunol* 2003; 133(1):30-37.

380. Ranjbar S, Boshoff HI, Mulder A, Siddiqi N, Rubin EJ, Goldfeld AE. HIV-1 replication is differentially regulated by distinct clinical strains of Mycobacterium tuberculosis. *PLoS One* 2009; 4(7):e6116.

381. Bifani PJ, Mathema B, Liu Z, Moghazeh SL, Shopsin B, Tempalski B, Driscol J, Frothingham R, Musser JM, Alcabes P, Kreiswirth BN. Identification of a W variant outbreak of Mycobacterium tuberculosis via population-based molecular epidemiology. *JAMA* 1999; 282(24):2321-2327.

382. Yaganehdoost A, Graviss EA, Ross MW, Adams GJ, Ramaswamy S, Wanger A, Frothingham R, Soini H, Musser JM. Complex transmission dynamics of

clonally related virulent Mycobacterium tuberculosis associated with barhopping by predominantly human immunodeficiency virus-positive gay men. *J Infect Dis* 1999; 180(4):1245-1251.

383. Bifani PJ, Plikaytis BB, Kapur V, Stockbauer K, Pan X, Lutfey ML, Moghazeh SL, Eisner W, Daniel TM, Kaplan MH, Crawford JT, Musser JM, Kreiswirth BN. Origin and interstate spread of a New York City multidrug-resistant Mycobacterium tuberculosis clone family. *JAMA* 1996; 275(6):452-457.

384. Bishai WR, Graham NM, Harrington S, Pope DS, Hooper N, Astemborski J, Sheely L, Vlahov D, Glass GE, Chaisson RE. Molecular and geographic patterns of tuberculosis transmission after 15 years of directly observed therapy. *JAMA* 1998; 280(19):1679-1684.

385. Braden CR, Templeton GL, Cave MD, Valway S, Onorato IM, Castro KG, Moers D, Yang Z, Stead WW, Bates JH. Interpretation of restriction fragment length polymorphism analysis of Mycobacterium tuberculosis isolates from a state with a large rural population. *J Infect Dis* 1997; 175(6):1446-1452.

386. van Soolingen D, de Haas PE, Hermans PW, Groenen PM, van Embden JD. Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of Mycobacterium tuberculosis. *J Clin Microbiol* 1993; 31(8):1987-1995.

387. Edlin BR, Tokars JI, Grieco MH, Crawford JT, Williams J, Sordillo EM, Ong KR, Kilburn JO, Dooley SW, Castro KG, . An outbreak of multidrug-resistant tuberculosis among hospitalized patients with the acquired immunodeficiency syndrome. *N Engl J Med* 1992; 326(23):1514-1521.

388. Wilkinson D, Crump J, Pillay M, Sturm AW. Nosocomial transmission of tuberculosis in Africa documented by restriction fragment length polymorphism. *Trans R Soc Trop Med Hyg* 1997; 91(3):318.

389. Pearson ML, Jereb JA, Frieden TR, Crawford JT, Davis BJ, Dooley SW, Jarvis WR. Nosocomial transmission of multidrug-resistant Mycobacterium tuberculosis. A risk to patients and health care workers. *Ann Intern Med* 1992; 117(3):191-196.

390. Godfrey-Faussett P, Githui W, Batchelor B, Brindle R, Paul J, Hawken M, Gathua S, Odhiambo J, Ojoo S, Nunn P, . Recurrence of HIV-related tuberculosis in an endemic area may be due to relapse or reinfection. *Tuber Lung Dis* 1994; 75(3):199-202.

391. Yang ZH, Mtoni I, Chonde M, Mwasekaga M, Fuursted K, Askgard DS, Bennedsen J, de Haas PE, van Soolingen D, van Embden JD, . DNA fingerprinting and phenotyping of Mycobacterium tuberculosis isolates from

human immunodeficiency virus (HIV)-seropositive and HIV-seronegative patients in Tanzania. *J Clin Microbiol* 1995; 33(5):1064-1069.

392. Chin DP, Crane CM, Diul MY, Sun SJ, Agraz R, Taylor S, Desmond E, Wise F. Spread of Mycobacterium tuberculosis in a community implementing recommended elements of tuberculosis control. *JAMA* 2000; 283(22):2968-2974.

393. Yang ZH, de Haas PE, Wachmann CH, van Soolingen D, van Embden JD, Andersen AB. Molecular epidemiology of tuberculosis in Denmark in 1992. *J Clin Microbiol* 1995; 33(8):2077-2081.

394. Classen CN, Warren R, Richardson M, Hauman JH, Gie RP, Ellis JH, van Helden PD, Beyers N. Impact of social interactions in the community on the transmission of tuberculosis in a high incidence area. *Thorax* 1999; 54(2):136-140.

395. Klovdahl AS, Graviss EA, Yaganehdoost A, Ross MW, Wanger A, Adams GJ, Musser JM. Networks and tuberculosis: an undetected community outbreak involving public places. *Soc Sci Med* 2001; 52(5):681-694.

396. Hughes AL, Friedman R, Murray M. Genomewide pattern of synonymous nucleotide substitution in two complete genomes of Mycobacterium tuberculosis. *Emerg Infect Dis* 2002; 8(11):1342-1346.

397. Borgdorff MW, Behr MA, Nagelkerke NJ, Hopewell PC, Small PM. Transmission of tuberculosis in San Francisco and its association with immigration and ethnicity. *Int J Tuberc Lung Dis* 2000; 4(4):287-294.

398. Barnes PF, Yang Z, Preston-Martin S, Pogoda JM, Jones BE, Otaya M, Eisenach KD, Knowles L, Harvey S, Cave MD. Patterns of tuberculosis transmission in Central Los Angeles. *JAMA* 1997; 278(14):1159-1163.

399. Jones TF, Craig AS, Valway SE, Woodley CL, Schaffner W. Transmission of tuberculosis in a jail. *Ann Intern Med* 1999; 131(8):557-563.

400. Glynn JR, Whiteley J, Bifani PJ, Kremer K, van Soolingen D. Worldwide occurrence of Beijing/W strains of Mycobacterium tuberculosis: a systematic review. *Emerg Infect Dis* 2002; 8(8):843-849.

401. Bifani PJ, Mathema B, Kurepina NE, Kreiswirth BN. Global dissemination of the Mycobacterium tuberculosis W-Beijing family strains. *Trends Microbiol* 2002; 10(1):45-52.

402. van der Spuy GD, Kremer K, Ndabambi SL, Beyers N, Dunbar R, Marais BJ, van Helden PD, Warren RM. Changing Mycobacterium tuberculosis population

highlights clade-specific pathogenic characteristics. *Tuberculosis (Edinb)* 2009; 89(2):120-125.

403. Cowley D, Govender D, February B, Wolfe M, Steyn L, Evans J, Wilkinson RJ, Nicol MP. Recent and rapid emergence of W-Beijing strains of Mycobacterium tuberculosis in Cape Town, South Africa. *Clin Infect Dis* 2008; 47(10):1252-1259.

404. Hanekom M, van der Spuy GD, Streicher E, Ndabambi SL, McEvoy CR, Kidd M, Beyers N, Victor TC, van Helden PD, Warren RM. A recently evolved sublineage of the Mycobacterium tuberculosis Beijing strain family is associated with an increased ability to spread and cause disease. *J Clin Microbiol* 2007; 45(5):1483-1490.

405. Bjune G. Tuberculosis in the 21st century: an emerging pandemic? *Norsk Epidemiologi* 2005; 15(2):133-139.

406. Reed MB, Gagneux S, DeRiemer K, Small PM, Barry CE, III. The W-Beijing lineage of Mycobacterium tuberculosis overproduces triglycerides and has the DosR dormancy regulon constitutively upregulated. *J Bacteriol* 2007; 189(7):2583-2589.

407. Frothingham R, Hills HG, Wilson KH. Extensive DNA sequence conservation throughout the Mycobacterium tuberculosis complex. *J Clin Microbiol* 1994; 32(7):1639-1643.

408. Cohn DL and O'Brien RJ. The use of restriction fragment length polymorphism (RFLP) analysis for epidemiological studies of tuberculosis in developing countries. *Int J Tuberc Lung Dis* 1998; 2(1):16-26.

409. Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rusch-Gerdes S, Willery E, Savine E, de Haas P, van Deutekom H, Roring S, Bifani P, Kurepina N, Kreiswirth B, Sola C, Rastogi N, Vatin V, Gutierrez MC, Fauville M, Niemann S, Skuce R, Kremer K, Locht C, van Soolingen D. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of Mycobacterium tuberculosis. *J Clin Microbiol* 2006; 44(12):4498-4510.

410. van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, Hermans P, Martin C, McAdam R, Shinnick TM, . Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. *J Clin Microbiol* 1993; 31(2):406-409.

411. Kremer K, van Soolingen D, Frothingham R, Haas WH, Hermans PW, Martin C, Palittapongarnpim P, Plikaytis BB, Riley LW, Yakrus MA, Musser JM, van Embden JD. Comparison of methods based on different molecular epidemiological markers for typing of Mycobacterium tuberculosis complex

strains: interlaboratory study of discriminatory power and reproducibility. *J Clin Microbiol* 1999; 37(8):2607-2618.

412. Chandler M. and Mahillon J. Insertion Sequences revisited. *Mobile DNA II.* Edited by: Craig N.L., Craigie R., Gellert M., and Lambowitz A.M. American Society for Microbiology, Washington D.C. 2002.

413. Thierry D, Cave MD, Eisenach KD, Crawford JT, Bates JH, Gicquel B, Guesdon JL. IS6110, an IS-like element of Mycobacterium tuberculosis complex. *Nucleic Acids Res* 1990; 18(1):188.

414. Kurepina NE, Sreevatsan S, Plikaytis BB, Bifani PJ, Connell ND, Donnelly RJ, van Sooligen D, Musser JM, Kreiswirth BN. Characterization of the phylogenetic distribution and chromosomal insertion sites of five IS6110 elements in Mycobacterium tuberculosis: non-random integration in the dnaA-dnaN region. *Tuber Lung Dis* 1998; 79(1):31-42.

415. van Soolingen D, de Haas PE, Hermans PW, van Embden JD. DNA fingerprinting of Mycobacterium tuberculosis. *Methods Enzymol* 1994; 235:196-205.

416. van Soolingen D. Molecular epidemiology of tuberculosis and other mycobacterial infections: main methodologies and achievements. *J Intern Med* 2001; 249(1):1-26.

417. van Embden JD, van Gorkom T, Kremer K, Jansen R, Der Zeijst BA, Schouls LM. Genetic variation and evolutionary origin of the direct repeat locus of Mycobacterium tuberculosis complex bacteria. *J Bacteriol* 2000; 182(9):2393-2401.

418. Kremer K, Glynn JR, Lillebaek T, Niemann S, Kurepina NE, Kreiswirth BN, Bifani PJ, van Soolingen D. Definition of the Beijing/W lineage of Mycobacterium tuberculosis on the basis of genetic markers. *J Clin Microbiol* 2004; 42(9):4040-4049.

419. Mazars E, Lesjean S, Banuls AL, Gilbert M, Vincent V, Gicquel B, Tibayrenc M, Locht C, Supply P. High-resolution minisatellite-based typing as a portable approach to global analysis of Mycobacterium tuberculosis molecular epidemiology. *Proc Natl Acad Sci U S A* 2001; 98(4):1901-1906.

420. Kremer K, Arnold C, Cataldi A, Gutierrez MC, Haas WH, Panaiotov S, Skuce RA, Supply P, van der Zanden AG, van Soolingen D. Discriminatory power and reproducibility of novel DNA typing methods for Mycobacterium tuberculosis complex strains. *J Clin Microbiol* 2005; 43(11):5628-5638.

421. Kremer K, Au BK, Yip PC, Skuce R, Supply P, Kam KM, van Soolingen D. Use of variable-number tandem-repeat typing to differentiate Mycobacterium tuberculosis Beijing family isolates from Hong Kong and comparison with IS6110 restriction fragment length polymorphism typing and spoligotyping. *J Clin Microbiol* 2005; 43(1):314-320.

422. Nguyen D, Brassard P, Menzies D, Thibert L, Warren R, Mostowy S, Behr M. Genomic characterization of an endemic Mycobacterium tuberculosis strain: evolutionary and epidemiologic implications. *J Clin Microbiol* 2004; 42(6):2573-2580.

423. Yeh RW, Ponce dL, Agasino CB, Hahn JA, Daley CL, Hopewell PC, Small PM. Stability of Mycobacterium tuberculosis DNA genotypes. *J Infect Dis* 1998; 177(4):1107-1111.

424. Niemann S, Richter E, Rusch-Gerdes S. Stability of IS6110 restriction fragment length polymorphism patterns of multidrug-resistant Mycobacterium tuberculosis strains. *J Clin Microbiol* 1999; 37(9):3078-3079.

425. de Boer AS, Borgdorff MW, de Haas PE, Nagelkerke NJ, van Embden JD, van Soolingen D. Analysis of rate of change of IS6110 RFLP patterns of Mycobacterium tuberculosis based on serial patient isolates. *J Infect Dis* 1999; 180(4):1238-1244.

426. Bauer J, Andersen AB, Kremer K, Miorner H. Usefulness of spoligotyping To discriminate IS6110 low-copy-number Mycobacterium tuberculosis complex strains cultured in Denmark. *J Clin Microbiol* 1999; 37(8):2602-2606.

427. Yang Z, Chaves F, Barnes PF, Burman WJ, Koehler J, Eisenach KD, Bates JH, Cave MD. Evaluation of method for secondary DNA typing of Mycobacterium tuberculosis with pTBN12 in epidemiologic study of tuberculosis. *J Clin Microbiol* 1996; 34(12):3044-3048.

428. Murray M. Sampling bias in the molecular epidemiology of tuberculosis. *Emerg Infect Dis* 2002; 8(4):363-369.

429. Glynn JR, Crampin AC, Ngwira BM, Mwaungulu FD, Mwafulirwa DT, Floyd S, Ponnighaus JM, Warndorff DK, Fine PE. Trends in tuberculosis and the influence of HIV infection in northern Malawi, 1988-2001. *AIDS* 2004; 18(10):1459-1463.

430. Asiimwe BB, Joloba ML, Ghebremichael S, Koivula T, Kateete DP, Katabazi FA, Pennhag A, Petersson R, Kallenius G. DNA restriction fragment length polymorphism analysis of Mycobacterium tuberculosis isolates from HIV-seropositive and HIV-seronegative patients in Kampala, Uganda. *BMC Infect Dis* 2009; 9:12.

431. Ellis BA, Crawford JT, Braden CR, McNabb SJ, Moore M, Kammerer S. Molecular epidemiology of tuberculosis in a sentinel surveillance population. *Emerg Infect Dis* 2002; 8(11):1197-1209.

432. Rhee JT, Tanaka MM, Behr MA, Agasino CB, Paz EA, Hopewell PC, Small PM. Use of multiple markers in population-based molecular epidemiologic studies of tuberculosis. *Int J Tuberc Lung Dis* 2000; 4(12):1111-1119.

433. Chan-Yeung M, Tam CM, Wong H, Leung CC, Wang J, Yew WW, Lam CW, Kam KM. Molecular and conventional epidemiology of tuberculosis in Hong Kong: a population-based prospective study. *J Clin Microbiol* 2003; 41(6):2706-2708.

434. Ferrazoli L, Palaci M, Marques LR, Jamal LF, Afiune JB, Chimara E, Martins MC, Silva Telles MA, Oliveira CA, Palhares MC, Spada DT, Riley LW. Transmission of tuberculosis in an endemic urban setting in Brazil. *Int J Tuberc Lung Dis* 2000; 4(1):18-25.

435. Tudo G, Gonzalez-Martin J, Obama R, Rodriguez JM, Franco JR, Espasa M, Simarro PP, Escaramis G, Ascaso C, Garcia A, Jimenez De Anta MT. Molecular epidemiology of tuberculosis in the Bata and Malabo districts of Equatorial Guinea. *Int J Tuberc Lung Dis* 2004; 8(12):1458-1463.

436. van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, Hermans P, Martin C, McAdam R, Shinnick TM, . Strain identification of Mycobacterium tuberculosis by DNA fingerprinting: recommendations for a standardized methodology. *J Clin Microbiol* 1993; 31(2):406-409.

437. Gutacker MM, Mathema B, Soini H, Shashkina E, Kreiswirth BN, Graviss EA, Musser JM. Single-nucleotide polymorphism-based population genetic analysis of Mycobacterium tuberculosis strains from 4 geographic sites. *J Infect Dis* 2006; 193(1):121-128.

438. van der Spuy GD, Kremer K, Ndabambi SL, Beyers N, Dunbar R, Marais BJ, van Helden PD, Warren RM. Changing Mycobacterium tuberculosis population highlights clade-specific pathogenic characteristics. *Tuberculosis (Edinb)* 2009; 89(2):120-125.

439. Anh DD, Borgdorff MW, Van LN, Lan NT, van Gorkom T, Kremer K, van Soolingen D. Mycobacterium tuberculosis Beijing genotype emerging in Vietnam. *Emerg Infect Dis* 2000; 6(3):302-305.

440. Drobniewski F, Balabanova Y, Nikolayevsky V, Ruddy M, Kuznetzov S, Zakharova S, Melentyev A, Fedorin I. Drug-resistant tuberculosis, clinical virulence, and the dominance of the Beijing strain family in Russia. *JAMA* 2005; 293(22):2726-2731.

441. Pfyffer GE, Strassle A, van Gorkum T, Portaels F, Rigouts L, Mathieu C, Mirzoyev F, Traore H, van Embden JD. Multidrug-resistant tuberculosis in prison inmates, Azerbaijan. *Emerg Infect Dis* 2001; 7(5):855-861.

442. Post FA, Willcox PA, Mathema B, Steyn LM, Shean K, Ramaswamy SV, Graviss EA, Shashkina E, Kreiswirth BN, Kaplan G. Genetic polymorphism in Mycobacterium tuberculosis isolates from patients with chronic multidrug-resistant tuberculosis. *J Infect Dis* 2004; 190(1):99-106.

443. Glynn JR, Vynnycky E, Fine PE. Influence of sampling on estimates of clustering and recent transmission of Mycobacterium tuberculosis derived from DNA fingerprinting techniques. *Am J Epidemiol* 1999; 149(4):366-371.

444. Vynnycky E, Nagelkerke N, Borgdorff MW, van Soolingen D, van Embden JD, Fine PE. The effect of age and study duration on the relationship between 'clustering' of DNA fingerprint patterns and the proportion of tuberculosis disease attributable to recent transmission. *Epidemiol Infect* 2001; 126(1):43-62.

445. van der Spuy GD, van Helden PD, Warren RM. Effect of study duration on the interpretation of tuberculosis molecular epidemiology investigations. *Tuberculosis (Edinb)* 2009; 89(3):238-242.

446. Dye C, Bassili A, Bierrenbach AL, Broekmans JF, Chadha VK, Glaziou P, Gopi PG, Hosseini M, Kim SJ, Manissero D, Onozaki I, Rieder HL, Scheele S, van Leth F, van der WM, Williams BG. Measuring tuberculosis burden, trends, and the impact of control programmes. *Lancet Infect Dis* 2008; 8(4):233-243.

447. Middelkoop K, Bekker LG, Myer L, Johnson L.F., Kloos M., Morrow C, Wood R. Antiretroviral therapy and TB notification rates in a high HIV prevalence South African community. *J Acquir Immune Defic Syndr* 2011; In press

448. McKeown T, Record RG, Turner RD. An interpretation of the decline of mortality in England and Wales during the twentieth century. *Popul Stud (Camb)* 1975; 29(3):391-422.

449. McKeown T and Record RG. Reasons for the Decline of Mortality in England and Wales during the Nineteenth Century. *Popul Stud (Camb)* 1962; 16(2):94-122.

450. Wilson LG. The historical decline of tuberculosis in Europe and America: its causes and significance. *J Hist Med Allied Sci* 1990; 45(3):366-396.

451. Selgelid M. Ethics, Tuberculosis and Globalization. *Public Health Ethics* 2008; 1(1):10-20.

452. Dye C. Tuberculosis 2000-2010: control, but not elimination. *Int J Tuberc Lung Dis* 2000; 4(12 Suppl 2):S146-S152.

453. Armstrong GL, Conn LA, Pinner RW. Trends in infectious disease mortality in the United States during the 20th century. *JAMA* 1999; 281(1):61-66.

454. Springett VH. An interpretation of statistical trends in tuberculosis. *Lancet* 1952; 1(6708):575-580.

455. Cobbett L. The Decline of Tuberculosis and the Increase in its Mortality during the War. *J Hyg (Lond)* 1930; 30(1):79-103.

456. United States Bureau of the Census. Historical Statistics of the United States, Colonial Times to 1970. 1975; US Government Printing Office, Washington DC.

457. Arthur Newsholme. The Prevention of Tuberculosis. *Edition 1.* Methuen & Co, London. 1908.

458. Davies RP, Tocque K, Bellis MA, Rimmington T, Davies PD. Historical declines in tuberculosis in England and Wales: improving social conditions or natural selection? *Int J Tuberc Lung Dis* 1999; 3(12):1051-1054.

459. Vynnycky E and Fine PE. Interpreting the decline in tuberculosis: the role of secular trends in effective contact. *Int J Epidemiol* 1999; 28(2):327-334.

460. McKeown T. The Role Of Medicine. Dream, Mirage or Nemesis. *Edition:2nd.* Basil Blackwell, Oxford. 1979.

461. World Health Organization: *The Global Plan to Stop TB, 2006-2015 Stop TB Partnership,* WHO Geneva, Switzerland. 2006; http://www.stoptb.org/globalplan/plan_p2main.asp?p=2http://www.stoptb.org/globalplan/plan_p2main.asp?p=2

462. World Health Organization. WHO Three I's Meeting: Intensified Case Finding (ICF), Isoniazid Preventive Therapy (IPT) and TB Infection Control (IC) for people living with HIV. 2008; http://www.who.int/hiv/pub/meetingreports/WHO_3Is_meeting_report.pdf

463. South African Department of Health. The South African Tuberculosis Control Programme. Practical Guidelines.Pretoria, South Africa. 2004; http://www.capegateway.gov.za/Text/2003/tb_guidelines2000.pdf

464. Health Systems Trust. Cape Town TB Control. Progress report 1997-2003. 2004; www.hst.org.za./publications/618

465. Actuarial Society of South Africa. ASSA2003 AIDS and Demographic model. 2005; http://aids.actuarialsociety.org.za/ASSA2003-Model-3165.htm

466. Breslow NE and Day NE. Statistical methods in cancer research. Volume II--The design and analysis of cohort studies. *IARC Sci Publ* 1987; (82):1-406.

467. Cochran WG. Some methods for strengthening the common X^2 test. *Biometrics* 1954; 10(4):417-451.

468. Zeinecker J, Morrow C, van Soelen N, Orrell C, Wood R. Missing the target despite high population levels of anti-retroviral therapy coverage in a South African community. *5th International AIDS Society Conference on HIV Pathogenesis, Treatment and Prevention* 2009; MOPED031; http://www.ias2009.org/mainpage.aspx?pageId=334

469. Clare van Halsema, Violet Chihota, James Lewis, Elizabeth Russell, Katherine Fielding, Gavin Churchyard, Alison Grant. The Effect of Antiretroviral Therapy on Tuberculosis Sputum Smear Status in HIV-infected Individuals in South Africa. *17th Conference on Retroviruses and Opportunistic Infections.* San Francisco. 2010; Abstract 775.

470. Akksilp S, Karnkawinpong O, Wattanaamornkiat W, Viriyakitja D, Monkongdee P, Sitti W, Rienthong D, Siraprapasiri T, Wells CD, Tappero JW, Varma JK. Antiretroviral therapy during tuberculosis treatment and marked reduction in death rate of HIV-infected patients, Thailand. *Emerg Infect Dis* 2007; 13(7):1001-1007.

471. Varma JK, Nateniyom S, Akksilp S, Mankatittham W, Sirinak C, Sattayawuthipong W, Burapat C, Kittikraisak W, Monkongdee P, Cain KP, Wells CD, Tappero JW. HIV care and treatment factors associated with improved survival during TB treatment in Thailand: an observational study. *BMC Infect Dis* 2009; 9:42.

472. Dye C, Lonnroth K, Jaramillo E, Williams BG, Raviglione M. Trends in tuberculosis incidence and their determinants in 134 countries. *Bull World Health Organ* 2009; 87(9):683-691.

473. Guwatudde D, Zalwango S, Kamya MR, Debanne SM, Diaz MI, Okwera A, Mugerwa RD, King C, Whalen CC. Burden of tuberculosis in Kampala, Uganda. *Bull World Health Organ* 2003; 81(11):799-805.

474. Pronyk PM, Kahn K, Tollman SM. Using health and demographic surveillance to understand the burden of disease in populations: the case of tuberculosis in rural South Africa. *Scand J Public Health Suppl* 2007; 69:45-51.

475. Corbett EL, Bandason T, Cheung YB, Makamure B, Dauya E, Munyati SS, Churchyard GJ, Williams BG, Butterworth AE, Mungofa S, Hayes RJ, Mason PR. Prevalent infectious tuberculosis in Harare, Zimbabwe: burden, risk factors and implications for control. *Int J Tuberc Lung Dis* 2009; 13(10):1231-1237.

476. Yimer S, Holm-Hansen C, Yimaldu T, Bjune G. Evaluating an active case-finding strategy to identify smear-positive tuberculosis in rural Ethiopia. *Int J Tuberc Lung Dis* 2009; 13(11):1399-1404.

477. Tupasi TE, Radhakrishna S, Co VM, Villa ML, Quelapio MI, Mangubat NV, Sarol JN, Rivera AB, Pascual ML, Reyes AC, Sarmiento A, Solon M, Solon FS, Burton L, Mantala MJ. Bacillary disease and health seeking behavior among Filipinos with symptoms of tuberculosis: implications for control. *Int J Tuberc Lung Dis* 2000; 4(12):1126-1132.

478. Needham DM, Bowman D, Foster SD, Godfrey-Faussett P. Patient care seeking barriers and tuberculosis programme reform: a qualitative study. *Health Policy* 2004; 67(1):93-106.

479. Kemp JR, Mann G, Simwaka BN, Salaniponi FM, Squire SB. Can Malawi's poor afford free tuberculosis services? Patient and household costs associated with a tuberculosis diagnosis in Lilongwe. *Bull World Health Organ* 2007; 85(8):580-585.

480. Glaziou P, van der Werf MJ, Onozaki I, Dye C, Borgdorff MW, Chiang CY, Cobelens F, Enarson DA, Gopi PG, Holtz TH, Kim SJ, van Leth F, Lew WJ, Lonnroth K, van Maaren P, Narayanan PR, Williams B. Tuberculosis prevalence surveys: rationale and cost. *Int J Tuberc Lung Dis* 2008; 12(9):1003-1008.

481. van der Werf MJ, Enarson DA, Borgdorff MW. How to identify tuberculosis cases in a prevalence survey. *Int J Tuberc Lung Dis* 2008; 12(11):1255-1260.

482. den Boon S, van Lill SW, Borgdorff MW, Enarson DA, Verver S, Bateman ED, Irusen E, Lombard CJ, White NW, de Villiers C, Beyers N. High prevalence of tuberculosis in previously treated patients, Cape Town, South Africa. *Emerg Infect Dis* 2007; 13(8):1189-1194.

483. Pronyk PM, Joshi B, Hargreaves JR, Madonsela T, Collinson MA, Mokoena O, Tollman SM, Hausler HR. Active case finding: understanding the burden of tuberculosis in rural South Africa. *Int J Tuberc Lung Dis* 2001; 5(7):611-618.

484. Jackson-Sillah D, Hill PC, Fox A, Brookes RH, Donkor SA, Lugos MD, Howie SR, Fielding KR, Jallow A, Lienhardt C, Corrah T, Adegbola RA, McAdam KP. Screening for tuberculosis among 2381 household contacts of sputum-smear-positive cases in The Gambia. *Trans R Soc Trop Med Hyg* 2007; 101(6):594-601.

485. Aluoch JA, Edwards EA, Stott H, Fox W, Sutherland I. A fourth study of case-finding methods for pulmonary tuberculosis in Kenya. *Trans R Soc Trop Med Hyg* 1982; 76(5):679-691.

486. Banda HT, Gausi F, Harries AD, Salaniponi FM. Prevalence of smear-positive pulmonary tuberculosis among prisoners in Malawi: a national survey. *Int J Tuberc Lung Dis* 2009; 13(12):1557-1559.

487. Habeenzu C, Mitarai S, Lubasi D, Mudenda V, Kantenga T, Mwansa J, Maslow JN. Tuberculosis and multidrug resistance in Zambian prisons, 2000-2001. *Int J Tuberc Lung Dis* 2007; 11(11):1216-1220.

488. Noeske J, Kuaban C, Amougou G, Piubello A, Pouillot R. Pulmonary tuberculosis in the Central Prison of Douala, Cameroon. *East Afr Med J* 2006; 83(1):25-30.

489. Nyangulu DS, Harries AD, Kang'ombe C, Yadidi AE, Chokani K, Cullinan T, Maher D, Nunn P, Salaniponi FM. Tuberculosis in a prison population in Malawi. *Lancet* 1997; 350(9087):1284-1287.

490. Rapid assessment of tuberculosis in a large prison system--Botswana, 2002. *MMWR Morb Mortal Wkly Rep* 2003; 52(12):250-252.

491. Girdler-Brown BV, White NW, Ehrlich RI, Churchyard GJ. The burden of silicosis, pulmonary tuberculosis and COPD among former Basotho goldminers. *Am J Ind Med* 2008; 51(9):640-647.

492. Day JH, Charalambous S, Fielding KL, Hayes RJ, Churchyard GJ, Grant AD. Screening for tuberculosis prior to isoniazid preventive therapy among HIV-infected gold miners in South Africa. *Int J Tuberc Lung Dis* 2006; 10(5):523-529.

493. Monkongdee P, McCarthy KD, Cain KP, Tasaneeyapan T, Nguyen HD, Nguyen TN, Nguyen TB, Teeratakulpisarn N, Udomsantisuk N, Heilig C, Varma JK. Yield of acid-fast smear and mycobacterial culture for tuberculosis diagnosis in people with human immunodeficiency virus. *Am J Respir Crit Care Med* 2009; 180(9):903-908.

494. Kali PB, Gray GE, Violari A, Chaisson RE, McIntyre JA, Martinson NA. Combining PMTCT with active case finding for tuberculosis. *J Acquir Immune Defic Syndr* 2006; 42(3):379-381.

495. Nachega J, Coetzee J, Adendorff T, Msandiwa R, Gray GE, McIntyre JA, Chaisson RE. Tuberculosis active case-finding in a mother-to-child HIV transmission prevention programme in Soweto, South Africa. *AIDS* 2003; 17(9):1398-1400.

496. Ngowi BJ, Mfinanga SG, Bruun JN, Morkve O. Pulmonary tuberculosis among people living with HIV/AIDS attending care and treatment in rural northern Tanzania. *BMC Public Health* 2008; 8:341.

497. Mtei L, Matee M, Herfort O, Bakari M, Horsburgh CR, Waddell R, Cole BF, Vuola JM, Tvaroha S, Kreiswirth B, Pallangyo K, von Reyn CF. High rates of clinical and subclinical tuberculosis among HIV-infected ambulatory subjects in Tanzania. *Clin Infect Dis* 2005; 40(10):1500-1507.

498. Mohammed A, Ehrlich R, Wood R, Cilliers F, Maartens G. Screening for tuberculosis in adults with advanced HIV infection prior to preventive therapy. *Int J Tuberc Lung Dis* 2004; 8(6):792-795.

499. Bakari M, Arbeit RD, Mtei L, Lyimo J, Waddell R, Matee M, Cole BF, Tvaroha S, Horsburgh CR, Soini H, Pallangyo K, von Reyn CF. Basis for treatment of tuberculosis among HIV-infected patients in Tanzania: the role of chest x-ray and sputum culture. *BMC Infect Dis* 2008; 8:32.

500. Were W, Moore D, Ekwaru P, Mwima G, Bunnell R, Kaharuza F, Rutherford G, Mermin J. A simple screening tool for active tuberculosis in HIV-infected adults receiving antiretroviral treatment in Uganda. *Int J Tuberc Lung Dis* 2009; 13(1):47-53.

501. Akhtar S, White F, Hasan R, Rozi S, Younus M, Ahmed F, Husain S, Khan BS. Hyperendemic pulmonary tuberculosis in peri-urban areas of Karachi, Pakistan. *BMC Public Health* 2007; 7:70.

502. Tupasi TE, Radhakrishna S, Quelapio MI, Villa ML, Pascual ML, Rivera AB, Sarmiento A, Co VM, Sarol JN, Beltran G, Legaspi JD, Mangubat NV, Reyes AC, Solon M, Solon FS, Burton L, Mantala MJ. Tuberculosis in the urban poor settlements in the Philippines. *Int J Tuberc Lung Dis* 2000; 4(1):4-11.

503. Datta M, Radhamani MP, Sadacharam K, Selvaraj R, Rao DL, Rao RS, Gopalan BN, Prabhakar R. Survey for tuberculosis in a tribal population in North Arcot District. *Int J Tuberc Lung Dis* 2001; 5(3):240-249.

504. Nsanzumuhire H, Lukwago EW, Edwards EA, Stott H, Fox W, Sutherland I. A study of the use of community leaders in case-finding for pulmonary tuberculosis in the Machakos district of Kenya. *Tubercle* 1977; 58(3):117-128.

505. Sekandi JN, Neuhauser D, Smyth K, Whalen CC. Active case finding of undetected tuberculosis among chronic coughers in a slum setting in Kampala, Uganda. *Int J Tuberc Lung Dis* 2009; 13(4):508-513.

506. Ayles H, Schaap A, Nota A, Sismanidis C, Tembwe R, de Haas P, Muyoyeta M, Beyers N. Prevalence of tuberculosis, HIV and respiratory symptoms in two

Zambian communities: implications for tuberculosis control in the era of HIV. *PLoS One* 2009; 4(5):e5602.

507. Kranzer K, Houben RM, Glynn JR, Bekker LG, Wood R, Lawn SD. Yield of HIV-associated tuberculosis during intensified case finding in resource-limited settings: a systematic review and meta-analysis. *Lancet Infect Dis* 2010; 10(2):93-102.

508. De Ville DG and Kleeberg HH. Comparison of tuberculosis case-finding in a high prevalence area. *S Afr Med J* 1974; 48(62):2582-2587.

509. den Boon S, White NW, van Lill SW, Borgdorff MW, Verver S, Lombard CJ, Bateman ED, Irusen E, Enarson DA, Beyers N. An evaluation of symptom and chest radiographic screening in tuberculosis prevalence surveys. *Int J Tuberc Lung Dis* 2006; 10(8):876-882.

510. World Health Organization. Antiretroviral therapy for HIV infection in adults and adolescents: recommendations for a public health approach. 2006; http://www.who.int/hiv/pub/guidelines/artadultguidelines.pdf

511. Murray CJ and Salomon JA. Modeling the impact of global tuberculosis control strategies. *Proc Natl Acad Sci U S A* 1998; 95(23):13881-13886.

512. South African Department of Health. National Antiretroviral Treatment Guidelines. 2010; http://www.doh.gov.za/docs/factsheets/guidelines/art.pdf

513. Reid MJ and Shah NS. Approaches to tuberculosis screening and diagnosis in people with HIV in resource-limited settings. *Lancet Infect Dis* 2009; 9(3):173-184.

514. Maher D, Borgdorff M, Boerma T. HIV-related tuberculosis: how well are we doing with current control efforts? *Int J Tuberc Lung Dis* 2005; 9(1):17-24.