An assessment of Canada's foreign-born tuberculosis surveillance strategy and insights gained into TB transmission

By

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Abstract

This thesis, comprising three published research papers, examines both Canada's tuberculosis (TB) surveillance strategies among foreign-born individuals and broader TB transmission dynamics.

In Canada, a country with low TB incidence, most TB cases occur among the foreign-born population. The global epidemiology of TB and the origins of Canada's TB migrants present significant challenges in reducing annual TB rates through current control programs.

The first two studies address Canada's TB surveillance strategy among foreign-born individuals, predominantly conducted through immigration medical exams. Utilizing data from Immigration, Refugees, and Citizenship Canada, along with provincial TB registry data, we established two cohorts of migrants in Alberta.

The first study, analyzing a cohort of migrants who arrived in Alberta between 2002 and 2013, compares TB incidence and transmission between those referred for post-arrival TB surveillance ("referrals") and those not required to undergo post-arrival TB surveillance ("non-referrals"). Our findings reveal that while referrals were more likely to be diagnosed with pulmonary TB, they had less severe disease and were significantly less likely (by 80%) to transmit. This could be interpreted as evidence of the program's effectiveness, where early identification of referrals potentially prevents progression to a more infectious disease state. However, an alternative hypothesis posits that unlike referrals, who have a history of TB disease treatment or radiographic evidence of old, healed TB, non-referrals with TB infection may not have developed an immune response as capable of controlling *M. tuberculosis*.

The second study uses chest radiographs from pre-arrival immigration medical exams to track the development of pulmonary TB disease in migrants diagnosed within two years of arriving in

Canada. We adjusted for the time difference between pre-arrival and post-arrival radiographs and determined that non-referrals progressed from TB infection to pulmonary TB disease more rapidly and aggressively than referrals. These findings imply a possible biological difference between the groups and suggest the benefit of moving beyond chest radiograph screening to latent TB infection screening and treatment among some non-referrals.

The third study investigates transmission differences based on sputum-smear microscopy status. Analyzing all pulmonary TB cases in Alberta from 2004 to 2016, we compared secondary cases arising from sputum smear-negative versus sputum smear-positive pulmonary TB cases. Using both DNA fingerprint clustering and conventional epidemiology, as well as genome sequencing among a subgroup of temporally and geographically linked cases, we found that smear-negative cases posed a 50% lower transmission risk than previously reported.

The thesis concludes by exploring potential changes to Canada's TB surveillance strategy. It also discusses the potential and limitations of whole genome sequencing, the influence of super-spreader events in TB transmission, and the utility of bioaerosol sampling in interrupting such transmission events.

Preface

This thesis is an original work by Leyla Asadi. The work presented herein has been previously published and are reproduced with the approval and support of my co-authors. Citations are provided below. Figures that are reproduced from publications are in those with Creative Commons Attribution non-Commercial Licenses; otherwise they are my own.

I have received ethics approvals from the University of Alberta Health Research Ethics Board for the three primary studies presented herein. Alberta Health Services (AHS) provided operational and administrative approvals. Grant support was provided by Alberta Innovates Health Solutions (AIHS) Clinician Fellowship and the Canadian Institute of Health Research.

Chapter 1: Portions were reproduced from Dr. Leyla Asadi's candidacy exam proposal as well as the paper published as *"*Asadi L, Heffernan C, Menzies D, Long R. Effectiveness of Canada's tuberculosis surveillance strategy in identifying immigrants at risk of developing and transmitting tuberculosis: a population-based retrospective cohort study. Lancet Public Health. 2017 Oct;2(10):e450-e457."

Chapter 2 was published as: *"*Asadi L, Heffernan C, Menzies D, Long R. Effectiveness of Canada's tuberculosis surveillance strategy in identifying immigrants at risk of developing and transmitting tuberculosis: a population-based retrospective cohort study. Lancet Public Health. 2017 Oct;2(10):e450-e457"

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Chapter 4 was published as: "Asadi L, Croxen M, Heffernan C, Dhillon M, Paulsen C, Egedahl ML, Tyrrell G, Doroshenko A, Long R. How much do smear-negative patients really contribute

to tuberculosis transmissions? Re-examining an old question with new tools. EClinicalMedicine. 2022 Jan 3;43:101250. doi: 10.1016/j.eclinm.2021.101250"

Some modifications were made from the published articles to this thesis. This was done to reduce duplication and improve clarity. No re-analyses were undertaken, or changes made to the results.

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Dedication and Acknowledgements

While I had expected the challenges of this journey to be purely academic, it was the confluence of external factors—a global pandemic, international political upheavals, and my own challenging pregnancies—that reshaped this process in ways I could not have anticipated. For that, my debt of gratitude extends to all who helped me navigate these complexities.

Dr. Richard Long, your unwavering support through maternity leaves, my COVID-19 responsibilities, and my clinical questions has been invaluable. Your patience, kindness, and commitment to ridding the world of tuberculosis are a guiding light in all my work. Dr. Menzies, your high standards have inspired me to deepen my own commitment to my work. Dr. Courtney Heffernan you've paved the way, providing answers before I even know the questions, consistently supporting my ideas and potential. To Cathy Paulsen, thank you for lending a listening ear. To Dr. Alexander Doroshenko Mary-Lou Egedahl and the rest of the TB Program Evaluation and Research Unit and the Edmonton TB clinic, thank you for all your support and teaching over the years.

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Tim, your love for knowledge and commitment to intellectual rigor are an inspiration. You see beauty in the pursuit of truth. We are a team in all things, including this venture.

Sam, Alex, Henry—you serve as my compass, reminding me of what really matters. I hope you see this thesis as the fruit of perseverance.

I chose to specialize in infectious diseases because the continued toll of diseases like HIV and TB seemed unacceptable to me. These "stupid deaths," to borrow Dr. Paul Farmer's phrase, are a glaring reflection of societal shortcomings, a call to action for all of us. This thesis is a product of a desire to contribute to the world in a meaningful way. I hope it reads that way, to everyone who made it happen.

Women, Life, Freedom

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Chapter 1: Introduction

1.1 Background

Prior to the onset of the COVID-19 pandemic in 2020, tuberculosis (TB) was the leading cause of infectious diseases mortality globally. While SARS-CoV-2 has now overtaken *Mycobacterium tuberculosis* as the primary infectious cause of death, the pandemic has also reversed years of tuberculosis control progress (1). Disruptions in TB-specific and general medical care, re-allocation of resources away from TB programmes, and the economic and social impacts of the pandemic all contributed to an increase in TB deaths in 2020, marking the first such increase since 2005 (2). Furthermore, there is growing awareness of the long-term sequelae of active TB among survivors, with almost half of the disability-adjusted life years (DALYs) lost to TB accruing beyond the acute period (3). Despite ongoing efforts to combat TB, the estimated 1.6 million TB deaths and 10.6 million cases in 2021 (2) serve as a reminder of the ongoing need for TB elimination.

This thesis comprises three original research papers that aim to enhance our understanding of TB surveillance and control practices. The first two studies focus on Canada's TB surveillance strategy among foreign-born individuals, a population group that accounts for over 70% of active TB cases in the country (4). These papers examine the effectiveness of the current TB surveillance strategy to identify areas for improvement. The third study takes a broader look at TB transmission patterns, specifically looking at the transmission risk related to sputum-smear status. The conclusion then explores potential changes to Canada's TB surveillance strategy as well as discussing whole genome sequencing, superspreader events, and bioaerosols can be used to better understand and interrupt transmission. To provide context for this research, the

introduction reviews *M. tuberculosis* microbiology, pathogenesis, and diagnostics, followed by an overview of TB surveillance in migrants.

1.2 *M. tuberculosis* overview

1.2.1 Microbiology

M. tuberculosis has afflicted humanity for millennia, leaving its imprint upon human history (5). Genome sequencing techniques estimate that an early predecessor of *M. tuberculosis* existed in East Africa around 3 million years ago, potentially infecting early hominids (6) and co-evolving with humans (7). Modern *M. tuberculosis* is more recent, having emerged from an ancestor dating between 20,000 and 15,000 years ago (8).

Tuberculosis disease is caused by the *M. tuberculosis* complex; however, only M. *tuberculosis* sensu stricto and *M. africanum* are the primary causes of human disease, with *M. africanum* responsible for about half of cases in West Africa(9). Humans are the sole reservoir for *M. tuberculosis*. It is a facultative anaerobe (an organism that makes ATP by aerobic respiration if oxygen is present but can convert to fermentation if oxygen is absent) and facultative intracellular bacterium with a slow growth rate in laboratory media. It has a distinct tripartite cell wall, primarily composed of a peptidoglycan and arabinogalactan complex that is covalently linked to a mycolic acids outer membrane(10). This thick, waxy, coat(11) forms a hydrophobic barrier against environmental and host defenses and is responsible for the characteristic acid-fast staining of the bacteria. The complete genome of *M.* tuberculosis consists of 4,411,429 base pairs and contains ~4,000 genes (12), many of which are devoted to lipogenesis, lipolysis, and cell wall biosynthesis.

1.2.2 Natural history and pathogenesis

The natural history of TB is characterized by a dynamic interaction between the host's immune response and *M. tuberculosis* (13). Upon inhalation of aerosolized M. tuberculosis, the bacilli are

confronted by alveolar macrophages in the host's lungs. If the macrophages phagocytose and effectively clear the bacteria, infection is prevented. If the bacilli evade clearance and continue replication, a state of infection is established. *M. tuberculosis* infection was classically dichotomized into either a latent or dormant state or active disease. More recent understanding, however, recognizes TB infection as a continuum of manifestations (14). In a small minority of individual—often the very young or severely immunocompromised— primary TB disease may develop. In the remainder of individuals, the spectrum of manifestations ranges from latent infection through to incipient TB, asymptomatic (subclinical) disease, and ultimately, to severe, life-threatening clinical disease (13, 14). An individual may move forward or backward between these states until the infection is controlled, either through the host immune system alone or with aid of anti-TB medication. Crucially for TB control programmes, only a small percentage of those with latent TB infection develop active TB disease(15). Risk factors that increase this likelihood include age and conditions that result in an immunocompromised state, such as HIV infection, organ transplantation, or dialysis requirement (14).

1.2.3 TB infection diagnosis and treatment: an opportunity for prevention

M. tuberculosis' persistence in a latent state presents a unique opportunity to prevent its progression to active disease. Two tests are used to identify this latent state: the tuberculin skin test (TST) and the interferon gamma release assay (IGRA), both of which detect immune responses to *M. tuberculosis* antigens (16). The TST measures the delayed hypersensitivity reaction to intradermal injection of tuberculin purified protein derivative (PPD). The IGRA is a laboratory-based test that measures the interferon-gamma produced by an individual's T-cells upon exposure to *M. tuberculosis*-specific antigens. IGRAs are more specific than TSTs, but both tests, unfortunately, have a low predictive value for the development of TB disease (15, 16).

This limitation underscores a significant challenge facing TB control programs, especially in countries like Canada, where most prevalent disease arises from latent TB reactivation. The current tests for latent infection are TB immunoreactivity tests which can identify an immune response to *M. tuberculosis* antigen but cannot determine who is at risk of reactivation (15). Since most infections do not progress to disease, widespread screening may be inefficient because individuals at low risk of disease are being treated. On the other hand, a narrow screening approach risks missing latent infections destined to become active disease. Thus, programs must identify the precise risk groups to test and treat.

Once a decision has been made to offer a patient TB preventive therapy, first-line regimens are four months of rifampin (4R) or a combination of weekly rifapentine and isoniazid for three months (3HP) (17, 18). These new TB preventative therapy regimens, changed from the previous standards of nine months of isoniazid, have higher completion rates and a reduced risk of hepatotoxicity(19-21). Consequently, the treatment of latent TB infection has become more acceptable and accessible than ever before.

1.2.4 TB disease diagnosis

Diagnosis of active pulmonary TB typically relies on sputum samples upon which smear microscopy, culture techniques, or nucleic acid-amplification can be conducted. The gold standard for diagnosing *M. tuberculosis* is culture from clinical specimens, which enables phenotypic drug resistance testing (22). However, the oldest and most widely used diagnostic tool is smear-microscopy. Sputum smear-microscopy remains the initial tool for the diagnosis of TB in most countries and continues to be the primary surrogate indicator of infectiousness (23). Mycobacteria, characterized by high mycolic acid content in their cell walls, resist decolorization during the staining process – a characteristic referred to as acid-fastness (24). The classical acid-

fast bacilli (AFB) stain techniques are the Ziehl-Neelsen and Kinyoun stains (25). However, where fluorescence or LED microscopes are readily available, auramine-rhodamine staining is the standard practice, as it allows for the bacilli to be seen at lower magnification and with a higher sensitivity. A semi-quantitative grading system is used to describe the number of bacilli seen per field. These are reported as negative, inconclusive/repeat, 1+,2+, 3+ or 4+.

Culture of *M. tuberculosis* requires extensive infrastructure and time. Smear-microscopy has lower sensitivity compared to cultures and provides no information on drug susceptibility. Nucleic acid amplification testing (NAAT), however, offers enhanced sensitivity and specificity over smear-microscopy and can provide some drug-resistance results, without the resource demands required for culture.

The Xpert MTB/RIF assay, performed on the GeneXpert platform, detects both *M.* tuberculosis and rifampin resistance. It was the first real-time, low-complexity, NAAT to be approved, with the WHO first endorsing its use in 2010(26). Since then, the Xpert MTB/RIF Ultra, along with six other WHO-recommended molecular rapid diagnostic testing platforms, have been recommended as the first test for most patients with suspected pulmonary TB (27). As of 2021, only 38% of TB cases worldwide had been identified via a WHO-recommended molecular rapid diagnostic (27) and in 2018, only 55% of pulmonary TB cases had been bacteriologically confirmed (defined as either: microscopy, culture or WHO-recommended rapid diagnostic) (22).

1.2.5 Strain typing

The technique currently used for classical genotyping is Mycobacterial Interspersed Repetitive Unit-Variable Number Tandem Repeat (MIRU-VNTR). With MIRU-VNTR, the number of variable number tandem repeats (VNTR) present in the genetic elements called MIRUs located in multiple loci (12, 15, or most commonly, 24) is analyzed to create a strain specific pattern(25,

28, 29). Before MIRU-VNTR, Restriction Fragment Length Polymorphism (RFLP) and spoligotyping were commonly employed. RFLP is based on the variability in the number of copies of the repetitive DNA sequence, IS6110. Isolates possess numerous copies of IS6110 at different chromosomal locations. Identical RFLP patterns are indicative of the same strain. Spoligotyping, used for further typing of isolates with five or fewer IS6110 bands, differentiates strains by analyzing spacer sequences in the clustered regularly interspaced short palindromic repeats (CRISPR) region of the genome.

The gold standard for genotyping is now Whole Genome Sequencing (WGS), which determines the complete DNA sequence (29, 30). This technique offers unique insight into strain diversity, transmission patterns, and phylogenetic relationships. Jurisdictions like the United Kingdom, the Netherlands, and Victoria, Australia have incorporated routine WGS for Mycobacterium tuberculosis isolates, with others, such as the USA and Germany, progressing towards its routine implementation.

1.3 Epidemiology: The Global and Canadian Context

In 2014, the World Health Organization's End TB strategy (31) established a global framework for TB control, setting ambitious targets for reducing TB incidence rate, TB related deaths, and patients' financial burdens. By 2035, the strategy aims to achieve a 95% reduction in TB deaths and a 90% reduction in TB incidence rate (to fewer than 100 per 1,000,000) compared to 2015. Low TB incidence countries (defined as having fewer than 100 cases per 1,000,000) should be progressing toward pre-elimination (fewer than 10 cases per million population) and, ultimately, elimination (fewer than 1 TB case per million) (32).

Regrettably, the world is far from reaching these elimination targets. The estimated 10.6 million people who were diagnosed with TB disease worldwide in 2021 represent a 3.6% increase in TB

as compared to 2020 (2). This contrasts with the 2% annual declines observed over the prior two decades. The 2022 WHO "Global Tuberculosis Report" identified 30 high TB burden countries (2). In most of these countries the incidence rate was 150-400 cases per 100,000 population. However, Central African Republic, Gabon, Lesotho, the Philippines, and South Africa continue to grapple with burdens exceeding 500 cases per 100,000 population. In terms of absolute case numbers, India (26%), the Russian Federation (8.5%), and Pakistan (7.9%) accounted for just over 40% of global cases.

Conversely, 47 countries reported a low TB incidence (less than 100 cases per 1,000,000 people annually), with Canada being one of them. Though Canada should be ideally situated for elimination, the overall TB incidence has plateaued at around 50 cases per 1,000,000 for the last 15 years (4). Moreover, TB disproportionately affects indigenous and foreign-born individuals in the country. Among the non-foreign born, non-indigenous Canadian population, the incidence rate of TB is 2/1,000,000. This is compared to a rate of 150/1,000,000 for foreign-born persons, with higher rates among certain sub-populations (33). Even though the foreign-born only make up 22% of Canada's population, 74% of Canada's active TB cases occur in this population (4). In the province of Alberta, this figure exceeds 90% (unpublished data, Alberta Health Services). Despite a constant incidence rate among foreign-born individuals in Canada, the absolute number of cases has risen due to increasing immigration from countries with intermediate or high TB incidence. Because so many of Canada's TB cases are due to reactivation of infection acquired prior to migration, if the global epidemiology of TB and the countries of origin of Canada's TB migrants remain unchanged, then Canada's TB control programming is unlikely to achieve a meaningful decline in annual TB rates. At our current pace, Canada will not achieve TB elimination until 2100 (34).

1.4 Infectious diseases and TB surveillance in migrants

The state of TB among the foreign-born population underscores the importance of robust surveillance systems. Concern about the spread of infectious diseases by immigrants has long prompted development of screening practices to minimize transmission risks and lessen burdens on health and social safety systems. In studying these strategies, we must recall the historical context and the stigma tied to infectious diseases, especially tuberculosis. History reveals prejudices often overshadowed scientific facts, imposing restrictive measures on immigrants without adequate healthcare provision (35).

Early efforts to control infectious diseases in migrants centered on quarantine. From the 14th century, ships at Mediterranean ports were held for 40 days (or "quarantino") to curb plague spread(36). By the late 19th and early 20th centuries, systematic screening at ports of entry like Ellis Island emerged (35). Countries like Canada and the USA then barred TB-affected immigrants from entering the country (36). As migrant flows increased and deportations were recognized to be overly burdensome, pre-departure screenings, including for TB, were introduced (36). A report from the Immigration Medical Services for Canada in the CMAJ in 1931 states that: "The system prevailing in the past was that of medical examination at the port of arrival. This method was unsatisfactory because it did not provide for as thorough an examination as was desirable, and also it imposed grave hardship on the immigrant, who, in many cases, after having burned his boats behind him at home, found himself denied admission to Canada. In order to overcome such difficulties, the medical service was extended overseas (37)." Predating both a full understanding of the germ theory of disease and the development of radiology, the initial screening processes consisted only of clinical examination and aimed to limit the burden of chronic disease rather than to prevent transmission.

Chest radiography for TB screening began during World War I (5) and became a routine part of Canada's immigration screening exam since World War II (38). These programs aimed to detect and treat active TB, and identify high-risk individuals for ongoing surveillance, not to identify latent TB or provide preventive therapy(39). As a consequence, until recently, no migrant population in Canada underwent routine TST or IGRA screening (4).

1.4.1 The Canadian TB medical surveillance system for migrants

The current Canadian foreign-born TB surveillance program occurs through the immigration medical examination, a mandatory health check for all permanent residents and some temporary residents before arrival to Canada. The immigration medical examination is also required of anyone in Canada seeking to change their immigration status, for instance, asylum seekers, or those applying to transition from a temporary visa to permanent residency status.

The immigration medical exam consists of a medical history, symptom inquiry, physical examination, and four age-related routine tests: urinalysis, chest radiograph, and syphilis and HIV serology (40). If there is radiographic evidence of tuberculosis, three sputum mycobacterial smears and cultures are obtained. Individuals with active TB must complete treatment before entering Canada. In 2019, 893,000 immigration medical examinations were performed, from which 885 individuals were found to have active TB (0.10% yield).

Applicants with inactive pulmonary TB are referred by Immigration, Refugees and Citizenship Canada to provincial or territorial public health authorities for a tuberculosis surveillance medical evaluation(4). Inactive pulmonary TB is defined as: "a history of treated active TB and/or an abnormal CXR suggestive of TB and two CXR taken at an interval of 3 months apart with stable appearance and 3 negative sputum smear cultures, or two CXR taken at an interval of 6 months apart with stable appearance (4)." On average, 2.0-2.5% of individuals who complete

an immigration medical examination prior to arrival are asked to undertake post-arrival surveillance(4). Individuals under surveillance are required to report to a public health authority within 30 days of arrival. The exact procedures conducted by each local public health authority varies considerably. In general, the surveillance medical evaluation begins with an assessment for active tuberculosis. If active disease is not identified, depending upon the jurisdiction and clinical provider, consideration is given to testing and treatment of TB infection, or annual surveillance with symptom inquiry, chest radiograph, and sputum (see **Figure 1.1**). The post-arrival assessment and treatment is highly jurisdiction dependent. Canada's foreign-born TB medical surveillance program's primary aim is to identify prevalent active TB in newly arrived migrants with the goal of ensuring they are non-infectious prior to arrival and thereby preventing transmissions.

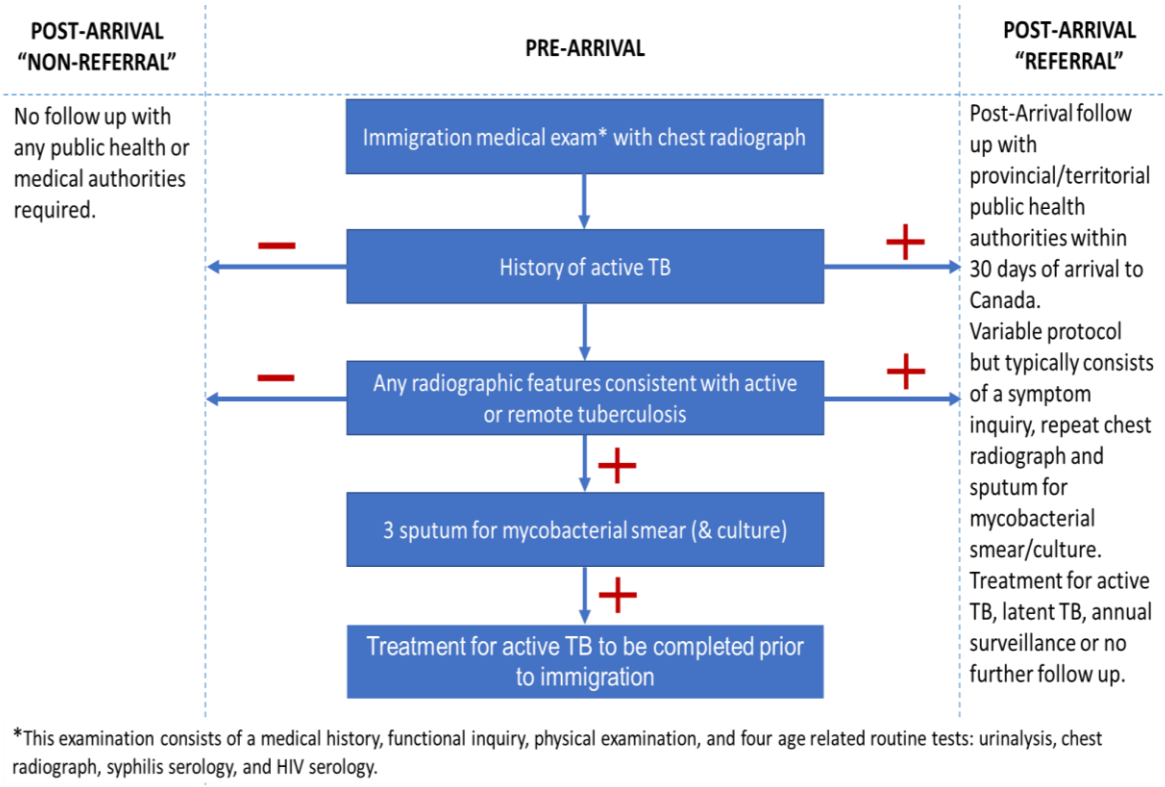

Figure 1.1 Canada's pre- and post-arrival immigrant surveillance system for tuberculosis

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Chapter 2 Preface

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Attribution: All authors participated in study conception, design, interpretation, critical revisions, and approved the final manuscript. LA did analyses and drafted the initial manuscript. CH, DM, and RL critically revised the manuscript. RL obtained funding and supervised the study.

Chapter 2: Effectiveness of Canada's TB surveillance strategy in identifying immigrants at risk of developing and transmitting TB: a population-based retrospective cohort study

2.1 Background

In this chapter, we look at the effectiveness of the TB control programme in identifying migrants with active TB disease. We also assess whether there is a difference in transmissions arising from migrants who undergo surveillance (referrals) versus those who are not required to undertake in-country surveillance (non-referrals). Previous studies (1, 2) have shown that referrals are four to five times more likely to have active tuberculosis than are non-referrals. Though the primary aim of the referral system is to prevent transmission and identify individuals who might pose a public health threat, there are no Canadian estimates of the differences in transmission arising from referrals versus non-referrals. International data for the efficacy of in-country surveillance in prevention of transmission are equally sparse (3). To address these gaps, we first determined the incidence of pulmonary tuberculosis in referrals and non-referrals, and then examined differences in transmission activity. We hypothesized that referrals would be less likely to transmit tuberculosis because of enhanced surveillance and earlier identification, despite being more likely to have active disease (4, 5).

2.2 Methods

2.2.1 Setting and datasets

Alberta is a province with a population of 4,108,300 people in 2014 (6), 10% of whom are foreign born (7). There is a low prevalence of HIV. Tuberculosis incidence in 2014 was 4·7 per 100,000 population (8). We used two distinct datasets. The IRCC dataset contains annual, aggregated information about year of arrival and country of citizenship for all foreign-born permanent residents and refugees (excluding refugee claimants) aged 15–64 years who arrived in

Alberta between Jan 1, 2002, and Dec 31, 2013. We also obtained TB data from the Alberta TB Registry, extracted from the Integrated Public Health Information System. These data included information about all tuberculosis cases and their contacts in the province.

This study was approved by the Health Research Ethics Board, Panel B, at the University of Alberta. Consent was not obtained because of the retrospective nature of these data. All patient data were anonymized and delinked before analysis.

2.2.2 Tuberculosis incidence

We used the IRCC dataset to determine the total number of foreign-born arrivals to Alberta from Jan 1, 2002, to Dec 31, 2013. The outcome of interest was culture-positive pulmonary TB and we identified all such cases diagnosed between 2002 and 2013 via the Alberta Tuberculosis Registry. From these data, we calculated crude incidence of culture-positive pulmonary tuberculosis and smear-positive pulmonary disease in Alberta during those 12 years within three groups: referrals, all non-referrals, and non-referrals from high- risk countries. Referrals were defined as individuals who had been referred for tuberculosis surveillance via the immigration medical examination before a diagnosis of active disease. High-risk countries were defined as countries of citizenship with a tuberculosis incidence of more than 150 per 100,000 population, or sub-Saharan Africa. This definition is the same as that used in UK tuberculosis programming recommendations and is largely in keeping with the Canadian TB Standards for latent infection screening in this subgroup (9, 10). The inclusion of sub-Saharan African countries, 16 of 45 of which had a TB incidence below 150/100,000, attempts to account for HIV co-infection rates in these settings as well as the possibility of concerns for epidemiologic data adequacy.

2.2.3 Culture-positive pulmonary TB case demographic data

These patients are variably infectious and constitute potential source cases. In addition to information about whether the potential source case was a tuberculosis surveillance referral, the provincial registry also included demographic, clinical, and laboratory information. Demographic information consisted of age, sex, and immigration status (permanent resident or Canadian citizen; refugee or refugee claimant; or on a visitor, student, work, or "other" temporary visa). By use of WHO estimates for incidence of active tuberculosis (11), incidence in the country of origin was defined as the average incidence of tuberculosis in the year of immigration and the 2 years before immigration. Clinical information included HIV status, active disease type (new *vs* relapse or retreatment), and disease site. We defined disseminated disease as per the Canadian tuberculosis standards, specifically, as either a miliary pattern on chest imaging or "the concurrent involvement of at least 2 non-contiguous organ sites of the body or involvement of the blood or bone marrow" (10). Laboratory information included smear and culture status and radiographic appearance. Treatment outcome describes whether an individual died before or during tuberculosis treatment and if death was related to tuberculosis.

2.2.4 Tuberculosis transmission

We applied conventional and molecular epidemiological (DNA fingerprint) techniques to identify transmission events arising from the subgroup diagnosed with culture-positive pulmonary tuberculosis between Jan 1, 2004, and Dec 31, 2013. Isolates of *Mycobacterium tuberculosis* from all culture-positive cases of tuberculosis diagnosed in Alberta were routinely DNA fingerprinted by use of standardised restriction fragment-length polymorphism, supplemented in isolates with five or fewer copies of the insertion sequence *6110*, by spoligotyping (12, 13).

We compiled contact lists for each potential source case. Routine contact tracing included the gathering of information about the number, type (close or casual), tuberculin skin test (TST), and disease status of contacts for all pulmonary tuberculosis cases. We defined close contacts as either household contacts or close, non-household contacts defined as "those who have regular, extensive contact with the index case and share breathing space daily or almost daily but do not sleep in the same household most of the time" (10). The determination of which contacts qualified as close contacts was made prospectively by the TB physicians and public health nurses involved in the management of each individual case. Assessment of close contacts included a symptom inquiry and TST 8–12 weeks after the final contact with the source case (if the contact was not already TST positive), a chest radiograph if symptomatic or TST positive, and sputum for acid-fast bacilli smear and culture if symptomatic or if chest radiograph was abnormal(14). Because of the relative infrequency of secondary cases arising from the foreign-born population(15) and the implications for tuberculosis perpetuation, we considered source cases to have resulted in transmission if at least one of their close contacts was found to have a TST conversion or was identified as a secondary case. We examined transmission only among close contacts because the effort that goes into identification and assessment of close contacts in Alberta is consistent across all cases. Additionally, because Alberta uses the stone-in-pond method of contact tracing (ie, the search among contacts for evidence of transmission by examination of close or high-risk contacts before casual or low-risk contacts), if transmission was not identified among close contacts, it would be unlikely that any would be seen among casual or "other" contacts(16).

We defined TST conversion as a TST of 10 mm or greater when a previous test resulted in a reaction of less than 5 mm. If the previous result was between 5 mm and 9 mm, we deemed

conversion to be an increase of 10 mm or more (10). If interferon gamma release assay (IGRA; typically, QuantiFERON-TB Gold) was done and the results were discrepant from the TST, IGRA results were used. Because of the little availability of IGRA during much of the study period, and the Canadian guideline endorsement of TST over IGRA in most cases, there was restricted use of IGRA. Tuberculosis cases were not included as TST converters.

We grouped transmission events (and the consequent secondary case) as **type-1** or **type-2** based on their conventional and molecular epidemiological links to the source case. Our group has previously reported on this method of transmission identification(17, 18). **Type-1** secondary cases were individuals who were listed as a contact of the source case, diagnosed with active tuberculosis within a transmission window extending from 6 months before to 24 months after the date of diagnosis of the source case, and culture positive with an isolate of *M tuberculosis* that was a genotypic match to the fingerprint of the putative source case. **Type-2** secondary cases were close contacts of the source case who were diagnosed with active tuberculosis within the 30-month transmission window, but were culture negative (or cultures could not be obtained). All paediatric type-2 pulmonary cases were independently verified by a paediatric pulmonary radiologist specialising in paediatric tuberculosis diagnoses and masked to referral status. Secondary cases diagnosed before the date of diagnosis of the source case were only counted as a transmission event if they had primary disease. We defined the date of diagnosis of the source case as the start date of treatment. To identify secondary cases, we cross-referenced contact lists against the Alberta Tuberculosis Registry from July 1, 2003, to Dec 31, 2015. Data were linked by use of tuberculosis registry number and name and were verified by date of birth and country of birth, as necessary.

2.2.5 Statistical analysis

We calculated the tuberculosis incidence per 100,000 person- years. The calculation of 95% CIs for rates assumed a Poisson distribution for case counts. We compared rates with incidence rate ratios and 95% CIs.

For transmission, we calculated demographic and clinical characteristics of referrals and non-referrals in addition to their respective findings from contact-tracing investigation. We used multivariate logistic regression to determine the independent association between referral for tuberculosis surveillance and transmission. We adjusted the analysis for age, immigration status (permanent resident or citizen *vs* refugee), risk of tuberculosis in country of birth (high risk *vs* lower risk), and number of months in Canada. We did not include clinical or contact characteristics of source cases in our model because we deemed them to be on the causal pathway between tuberculosis surveillance referral and a transmission event.

We did a sensitivity analysis assessing three different groups: individuals from high-risk countries, individuals who had been in Canada for 2 years or less, and a more inclusive cohort of temporary and permanent residents. All statistical analyses were done with SAS (version 9.4).

2.3 Results

Between 2002 and 2013, there were 223,225 foreign-born migrants to Alberta, of whom 5500 (2%) were referrals and 217,657 (98%) were non-referrals. Many referrals (n=3805) and non-referrals (n=115226) were from high-risk countries. The proportion and absolute number of foreign-born arrivals from high-risk countries increased over time (see **Figure 2.1**). Both referrals and non-referrals were most frequently from the Philippines, India, or China **(**refer to **Appendix 2A tables 2A.1** and **2A.2).**

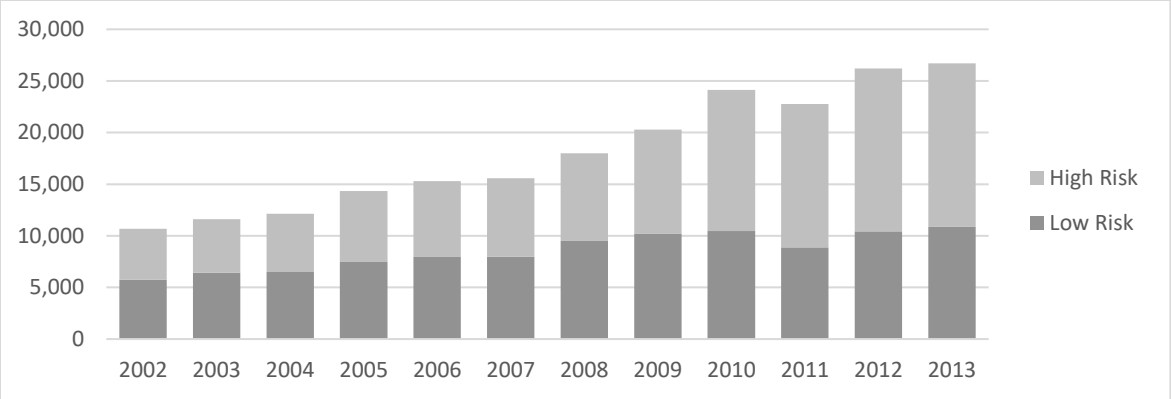

Figure 2.1. Foreign-born migrants to Alberta according to country of citizenship TB incidence risk category (high-risk)

234 foreign-born individuals were diagnosed with culture-positive pulmonary tuberculosis between 2004 and 2013. There were 50 (21%) referral and 184 (79%) non-referral source cases. The overall incidence of pulmonary tuberculosis for all foreign-born cases was 19 per 100,000 person-years (95% CI 17–22). The highest incidence was among referrals, followed by non-referrals from high-risk countries **(**see **Table 2.1).** Whereas the rate of culture-positive pulmonary tuberculosis was five times higher in referrals than non- referrals from high-risk countries, the rate of smear-positive pulmonary disease was only two times higher **(**see **Table 2.1).**

Table 2.1: Incidence of culture-positive pulmonary TB in 223,225 foreign-born migrants to the province of Alberta

	Culture-Positive pulmonary TB Cases	**Culture Positive pulmonary TB Incidence (per 100,000 person-years)**	**Rate* (95% CI)**	**Smear-Positive pulmonary TB Cases**	**Smear-Positive pulmonary TB Incidence (per 100,000 person-years)**	**Rate° (95% CI)**
Referrals (n=5500)	50	141	*	11	31	°
Non-Referrals (n=217,675)	184	15	9.1 (6.7-12.5)	103	9	4.0 (1.9-6.7)

Non-Referrals from High-Risk Countries (n=115,226)	167	28	5.0 (3.6-6.8)	93	16	2.0 (1.0-3.7)

*Incidence Rate Ratio of culture-positive pulmonary TB (referrals group as numerator)
°Incidence Rate Ratio of smear-positive pulmonary TB (referrals group as numerator)

2.3.1 Characteristics

Referrals had been in Canada for a median of 11 months before diagnosis, compared with 33 months in the non-referral group **(**see **Table 2.2).** 39 (78%) referrals were diagnosed during routine surveillance follow-up. Roughly 90% of individuals in both groups were from a high-risk country**.** Referrals presented with less advanced disease and with characteristics associated with decreased infectivity. No referrals had disseminated disease compared with nine (5%) non-referrals. Only three (6%) referrals had cavitary and only ten (20%) had smear- positive disease compared with 67 (36%) and 103 (56%) cases, respectively, among referrals.

Table 2.2: Baseline characteristics of 234 foreign-born patients with culture-positive pulmonary TB

	Referrals (n=50)	**Non-referrals (n=184)**
Male	19 (38)	103 (56)
Age in years, Mean (SD)	37 (13)	36 (13)
Months in Canada, Mean (SD) & Median	19 (23), 11	39 (31), 33
HIV-positive	2 (4)	20 (11)
Immigration Status		
Canadian citizen/Landed immigrant	46 (92)	161 (88)
Refugee	4 (8)	23 (13)
TB Incidence in Country of Birth (per 100,000)		
<30	0 (0)	4 (2)
30-99	5 (10)	11 (6)
100-149	5 (10)	18 (10)
150-199	13 (26)	20 (11)
>200	27 (54)	131 (71)
Cavitation on CXR	3 (6)	67 (36)
Sputum Smear Positivity	10 (20)	103 (56)
Disseminated TB	0 (0)	9 (5)
TB Caused or Contributed to	0 (0)	0 (0)

Death		

2.3.2 Transmissions

Referrals had fewer total contacts and fewer close contacts than did non-referrals, 842 (IQR -2 to 8) vs 3098 (IQR -7 to 18) **(**refer to **Appendix Table 2A.3).** 71 total transmission events arose from the foreign-born individuals with culture-positive pulmonary tuberculosis—three (4%) from referrals and 68 (96%) from non-referrals (see **Figure 2.2).** Whereas no secondary cases were attributable to a referral source case, 18 secondary cases were attributable to 11 different non-referral source cases **(**see **Figure 2.2).** Eight type-1 secondary cases and ten type-2 secondary cases were identified. As a reminder, type-1 secondary cases were defined as individuals diagnosed with active TB within a transmission window that extended from 6 months before to 24 months after the date of diagnosis of the putative source case, listed as a contact of the putative source case, and was culture-positive with an isolate of *M. tuberculosis* that was genotypically identically to that of the putative source case. Type-2 secondary cases were listed contacts with active TB within the same transmission window but who were culture-negative. All but one of the type-2 secondary cases were paediatric cases. Three TST conversions were attributable to three different referral source cases **(Figure 2.2).** All these referral source cases had been diagnosed through the surveillance process. 50 TST conversions arose from 31 different non-referral source cases **(Figure 2.2).**

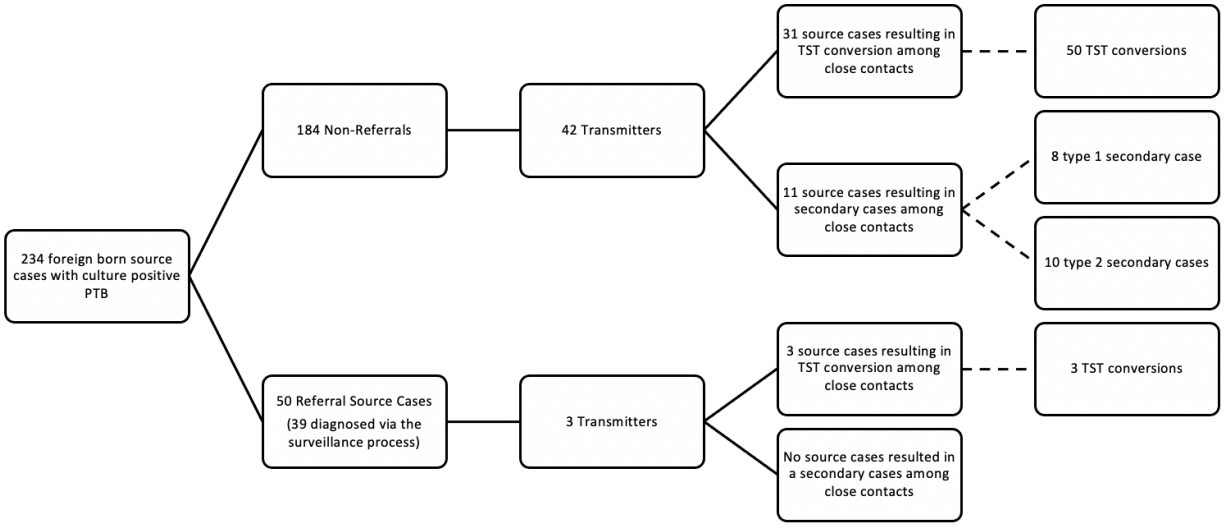

Figure 2.2: Transmission events (TST conversions and secondary cases) arising from the 234 permanent residents who developed culture-positive pulmonary TB
---- represents transmissions to close contacts
*****secondary cases were not also counted as TST conversions.

That is, among the tuberculosis surveillance referrals, three (6%) of 50 transmitted *M tuberculosis* compared with 42 (23%) of 184 non-referrals (unadjusted odds ratio 0.22, 95% CI 0·065–0·75; p=0·01; **Table 2.3).** The independent association between being a referral and the lower likelihood of transmission persisted in multivariable adjusted analyses. **Table 2A.4** in Appendix 2A shows the results of univariate and multivariate analyses for the remainder of the variables included in the final model. 37 (83%) of 45 source cases resulting in any transmission and 11 (100%) of source cases resulting in a secondary case were from high-risk countries. Our sensitivity analysis showed that, among foreign-born individuals in Canada for 2 years or less, referrals were significantly less likely to transmit tuberculosis than were non-referrals **(Table 2.3).** The association persisted for individuals from high-risk countries and with inclusion of temporary residents **(Table 2.3).**

Table 2.3: Odds ratios for transmission comparing referrals vs non-referrals (including sensitivity analyses)

	Odds Ratio for Transmission
Unadjusted	0.22, 95% CI (0.065-0.75), p=0.01
Adjusted	0.19, 95% CI (0.054-0.66), p=0.009
<2 years since arrival to Canada [3/38 referral v 23/75 non-referral]	0.19, 95% CI (0.049-0.70), p=0.01
From Countries with TB incidence >150 or Sub-Saharan Africa [3/45 referral v 36/167 non-referral]	0.23, 95% CI (0.064-0.80), p=0.02
All Foreign-Born Patients with pulmonary TB (including temporary residents) [6/78 referrals vs 50/240 non-referral]	0.31, 95% CI (0.12-0.76), p=0.01

2.4 Discussion

Our analysis showed that foreign-born individuals referred for further in-country surveillance were more likely to be diagnosed with pulmonary tuberculosis than were all non-referrals and the subset of non-referrals from high-risk countries. However, referrals had a roughly 80% decreased risk of transmitting tuberculosis and did not result in any secondary cases.

Referrals, perhaps because they had lived in Canada for a shorter time before diagnosis, had fewer contacts than did non-referrals, which might be one reason why they were less likely to transmit tuberculosis. However, it is more likely that the decreased transmission is attributable to the lower incidence of smear-positive disease in referrals than non-referrals (19). Why then were referrals less likely than non-referrals to be smear positive? One explanation might be earlier time to diagnosis. About 80% of the referrals were asymptomatic and were diagnosed during routine surveillance. Their time from symptom onset to diagnosis would have been much shorter than in non-referrals. Another Canadian study (5) found that time to diagnosis from symptom onset was 18 days earlier for compliant referrals than for non-referrals. Studies of nosocomial transmissions and outbreaks also point to an association between delayed diagnosis and increased transmission (20, 21). However, longer time to diagnosis did not predict tuberculosis

transmission in contacts of US-born patients with pulmonary disease (22) nor was it associated with increased transmission to close contacts of foreign-born US patients (23).

An alternative possibility is that the referral group represents a distinct disease entity. Referrals either had evidence of old, healed tuberculosis on chest radiograph or a history of tuberculosis. They might have achieved some degree of accommodation(24) with their tuberculosis infection, which might render their active disease less severe and less infectious. A review of about 159,000 pulmonary tuberculosis cases showed that those with smear-negative disease were more likely to be foreign born(25). The findings were attributed to the foreign-born population being more likely than the native-born population to have been diagnosed via screening. The possibility that reactivation of disease in foreign-born individuals who had radiographic evidence of old healed TB might be of a different—and perhaps less severe—manifestation was not explored. However, the concept that patients with smear-negative microscopy uniformly, and in a linear fashion, progress to smear-positive disease was questioned as far back 1979 (26). And I will explore this idea further in the subsequent chapter.

Our study has several limitations. The inability to reliably track the number of temporary residents arriving into the province forced their exclusion from the main analyses. Nevertheless, we did sensitivity analyses including temporary residents and our transmission findings were unchanged. The IRCC dataset provided information about country of citizenship and the assumption was made that, in most cases, country of citizenship at the time of immigration corresponded with country of birth. These definitions were used interchangeably. The assumption that country of citizenship is equivalent to country of birth might overestimate the

number of foreign-born individuals from low-risk countries (to which migrants might have immigrated before entering Canada).

There may also be concerns about our definition of transmission. Unfortunately, no standardised definition of tuberculosis transmission exists in the literature. Our chosen definition could potentially exclude secondary cases who were not named as contacts of the source case. Similarly, some might call the 30-month transmission window into question. However, our method has been previously published and described (17, 18), with sensitivity analyses corroborating its appropriateness. Findings from other studies also suggest that this length of time is likely to capture most transmission events (27, 28).

Another limitation is that use of TST conversion as a marker of transmission in a population with a high proportion of BCG-vaccinated individuals might have identified BCG boosting rather than true conversion. If IGRA had been more widely used, or if we had chosen a cut-off of more than 15 mm for TST conversion, we might have identified fewer transmissions. However, we have adhered to the Canadian tuberculosis standards' definition of TST conversion and would not expect differential outcome misclassification between the two groups. Unfortunately, our data does not include information on the use of TB preventative therapy for contacts. While we would not anticipate a differential latent TB therapy completion rate among contacts of referrals versus non-referrals, it is pertinent for understanding the risk of progression to active disease. The same concern applies to lack of data on the underlying health status of contacts.

We also appreciate that in our analysis of tuberculosis incidence, the denominator assumes no substantial emigration from or immigration into the province from elsewhere in Canada. Unfortunately, no national registries exist to allow us to consider in-country migration patterns

after arrival. Finally, our data are specific to the province of Alberta. Although Quebec is more inclined to accept French speakers, immigration patterns and foreign-born tuberculosis epidemiology is similar across the major immigrant receiving provinces of Quebec, Ontario, Alberta, and British Columbia. However, implementation of the surveillance medical evaluation varies from province to province; therefore, some caution should be applied in extrapolation of our findings to the whole country.

To our knowledge, this was the first study assessing the impact of the national tuberculosis surveillance programme on transmission of tuberculosis in Canada. A large study of migrants to the UK found that those born in countries with higher tuberculosis incidence had a higher risk of reactivation. The investigators also reported minimal transmission (29). The study did not examine differences between individuals who had undergone screening and those who had not been screened, instead focusing on the out-of-country screening process. Verver et al. (2002) (3) found that absence of tuberculosis screening and increased duration of stay in the Netherlands were risk factors for transmission, but that these risk factors were strongly correlated in multivariate analysis. By contrast, we found that even with adjustment for length of stay in Canada and even with sensitivity analyses assessing only individuals who had been in Canada for less than 2 years, referrals were less likely to transmit tuberculosis than non-referrals.

When Canada's surveillance system identifies an individual to be at risk for pulmonary tuberculosis, they are indeed at an increased risk of being diagnosed with the disease. However, the surveillance system did not identify roughly 80% of the foreign-born population who developed disease, nor did it identify the cases that accounted for most transmissions. Our findings could be used to lend support to the UK recommendations to undertake screening for

latent tuberculosis infection in individuals from countries with a tuberculosis incidence of more than 150 per 100,000 population or sub-Saharan Africa, since this high-risk group accounted for 100% of secondary cases. Still, although more than 100,000 migrants were high risk, the number of secondary cases (n=18) was relatively low. Further research could improve the feasibility of screening this large group by clarifying who is at highest risk of developing and transmitting tuberculosis and should be supplemented with economic evaluations examining the resource implications.

2.5 Chapter 2 References

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2.6 Chapter 2 Appendix A

Table 2A.1 Most frequent country of origin for 223,225 permanent residents arriving to Alberta between 2002-2013 (includes referrals and non-referrals)

Country	Number (%)
Philippines	43,025 (19)
India	31,315 (14)
China	20,455 (9)
United Kingdom	11,320 (5)
Pakistan	9,655 (4)
United States	7,250 (3)
Korea, Rep	4,950 (2)
Nigeria	4,525 (2)
Mexico	3,705 (2)

Table 2A.2. Most frequent country of origin for 5500 referral permanent residents arriving between 2002-2013

Country	Number (%)
Philippines	1769 (32)
India	680 (12)
China	509 (9)
South Korea	290 (5)
Ethiopia	226 (4)
Vietnam	222 (4)
Pakistan	188 (3)
Somalia	158 (3)
Sudan	120 (2)

Table 2A.3 Characteristics of contacts of foreign-born migrants with culture-positive pulmonary TB

	Referral contacts	Non-referral contacts
All named contacts (median/IQR)	841, 3 (-2 to 8)	3098, 6 (-7 to 18)
Close Contacts (mean/SD), (median/IQR)	216, 3 (0 to 6)	1201, 4 (-1 to 9)
Documented TST Conversion	3 (1)*	68 (6) [+]
Newly Identified TST Positive	10 (29)	416 (35)
Previous Positive	10 (5)	77 (6)
Incomplete**	16 (7)	139 (12)

*% of close contacts

[+] In this table, secondary cases were included among the TST converters

****These are named close contacts who could not be contacted, did not return phone calls or letters, or did not fully complete assessment.

Table 2A.4 Univariate and multivariate analyses for variables included in the final model

Variable	Univariate OR (OR, 95% CI, p-value)	Multivariate OR (OR, 95% CI, p-value)
Referral Status	0.220, 95% CI 0.065-0.745, p=0.015	0.190, 95% CI 0.054-0.663, p=0.009
Immigration Status (Canadian citizen or landed immigrant vs refugee)	1.548, 95% CI 0.611-3.923, p=0.0357	1.247, 95% 0.472-3.294, p=0.656
Age at diagnosis	0.989, 95% CI 0.964-1.016, p=0.426	0.992, 95% CI 0.965-1.020, p=0.585
Months in Canada	0.995, 95% CI 0.984-1.007, p=0.412	0.992, 95% CI 0.980-1.005, p=0.236
High risk country of birth	0.565, 0.206-1.545, p=0.265	0.580, 95% CI 0.199-1.689, p=0.318

Chapter 3 Preface

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Chapter 3: Is there a fundamental flaw in Canada's post-arrival immigrant surveillance system for tuberculosis?

3.1 Background

As discussed in Chapter 2, Canada's TB surveillance strategy relies on the chest radiograph to identify persons at risk of developing and transmitting tuberculosis. Applicants with a history of treated TB or evidence of old healed TB on pre-arrival chest radiograph, "referrals", undergo follow-up with public health authorities after arrival (see **Figure 1.1**, Chapter 1)(1, 2) Referrals are at higher risk of developing active pulmonary TB (3), but our group (4) and others (5, 6) have challenged the commonly held belief that they pose a serious public health threat.

In Chapter 2, we showed that referrals who develop active TB are far less likely to transmit TB than "non-referrals". This lower risk of transmission has been assumed to reflect earlier diagnosis of TB among referrals. However, that hypothesis relies upon a linear progression from smear-negative to smear-positive disease. An alternative explanation is that the referral group represents an immunologically distinct group with distinct disease manifestations. As early as 1979, Kurt Toman, posited this hypothesis and questioned the merits of routine radiographic screening (7). As evidence, he pointed to the lack of reduction in the proportion of sputum smear-positive, pulmonary disease cases during longitudinal, chest-radiographic based mass case-finding studies in Czechoslovakia (8) and the Netherlands (9). Specifically, he stated that radiographic screening assumes that "TB in adults starts as a rule with a minimal lesion 'early infiltrate' that—without treatment— would all develop step by step into advanced, smear-positive tuberculosis…However, studies in populations under surveillance have shown that newly detected, smear-positive TB usually develops fast—ie, without passing through a

clinically perceptible initial stage". That is, the TB infection identified via screening radiography may not develop into active TB disease with the high morbidity or infectiousness most concerning to public health programs. Indeed, the more dangerous, and infectious smear-positive TB may be more likely to develop in those lacking radiographic evidence of latent TB. After the Second World War, chest radiography became the standard screening practice (10-13), but given the more widely available alternate screening methods (i.e. interferon-gamma release assays), this time-honoured radiograph-based screening strategy may at best be insufficient or at worst, creating a false sense of assurance (13-17).

To explore whether referrals and non-referrals have different disease manifestations, we undertook a case-control study to examine pre-arrival and incident (at time of TB diagnosis) chest radiographs of referrals and non-referrals who developed active pulmonary TB within two years of arrival to Canada. We hypothesized that chest radiographs among non-referrals would show more rapid disease progression.

3.2 Methods

3.2.1 Population

This retrospective case-control study was undertaken in Alberta, Canada, where all TB services are provided out of three public health clinics, one clinic in each of the two major cities, Edmonton and Calgary, and one provincial ("virtual") clinic serving rural Alberta(18). The provincial clinic is the steward of the TB Registry and receives notifications from Immigration, Refugee, and Citizenship Canada (IRCC) of all immigrants (and some temporary residents) whose admission to the province is conditional on compliance with TB surveillance. These referrals are estimated to be 2-3% of all new immigrants to the province (4, 19).

Over a 160-month period beginning January 1st, 2004, all foreign-born, culture-positive pulmonary TB patients managed out of the Edmonton and Provincial TB Clinics were identified in the TB Registry. Only patients who were ≥12 years of age on arrival and whose date of diagnosis (start date of treatment) was within 2 years of the date of arrival were eligible for study. The age criterion is based on the age at which a chest radiograph is required at Canada's immigration medical exam (≥ 11 years), in turn, based on when children begin to develop adult-type (potentially infectious) pulmonary TB (2, 20), plus the period of time that the immigration medical exam (IME) is considered valid (1 year). Patient who had undergone an overseas IME were grouped into those who had, and those who had not, been referred. A small subset of the cohort were diagnosed at an in-Canada IME (the processes for which are the same as the overseas IME). These included refugee claimants (asylum seekers) and those seeking an extension to a visitor visa or a change in immigration status. Another small subset had no IME, either overseas or in-Canada.

3.2.2 Variables of Interest

The demographic and clinical characteristics, immigration status and country-of-birth (high vs low incidence) (21), were abstracted from the TB Registry, individual public health, and hospital records. A high incidence country was: i) a country with an average incidence of TB (all forms) in the year of arrival of the case and the two years preceding it, of ≥150 per 100,000 persons (for cases diagnosed in 2017 the average incidence in 2014–2016, the most current incidence data at the time of writing, was used), or ii) any country of sub-Saharan Africa. The number of pre-treatment specimens and the average time to liquid culture positivity of all culture-positive specimens was confirmed in the Provincial Laboratory for Public Health where all mycobacteriology is performed . First-line antituberculosis drug susceptibility testing and DNA

fingerprinting was performed on all initial isolates of Mycobacterium tuberculosis. DNA fingerprinting was performed using either restriction fragment-length polymorphism (RFLP), supplemented in those isolates with five or fewer copies of the insertion sequence 6110 by spoligotyping, or by 24 loci Mycobacterial Interspersed Repetitive Units (MIRU)–Variable Number Tandem Repeats (VNTR) (22-24).

3.2.4 Primary outcome of radiographic progression

For all referrals and non-referrals, posterior-anterior (PA) and lateral (LAT) chest images acquired at the time of diagnosis (the "incident radiographs") were assembled and re-read independently by three experts (two chest radiologists and a pulmonologist) blinded to the referral status of the patients. For those patients with infiltration localized to, or predominantly in, the upper lung zones, with or without cavitation—a gas filled space within pulmonary consolidation, a mass or a nodule—but with no discernable intrathoracic adenopathy, the radiograph was categorized as "typical" for adult-type pulmonary TB (25). All other radiographic patterns were categorized as "atypical" for adult-type pulmonary TB. The radiograph was coded as being normal, exhibiting minimal, moderately-advanced, far-advanced, or miliary TB according to criteria established by the US National Tuberculosis and Respiratory Disease Association (26). The details of this classification system are available in **Appendix 3A, Table 3A.1.** Inter-reader variability analysis was performed, and discrepant readings were resolved by consensus.

All pre-arrival radiographs in referrals were obtained from IRCC; in accordance with Canadian immigration TB screening requirements for the foreign-born, multiple overseas films had been performed in most. The extent of disease on each of these past radiographs was coded as above for the incident case films. For these individuals, sequential overseas, and for referrals with an

in-Canada IME only, sequential in-Canada radiographs (still considered "pre-arrival" as they were prior to the incident film), were then reported as being **unchanged**, **subtly changed** (a less than minimal change that was considered to be unrelated to projection, technique or composite shadows), **minimally changed** (an unequivocal increase in extent or density of abnormality that did not result in the "extent of disease" being re-categorized as greater than "minimal"), or **substantially changed** (an unequivocal increase in extent or density of abnormality that resulted in the "extent of disease" being re-categorized from normal or minimal to moderately-advanced or greater). For non-referrals, whose overseas chest radiographs had been reported as normal by IRCC panel physicians at the time of their IME, we were unable to re-interpret their films. This was because normal films are not shared with the province or territory of destination of the immigrant, nor are they archived by IRCC beyond a period of 2 years. IRCC was, however, able to: i) confirm that their records did indeed show that these non-referral, pre-arrival films had been read as normal, and ii) provide us with the date the film had been performed.

For purposes of reporting on change in the extent of disease on referral and non-referral radiographs, change was dichotomized into **"minimal"** (unchanged, subtly changed or minimally changed as above) vs. **"substantial"** (as above). Consensus opinion on change status is reported.

3.2.5 Secondary outcome of transmission events

As a secondary analysis, we examined transmission events. In this analysis, we used the same definition of secondary cases that were described in Chapter 2 (type-1 and type-2 transmission events)). Type-1 secondary cases are individuals diagnosed with active TB within a transmission window that extends from 6 months before to 24 months after the date of diagnosis of the case to whom they had been identified as a contact. They must also have an isolate

of *M. tuberculosis* that is a 100% genotypic match with the putative source case. Type-2 are individuals notified with active TB within the same transmission window who are listed as a contact, but who were culture-negative (mainly children).

In this analysis, however, we also included spatiotemporally associated secondary cases (individuals who were not named contacts with the putative source case). We called these cases type-3 secondary cases and included them to account for the possibility that members of the study cohort had incomplete contact lists. Secondary cases were searched for among notified cases of TB in the province who were culture-positive, had a genotypically matched isolate of *M. tuberculosis*, and were temporally (diagnosed in the same transmission window) and spatially (lived in the same forward-sortation-area—a geographic unit associated with a postal facility from which mail delivery originates—as determined by the first three digits of their postal code) linked to the source case. Secondary cases who were index cases, i.e. were diagnosed before the date of diagnosis of the cohort case, were only included if they had primary disease.

3.2.5 Statistical analysis

We report generalized kappa statistics to quantify the level of agreement between readers for incident radiographs. The formula proposed by Abraira and Pérez de Vargas was used when weighted categories were necessary (27). We used multivariate logistic regression to determine the independent association between referral for tuberculosis surveillance (referral status) and degree of chest radiograph progression (minimal vs substantial). We adjusted for variables known to alter the radiographic presentation of pulmonary TB, including, age, HIV status and diabetes(28-32). We also adjusted for other potential confounders including “time between films” defined as the number of days between the oldest pre-arrival radiograph and incident

radiograph, immigration status (permanent resident or citizen vs refugee), and risk of tuberculosis in country-of-birth (high risk vs lower risk). This main analysis was carried out only on the patients for whom pre-arrival chest radiographs were available (see **Figure 3.1**). We also carried out two sensitivity analyses. For the first, we considered referrals who had any instability in their chest radiograph and used the time from their newest pre-arrival radiograph to incident radiograph. Second, we included all referrals and non-referrals, by imputing the average "time between films" of non-referrals without past chest radiographs. We also assumed that these non-referrals would have had "minimal" change between their pre-arrival radiograph and their incident radiograph. Data were analyzed using SAS 9.4. Study approval was obtained from the University of Alberta Health Research Ethics Board (HREB), Panel B, which reviews all non-invasive research projects (PRO-00070701).

3.3 Results

Between January 1, 2004, and April 30, 2017, 174 foreign-born persons ≥ 12 years of age were diagnosed with culture-positive pulmonary TB within 24 months of arrival. Of these, 61 (35.1%) were referrals and 113 (64.9%) were non-referrals. Of the referrals, 6 (9.8%) were diagnosed at an in-Canada IME and had past abnormal in-Canada chest radiographs; of the non-referrals, 15 (13.3%) were diagnosed at an in-Canada IME or had no past chest radiographs **(Figure 3.1).**

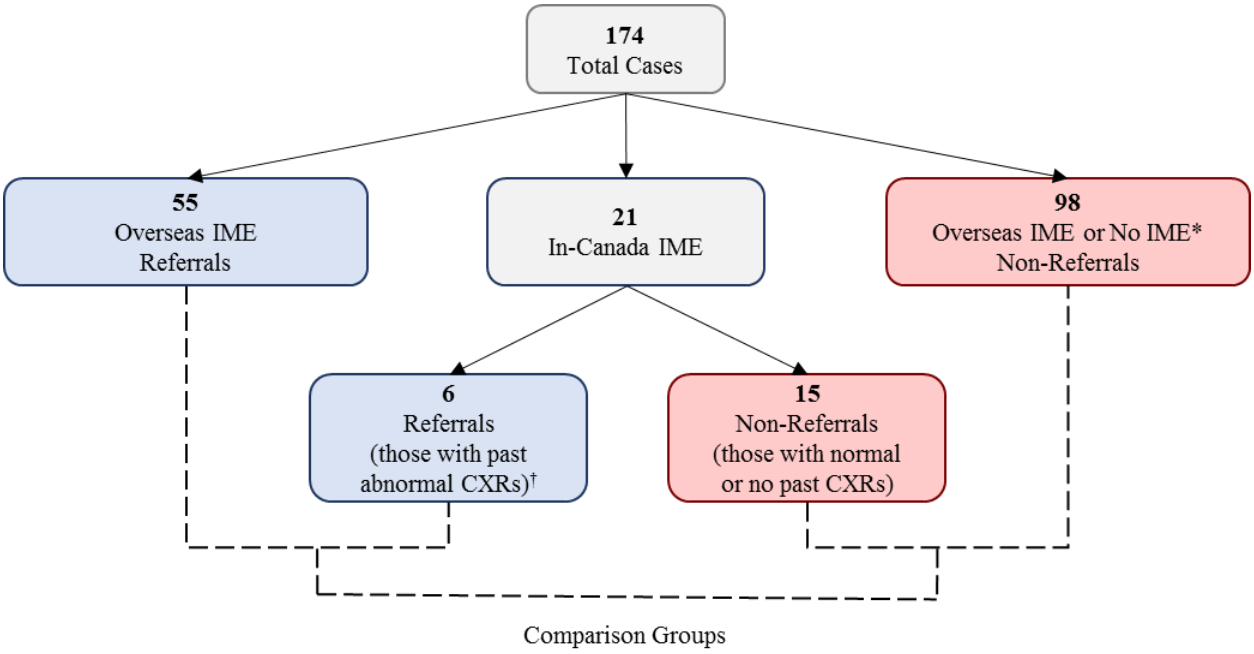


Figure 3.1: Cohort of recently arrived foreign-born pulmonary tuberculosis cases.
Abbreviations: CXR chest x-ray; IME. * Of the 98 "overseas IME or no IME" non-referrals, 83 had an overseas IME and 15 had neither an overseas nor an in-Canada IME. Of the 83 non-referrals with an overseas IME, 80 had a normal overseas CXR and 3 had an abnormal overseas CXR. † Past CXRs refers to a CXR >3 months before their incident case film.

Most referrals (93.4%) were diagnosed at an initial or one-year follow-up visit; of those that were overseas IME referrals, most (90.6%) were documented to have negative sputum mycobacteriology at their IME. On average, referrals were diagnosed sooner after arrival than non-referrals (mean ± SD 21.6 ± 19.1 vs 47.0 ± 28.5 weeks; median 12.0 vs 41.9 weeks).

Referrals were more likely than non-referrals to be older (mean [±SD] and median age 46.9 ± 18.2; 45 years and 35.3 ± 15.3; 31 years, respectively), to have a history of TB, and to be asymptomatic, see **Table 3.1.** Cough was reported in 12 (19.7%) referrals and 79 (69.9%) non-referrals. Referrals and non-referrals did not differ by sex, co-morbidity status, immigration status, or high vs low incidence country-of-birth. Nobody had dialysis dependent renal failure.

The five leading countries of birth of referrals were the Philippines, China, India, Vietnam, and Somalia; of non-referrals the Philippines, India, Ethiopia, Congo, and Somalia.

Table 3.1: Demographic and clinical characteristics of foreign-born pulmonary TB patients by referral status.

	Referrals (n=61) n (%)	Non-Referrals (n=113) n (%)	p-value
Male Sex	35 (57)	59 (52)	0·514
Age >64	14 (23)	8 (7)	**0.003**
Disease Type			
New active	45 (74)	105 (93)	**0·001**
Relapse/retreatment	16 (26)	8 (7)	
Symptomatic	16 (26)	91 (81)	**<0·001**
HIV Positive	2 (3)	8 (7)	0·497
Diabetes	9 (15)	10 (9)	0·233
Immigration Status			
Permanent Resident	35 (57)	51 (45)	
Temporary resident	23 (38)	49 (43)	0·185
Refugee	3 (5)	13 (12)	
Country-of-Birth*			
High Incidence	53 (87)	96 (85)	1·729
Low Incidence	8 (13)	17 (15)	

* high incidence = a country with an average incidence of TB (all forms) in the year of arrival of the case and the two years preceding it, of ≥ 150/100,000 persons or sub-Saharan Africa; for cases diagnosed in 2017 the average incidence in 2014-16, the most current incidence data at the time of writing, was used.

Referrals were less likely than non-referrals to be smear-positive and they had longer times to culture-positivity, indicative of lower bacillary loads. There was no difference in drug susceptibility test results, see **Table 3.2.** Referrals were also less likely to have cavitation or extensive disease (moderately-advanced, far-advanced or miliary) on chest radiograph. Referrals and non-referrals did not differ by incident case chest radiograph category (typical vs. atypical), or laterality (unilateral vs. bilateral). One asymptomatic, smear-negative referral was pregnant and declined an incident case chest radiograph. Six non-referral incident case chest radiographs

had been purged and reports alone were available for interpretation. Incident case chest radiographs were performed within 7.8 ± 10.0 (median 2.5) and 5.9 ± 8.5 (median 3.0) days of the date of diagnosis in referrals and non-referrals, respectively (p=0.20). Expert inter-reader agreement was good **(**refer to **Appendix 3A, Table 3A.2).**

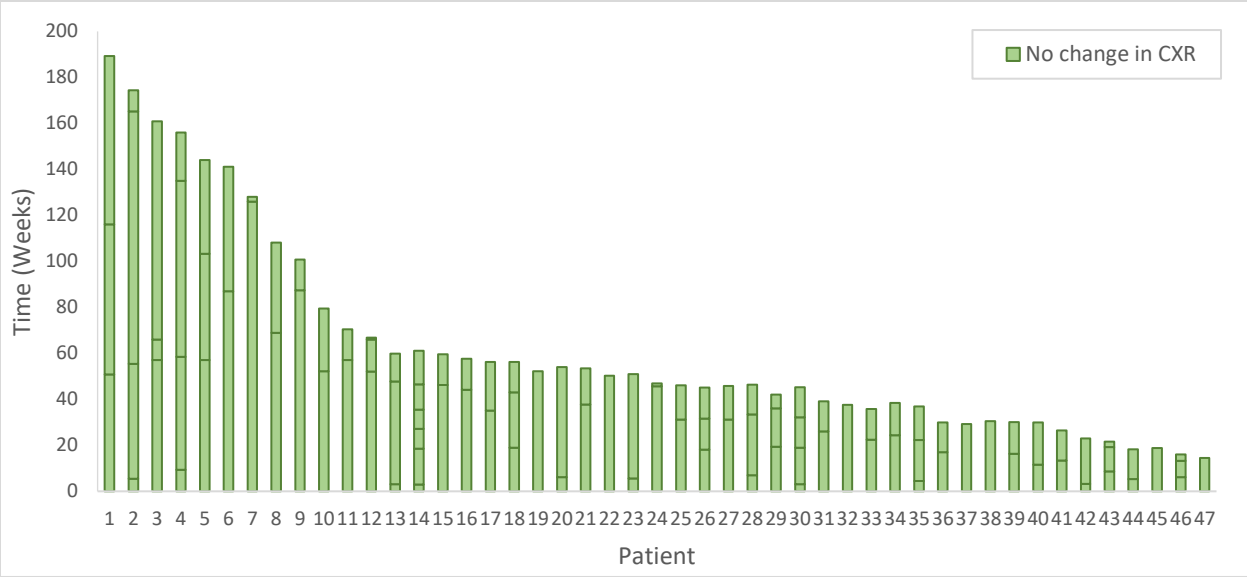
Table 3.2: Mycobacteriologic and incident case chest radiograph (CXR) characteristics in foreign-born, pulmonary tuberculosis patients by referral status.

Characteristic	**Referrals (n=61) n (%)**	**Non-Referrals (n=113) n (%)**	**p-value**
Sputum smear-positive*	9 (15)	56 (50)	p <0·001
Time-to-culture positivity* (mean ± SD days)	19·8 ± 8·6	13·2 ± 6·9	p <0·001
First-line drug resistance	8 (13)	15 (13)	0·976
CXR Category†			
Typical	43 (72)	84 (74)	0.860
Atypical	12 (20)	22 (19)	
Normal	5 (8)	7 (6)	
CXR Laterality†			
Normal	5 (8)	7 (6)	0·268
Unilateral	38 (63)	60 (53)	
Bilateral	17 (28)	46 (41)	
CXR Cavitation†	0 (0)	39 (35)	p <0·001
CXR Extent of Disease†			p <0·001
Normal	5 (8)	7 (6)	
Minimal	46 (77)	40 (35)	
Moderately-advanced	8 (13)	44 (39)	
Far-advanced	1 (2)	18 (16)	
Miliary	0 (0)	4 (4)	

***** Among referrals and non-referrals, respectively, 58 and 80 submitted three, 3 and 13 submitted two, and 0 and 18 submitted one specimen. If multiple pre-treatment specimens were culture-positive, the average time-to-culture positivity was used.
† One referral did not have an incident case chest radiograph.

Among the 60 referrals who had undergone both an incident case as well as one or more past chest radiographs, there was a total of 213 performed radiographs for an average of 3.54 radiographs per referral. In 47 referrals (78.3%), the radiographs were unchanged over an average of 62.2 ± 44.7 weeks (median 47.0 weeks; IQR: 30.6–66.9 weeks), see **Figure 3.2.**

Figure 3.2. Stable chest radiograph histories in 47 referral cases



Each column represents an individual referral; horizontal lines on the columns indicate when CXRs were performed relative to the date of diagnosis. For example, patient #1 had three CXRs at 51, 116 and 189 weeks prior to diagnosis. One referral did not undergo an incident case CXR and is not included.

In 13 referrals (21.7%), the radiograph changed over time. In 9, the final extent of disease remained unchanged; in 4, it progressed from minimal to moderately-advanced, see **Figure 3.3**

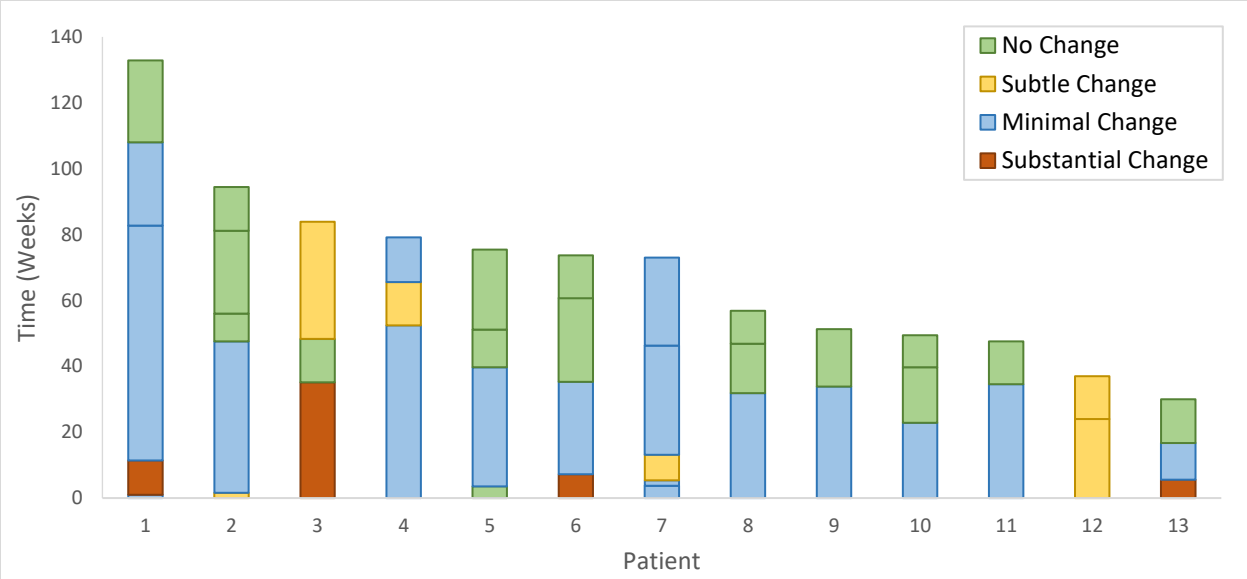


Figure 3.3. Unstable chest radiograph histories in 13 referral cases

Each column represents an individual referral; horizontal lines on the columns indicate when CXRs were performed relative to the date of diagnosis. For example, patient #2 had 5 CXRs at 2, 48, 56, 81 and 94 weeks prior to diagnosis. On the 2nd (at week 81), 3rd (at week 56) and 4th (at week 48) there was no change from the first, but on the next film (at week 2) the independent readers judged there was a minimal change from the 4th, and on the next film after that (the incident case film at week 0) there was subtle change from the 5th. In these 13 referrals the extent of disease remained unchanged over time in nine (8 remained minimal, 1 remained moderately-advanced). In four, patients #1, 3, 6 and 13, the extent of disease changed from minimal to moderately-advanced.

Among the non-referrals with overseas IMEs, 80 had normal and 3 had abnormal radiographs. In the 80 who had a normal overseas radiograph, the time between the last normal chest radiograph and the incident case film averaged 77.4 ± 35.2 weeks (median 78.1 weeks; IQR: 51.1–100.4 weeks). At diagnosis, the extent of disease was as follows: 5 (6%) normal, 27 (34%) minimal, 31 (39%) moderately-advanced, 13 (16%) far-advanced, 4 (5%) miliary. Thus, 4 (7%) of 60 referrals had substantial changes as compared with 48 (60%) of the 80 non-referrals; adjusted odds ratio 0.058; 95% Confidence Interval (0.018–0.199). This difference persisted in sensitivity analyses **(**see **Table 3.3).**

Table 3.3: The independent association between referral status and degree of chest radiograph (CXR) progression (minimal or substantial)

Referrals vs Non-Referrals with Substantial Radiograph Progression	Unadjusted OR	Adjusted OR
Main analysis (n=134) (4/54 vs 48/80)*	OR: 0.053 95% CI (0.018-0.162) p<0.0001	OR: 0.058 95% CI (0.018-0.199) p<0.0001
Sensitivity Analysis 1: Main analysis using most recent pre-arrival CXR for referrals with unstable CXR (n=134) (4/54 vs 48/80) †	OR: 0.053 95% CI (0.018-0.162) p<0.0001	OR: 0.047 95% CI (0.013-0.173) p<0.0001
Sensitivity Analysis 2: Main analysis, in addition to referrals and non-referrals with in-Canada IMEs (n=6 and n=15, respectively), non-referrals with an overseas IME and abnormal CXR (n=3), and non-referrals with no IME (n=15) (Total n=173) (4/60 vs 48/113) ‡	0.097, 95% CI 0.033-0.285, p<0.0001	OR: 0.112 95% CI (0.035-0.355) p=0.0023

*Adjusted for referral status, time from oldest (that is, earliest) pre-arrival CXR to diagnosis CXR "time between films", immigration status, low or high incidence country-of-birth, age, HIV and diabetes
† Adjustments were as above except that for referrals with any instability in their pre-arrival CXRs, the "time between films" was time from newest (that is, closest to arrival) pre-arrival CXR to diagnosis CXR
‡ Adjustments as per main analysis; for non-referrals without pre-arrival radiograph information (15 with an in-Canada IME; 15 with no IME) or abnormal pre-arrival radiographs (n=3), we imputed the average "time between films" (from oldest pre-arrival CXR to diagnosis CXR) for non-referrals and also assumed the degree of change was minimal

Two of 174 (1.1%) cohort (both non-referrals) and 53 of 1863 (2.8%) non-cohort case isolates during the study period had not been fingerprinted; most of these unfingerprinted isolates were *M. tuberculosis* complex species other than *Mycobacterium tuberculosis*. More non-referrals than referrals had transmission events (40/113 [35%] vs. 12/61 [20%], p<0.04) with the vast majority of transmission events (100% of secondary cases and 81.7% of TST conversions) occurring among contacts of non-referrals (see **Table 3.4** and **Appendix 3, Table 3A.3**). Among referral and non-referral converters, only 4 and 16, respectively, had a second TST of ≥ 15mm induration–a result that is known to correlate better with a positive IGRA(33). There were 16 secondary cases; 13 foreign-born; 3 Canadian-born children of foreign-born parents. All but one was diagnosed within 6 months of the source case.

Table 3.4: Transmission events among contacts of recently arrived, foreign-born, pulmonary TB cases by referral status.

Characteristic	Total	Referrals n (%)	Non-Referrals n (%)
Number of contacts	2012	352	1660
Number of contacts completely assessed	1606 (79·8)	299 (84·9)	1307 (78·7)
Number of contacts completely assessed per case	9·2	4·9	11·6
Number of contacts completely assessed with:			
Previous Positive TST	91 (5·7)	16 (5·4)	75 (5·7)
New Positive TST	551 (34·3)	118 (39·5)	433 (33·1)
TST Conversion*	82 (5·1)	15 (5·0)	67 (5·1)
Secondary Case	15 (0·9)	0 (0·0)	15 (1·1)
Negative TST	867 (54·0)	150 (50·2)	717 (54·9)

Abbreviation: TST tuberculin skin test
* Of the contacts of referrals that were converters, 4 had a second TST of 15 mm or more; of the contacts of non-referrals that were converters 16 had a second TST of 15 mm or more.

3.4 Discussion

The disease manifested by referrals is relatively benign from an individual and public health perspective (4-6). Referrals were more likely to be asymptomatic, smear-negative, and to have minimal or no disease on chest radiograph. At the time of diagnosis, the chest radiographic abnormalities in most (78.3%) referrals had been stable for an average of 62.2 weeks and even in referrals whose chest radiographic abnormalities worsened over time (21.7%), the changes were usually subtle or minimal. By contrast, over an average of ~77 weeks, non-referrals progressed from no disease to disease that was often both a threat to themselves and others. Adjusted for relevant demographic and clinical features, non-referrals were much more likely to have substantial changes on their chest radiographs over a similar period. Further, almost all transmission events occurred among contacts of non-referrals.

The observation that immigration referrals are often asymptomatic with minimal and stable radiographic abnormalities was first made by Wang et al in 1991, but its significance was unappreciated at the time(34). The stability of the chest radiograph together with the absence of symptoms strongly suggests that, despite being diagnosed earlier, the paucibacillary nature of referral's disease is not a reflection of early diagnosis but rather an accommodation having been reached between the host and pathogen. The immuno-pathologic correlates of this accommodation, once defined, can be expected to impact not only the accuracy estimates for novel TB biomarkers and diagnostics but vaccine development(35, 36). With respect to non-referrals, while there is abundant literature on the sensitivity and specificity of immunological tests for LTBI (TST and IGRA), the more important measure from an individual perspective and from a public health perspective is the progression rate, the likelihood that a person with a positive test will go on to develop active TB. Currently known risk factors for progression, including the incidence of TB in the country-of-birth, chest radiographic abnormalities (without active TB), moderate or high-risk medical conditions, recent TB contact, immigration status (refugees vs others), and slight but significant variation across immunological tests, are limited in their capacity to predict reactivation(6, 17, 37-39). There is an urgent need for a diagnostic test that would predict progression, or alternatively, better identify those at high risk of developing symptomatic and contagious disease.

Strengths of this study include the detailed clinical, radiologic, microbiologic, and transmission information, some of it not readily accessible, such as the referral radiographs, and little of it missing. To our knowledge, this is the first study that compares pre-arrival and post-arrival radiographs and systematically re-interprets pre-arrival films. Shorter time to diagnosis is the main alternate hypothesis for the milder disease in referrals. We therefore not only adjusted for

time between radiographs but also undertook sensitivity analyses that considered different definitions of "time between films". The fact that our finding was unchanged with these analyses lends support to referrals representing a different and less rapidly progressing disease course. Weaknesses of our study include its retrospective design and the relatively small sample size; accordingly, it is best understood as a proof-of-concept.

In conclusion, while the value of the overseas IME in detecting prevalent active disease and the need for pre-arrival treatment is undisputed, the value of the post-arrival surveillance system in further protecting the health of the Canadian public is less clear. Referrals, a relatively small group, are at increased risk of disease and ought to continue to be flagged for post-arrival surveillance. However, the disease they manifest constitutes relatively little risk to themselves or others. Non-referrals on the other hand, while a much larger group, appear to include a subgroup of individuals who rapidly progress from LTBI to overt disease that constitutes a risk to both themselves and others. The challenge going forward, is to identify, in a cost-effective manner, this subgroup and to provide them with preventative therapy.

3.5 Chapter 3 References

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3.6 Chapter 3 Appendix

Table 3A.1 Radiographic Extent of Disease (Replicated from Falk et al, 1969)

A. Normal This classification applies to lesions that cannot be seen on the roentgenogram but are associated with positive cultures for *M. tuberculosis* complex.
B. Minimal lesions include those that are of slight to moderate density but do not contain demonstrable cavitation. They may involve a small part of one or both lungs, but the total extent, regardless of distribution, should not exceed the volume of lung on one side, which is present above the second chondrosternal junction and the spine of the fourth or the body of the fifth thoracic vertebra. The term minimal is not to be interpreted as minimizing the activity or hazards of the disease in this stage.
C. Moderately advanced lesions may be present in one or both lungs, but the total extent should not exceed the following limits: disseminated lesions of slight to moderate density that may extend throughout the total volume of one lung or the equivalent in both lungs; dense and confluent lesions that are limited in extent to one-third the volume of one lung; total diameter of cavitation if present must be less than 4 cm.
D. Far advanced is used to describe lesions that are more extensive than moderately advanced.
E. Miliary Diffuse micronodular pattern.

Table 3A.2 Expert Inter-Reader Variability of Incident Case Diagnostic Chest Radiograph Interpretations

	Expert Reader Interpretation*	Agreement†	Kappa Statistic	95% Confidence Interval
Referrals	**Category**	Substantial	0.715	[0.543, 0.863]
	Laterality	Substantial	0.661	[0.519, 0.826]
	Cavitation	n/a‡	n/a‡	n/a‡
	Extent of Disease	Substantial	0.751	[0.609, 0.912]
Non- Referrals	**Category**	Almost Perfect	0.870	[0.780, 0.945]
	Laterality	Substantial	0.775	[0.679, 0.850]
	Cavitation	Substantial	0.768	[0.658, 0.843]
	Extent of Disease	Substantial	0.727	[0.650, 0.803]
Both	**Category**	Almost Perfect	0.814	[0.744, 0.878]
	Laterality	Substantial	0.741	[0.666, 0.822]
	Cavitation	Substantial	0.767	[0.656, 0.861]
	Extent of Disease	Substantial	0.754	[0.694, 0.818]

* See text for definition of chest radiograph category and cavitation; see reference #24 and the online supplement for definition of extent of disease. Only 10 radiographs (4 in referrals, 6 in non-referrals) required deferring to a process of consensus.
† Kappa statistics were defined as follows: less than 0.00 as poor; 0.00–0.20 as slight; 0.21–0.40 as fair; 0.41–0.60 as moderate; 0.60–0.80 as substantial; 0.81–1.00 as almost perfect.
‡ Not available. Because none had cavitation in the referral group, a kappa statistic could not be calculated. Percent agreement was high, with all three readers agreeing that the CXR was non-cavitary in 56 of 60 (93%) referral cases.

Table 3A.3. Secondary cases among reported and "unreported" contacts of recently arrived foreign-born pulmonary TB cases by referral status.

Secondary Case by Type*	Total	Source Case	
		Referrals n (%)	Non-Referrals n (%)
Type 1	6	0 (0)	6 (100)
Type 2	9	0 (0)	9 (100)
Type 3	1	0 (0)	1 (100)
All Types	16	0 (0)	16 (100)

* *Type 1*: Individuals diagnosed with active TB within a transmission window that extended from 6 months before to 24 months after the date of diagnosis of the putative source case, listed as a contact of the putative source case and culture-positive with an isolate of *M. tuberculosis* that matched genotypically that of the putative source case.
Type 2: Individuals notified with active TB within the same transmission window, listed as a contact but who were culture-negative (mainly children).
Type 3: Individuals who were not reported as a contact but who were culture-positive, had a genotypically matched isolate of *M. tuberculosis*, and were temporally (diagnosed within the same 30 month transmission window) and spatially (lived in the same forward sortation area – a geographical unit associated with postal facility from which mail delivery originates – as determined by the first three digits of their postal code).
Secondary cases diagnosed before the start date of treatment of the putative source case had to have primary disease.

Chapter 4 Preface

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Chapter 4: How much do smear-negative patients really contribute to tuberculosis transmissions? Re-examining an old question with new tools

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As we examined the difference in transmissions arising from referrals and non-referrals, we observed that referrals were more likely to have smear-negative sputum microscopy. It has long been known that TB patients with smear-negative disease are less infectious than those with smear-positive disease; however, our observations suggested even fewer transmissions arising from smear-negative individuals than would be estimated using the available literature. This observation led to the study undertaken in this chapter which examines the difference in secondary cases arising from active pulmonary TB patients with smear-negative vs smear-positive sputum disease.

4.1 Background

Sputum smear microscopy remains the most commonly used surrogate marker for estimating infectiousness(1). Historical studies observed that household contacts of smear-positive patients were between 2 and 12 times more likely than household contacts of smear-negative patients to be infected with *Mycobacterium tuberculosis (M.tb)(2)*. More recent studies have examined the question of transmission from smear-negative patients by comparing clusters of DNA fingerprints arising from smear-negative vs smear-positive source patients (3-5). These studies, themselves now between 10 and 20 years old, estimated that the relative transmission rate of smear-negative compared with smear-positive patients was 0.22–0.24, or roughly 20 to 25% the likelihood of transmission.

In some studies in low-incidence settings, conventional epidemiology alone, without DNA fingerprinting, may underestimate transmissions(6). By contrast, DNA fingerprint clustering alone appears to overestimate the number of recent transmissions(7, 8). When France et al. compared field-based (conventional and molecular investigation) evidence of recent transmission with genotype-based (molecular) estimates, they found that genotype-based estimates could overestimate recent transmission by as much as 75% (8). However, it is also well-established that compared with traditional molecular methods, genome sequencing allows for greater resolution in identifying transmissions(9-11). Unfortunately, for most jurisdictions, genome sequencing has often remained prohibitively expensive or has been deemed unnecessary for TB control efforts. Given the reliance on sputum smear microscopy for triaging contact-tracing efforts and establishing infection control protocols, estimates of the infectiousness of smear-negative cases need to be updated. Therefore, we re-examined the contribution of smear-negative source patients to tuberculosis transmissions in a low TB incidence, low HIV prevalence, high-income setting. We compared previously described DNA fingerprint clustering techniques with an approach combining molecular and conventional epidemiology supplemented with genome sequencing.

4.2 Methods

4.2.1 Population

We included all culture-positive pulmonary TB patients diagnosed in the province of Alberta, Canada from January 2004 to December 2016 as notified in the Alberta TB Registry. For each patient, we extracted routinely and systematically collected (in real-time) demographic, clinical, laboratory, and contact-tracing information from the Integrated Public Health Information System—the location of the provincial tuberculosis database.

4.2.2 Smear-Status definition

We used auramine-rhodamine stains for screening respiratory sputum samples and followed this with confirmatory Ziehl-Neelsen staining. A smear-positive individual had at least one positive respiratory sample prior to the initiation of anti-tuberculosis therapy. A smear-negative individual had to have at least three respiratory samples submitted for analysis and all samples submitted prior to initiation of therapy had to be negative. All mycobacteriology in the province is performed in a single laboratory: Provincial Laboratory for Public Health.

4.2.3 Molecular genotyping

From July 2003 to June 2016, isolates of *Mycobacterium tuberculosis* from all culture-positive cases of TB diagnosed in Alberta were routinely DNA fingerprinted by use of standardised restriction fragment-length polymorphism (RFLP), supplemented in isolates with five or fewer copies of the insertion sequence *6110*, by spoligotyping(12, 13). From January 2014 onwards, isolates had 24 loci mycobacterial interspersed repetitive units (MIRU) typing(14).

4.2.4 Genome sequencing of *M. tuberculosis*

M. tb was grown on Lowenstein-Jensen media, and heat killed at 90 °C for 30 min prior to DNA extraction. Routine methods of extraction, sequence quality control and assessment were then performed.

Following bead beating with 0.5 mm glass beads (Sigma Z763748) for 5 min, lysozyme (Sigma L6876) was added and incubated at 37 °C for 1 h. Further lysis of the cells was done with the MagaZorb® DNA Miniprep kit proteinase K, lysis buffer (Promega MB1004) and RNAse A (Qiagen #19,101) digestion at 60 °C overnight. Extraction was completed using the MagaZorb® kit on the Kingfisher mL Purification System. Extracted DNA was checked on a 1% agarose gel and quantified using the Qubit BR kit.

Illumina-compatible libraries were generated using the Illumina Nextera DNA Flex Library Prep Kit (Illumina 20,018,705), and sequenced on an Illumina MiSeq with the 600-cycle MiSeq Reagent Kit v3 (Illumina MS-102–3003).

4.2.5 Sequence quality control and assessment

Raw Illumina sequences were assessed for quality with FastQC v0.11.8 (bioinformatics.babraham.ac.uk/projects/fastqc/) and MultiQC v1.7 (15) . Adapters were trimmed with trimmomatic v0.39, as well as quality filtering and keeping any sequences greater than 75 bp in length. Kraken 2.0.8-beta (16)

with minikraken2_v2_8GB_201,904_UPDATE was used to classify the trimmed reads, allowing us to identify and keep reads classified as *M. tb* complex (MTBC) or the genus *Mycobacterium* – all other reads were discarded. Of the 49 *M. tb* genomes sequenced, two (Mtb34 and Mtb42) had significant non-Mycobacterium sequences (26% and 41%, respectively) that were removed by this taxonomic filtering. The remaining 47 genomes were > 99% classified as Mycobacterium. These quality-filtered classified sequences were used in downstream analysis. Estimated depth of coverage after quality filtering ranged from 40 to 454x. Biohansel 2.4.0 (17) was used to determine the lineages of each data set, indicating a mix of lineage 1, 2 and 4.

Phylogenetic tree and pairwise single nucleotide variant distances

H37Rv (NC_000962.3) was used as a reference for mapping and single nucleotide variant (SNV) calling with Snippy 4.5.0 (github.com/tseemann/snippy) using default settings (minimum mapping quality 60, minimum base quality 13, minimum depth 10). Core SNVs were determined with Snippy, masking the variable regions with the packaged masking file resulting in 93–95%

coverage of the H37Rv reference genome. A core SNV phylogeny was generated using IQ-TREE 1.6.12

(18) with a generalized-time reversible model and with discrete Gamma rate heterogeneity (GTR+G4), one thousand bootstraps, (19, 20), one thousand single branch test replicates(21), and using constant sites as determined by snp-sites 2.5.1 (22). The resulting phylogenetic tree was visualized with FigTree v1.4.4 (github.com/rambaut/figtree), displayed using a midpoint-root. Pairwise SNV distances were calculated using snp-dists 0.6.3 (github.com/tseemann/snp-dists).

Since three different lineages were used to generate the pairwise SNV distances and the phylogenetic trees, to provide further resolution on the pairwise distances between known MIRU pairs, we regenerated the core SNVs for each individual pair using Snippy and re-calculated the pairwise distance using snp-dists.

4.2.6 Outcome

The primary outcomes of interest were the proportion of TB transmission attributable to smear-negative cases and the relative transmission rate. The **proportion of TB transmission attributable to smear negative sources** was defined as: [number of transmissions arising from smear-negative sources]/[total number of transmissions]. The **relative transmission rate** is defined as: [Smear-negative transmission events/total number of smear-negative patients with culture-positive pulmonary TB]/[smear-positive transmission events/total number of smear-positive patients with culture-positive pulmonary TB].

4.2.7 Statistical methods

The Wald method was used to estimate 95% confidence intervals (CI) for the proportions of transmission attributable to smear-negative cases; we also calculated 95% CI for relative transmission rate using the Clopper-Pearson (exact) method. Chi-square testing was used to

examine whether there was a statistically significant difference in the proportion of transmissions attributable to smear negative cases between method #1 (molecular epidemiology using DNA fingerprint clusters) and method #2 (combined conventional epidemiology, molecular epidemiology, and genome sequencing). Chi-square testing was also used to examine whether there was global difference between method #1, method #2, and the two sensitivity analyses. SAS version 9.4 was used for statistical analysis.

4.2.8 Method #1: molecular epidemiology (DNA fingerprint cluster)

The goal of this analysis was to replicate the methodology of previous studies (3-5). The analysis was carried out on all TB patients from July 2003 to June 2016. Given there was no RFLP typing after June 2016, clustering could no longer be determined beyond this date. We defined a cluster as two or more patients having 100% matched DNA fingerprints and ordered them chronologically. Within the cluster, if the first patient had only extra-pulmonary TB, they were excluded and the subsequent patient (ordered chronologically), would be considered the index patient. We employed the methodology described in the seminal paper by Behr et al.: "Secondary cases that were preceded only by cases of smear-negative TB were attributed to smear-negative transmission. All cases that occurred after any case of smear-positive TB were attributed to smear-positive transmission (4)." Tostmann et al. also used this method (5). We also carried out an analysis where we restricted the time elapsed between a transmission event to 2-years.

4.2.9 Method #2: combined conventional and molecular epidemiology supplemented with genome sequencing

For this method, we identified all contacts of pulmonary TB patients diagnosed from January 2004 to December 2016, see **Figure 4.1**. The province of Alberta has a rigorous, prospective, routine contact tracing system that relies upon the stone-in-pond principle (i.e., contact tracing begins with close or high-risk contacts before casual or low-risk contacts)(2). As per the

Canadian TB standards, for smear-negative cases, household and other high-priority contacts are assessed in the initial round of contact investigations; however, medium-priority contacts (e.g. other close non-household contacts) are only assessed if there is evidence of transmission in the first round(2). For smear-positive cases, both high and medium priority are assessed from the outset. Contacts in places of social aggregation (i.e. educational or work settings, places of worship, or homeless shelters) are routinely identified(23).

Once the contact list for each case was assembled, we matched contacts by name, date-of-birth, and their unique TB registry number to a list of all known cases of TB in the province that occurred 6 months before or 24 months after the diagnosis of the putative source case. Type-1, Type-2 and Type-3 secondary cases were determined as described in Chapter 2 and 3. To further confirm that putative source and Type-3 secondary case were linked, their isolates were sequenced and required to show no more than 10 SNVs differences. The study in Chapter 2 did not include type-3 secondary cases. The study in Chapter 3 included type-3 secondary cases with identical DNA fingerprints. And this study then further clarified whether the type-3 secondary cases were attributable to the putative source case by undertaking genome sequencing.

We ordered conventional epidemiologically linked cases with identical DNA fingerprints chronologically and assumed the first case was the source case. As in the DNA fingerprint clustering analysis, “secondary cases that were preceded only by cases of smear-negative TB were attributed to smear-negative transmission. All cases that occurred after any case of smear-positive TB were attributed to smear-positive transmission”(4).

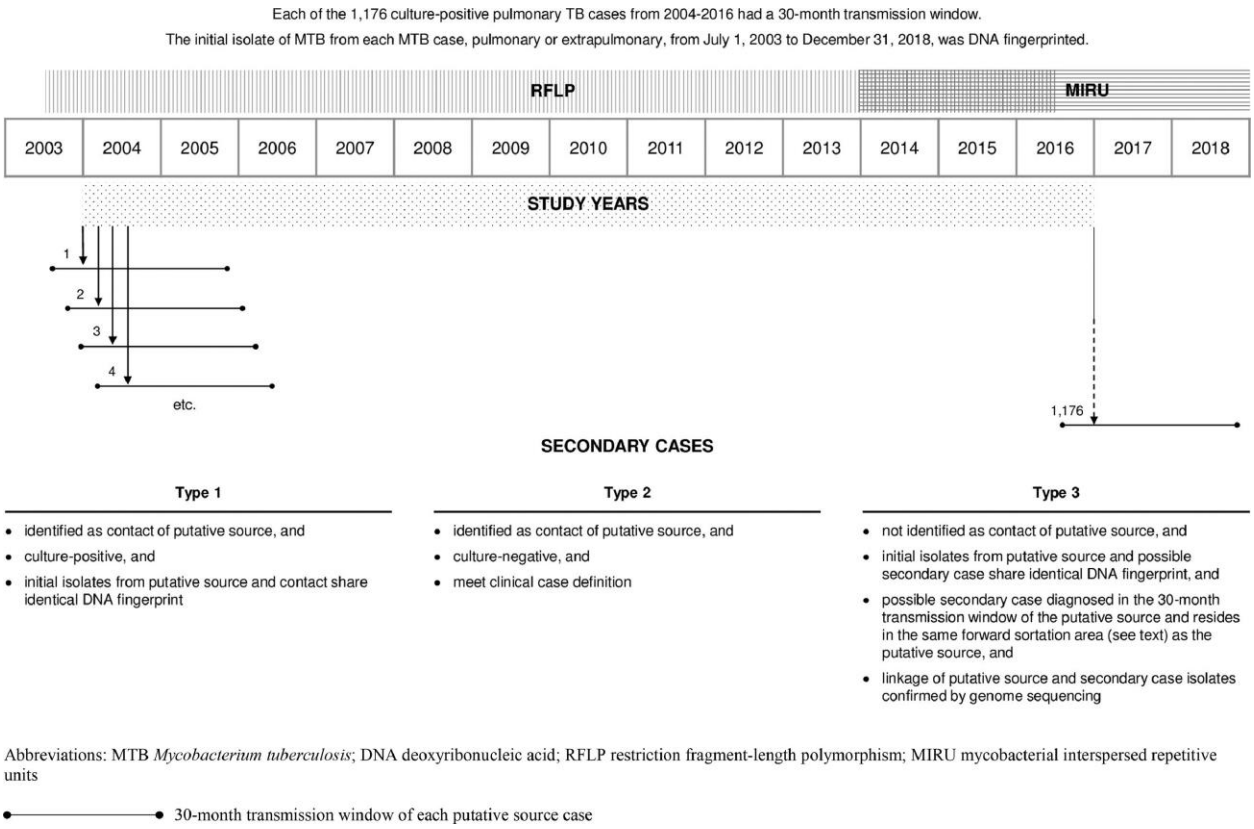


Figure 4.1: Summary of the TB transmission ascertainment process by Method #2

We also undertook four sensitivity analyses. First, we recognized that the assumption that all cases occurring after a smear-positive case were attributable to the smear-positive source could, in some chains of transmission, lead to an underestimation of transmissions arising from smear-negative cases. For instance, underestimation could occur where the first case is smear-positive and the second and third cases are smear-negative, and the third case is a contact of both the first and the second case. Therefore, we undertook a sensitivity analysis where cases that were linked to both a smear-positive and smear-negative case but occurred chronologically immediately after the smear-negative case were attributed to the smear-negative case. That is, in the sensitivity analysis, all cases that occurred after the smear-positive TB case were *not* attributed to the smear-positive individual. Next, we undertook a sensitivity analysis where we excluded all

transmissions in contacts who had been diagnosed within 6 months of arrival to Canada. This was done to account for the possibility that the contact may have acquired their disease prior to coming to Canada. That is, their disease may have been acquired from a source who was not residing in Alberta. For the final sensitivity analysis, we only considered transmissions that arose in close contacts.

The University of Alberta Health Research Ethics Board (HREB) Biomedical Panel for review of non-invasive studies involving humans provided approval for this study, protocol ID Pro00088408. Patient consent was not obtained as routinely collected data was used.

4.3 Results

4.3.1 Method #1: molecular epidemiology (DNA fingerprint cluster)

There were 1767 patients diagnosed with culture-positive TB (pulmonary or extra-pulmonary) between July 2003 and June 2016. From this cohort, 633 individuals were included in 151 unique clusters, ranging in size from 2 to 90. The cluster of 90 individuals was due to a low-copy number and these isolates underwent spoligotyping. Upon spoligotyping, there were 29 unique transmission chains—all of which were initiated by a smear-positive case. 62% of clusters consisted of only 2 individuals. There were 372 secondary events. From these secondary events, 314 arose from smear-positive patients and 58 from smear-negative patients. That is, the proportion of cases arising from a smear-negative source was 16% (58/372), 95% CI, 12–19%. In this cohort, there were 1135 pulmonary TB cases; 554 were smear-negative and 581 were smear-positive. Therefore, the relative transmission rate was (58/554)/(314/581)=0.19, 95% CI, 0.14–0.26 (see **Table 4.1)** These findings were unchanged when we restricted transmissions to a 2-year window.

Table 4.1. A comparison of the relative transmission rate and proportion of transmissions[a] attributable to smear-negative sources when replicating previous methodology versus our combined approach

	Relative Transmission Rate	Proportion of Transmissions Attributable to Smear-Negative Source
Replication of DNA fingerprint clustering methods (Method #1)	0.19 (95% CI, 0.14-0.26)	16% (95% CI, 12-19%)
Molecular and conventional epidemiology supplemented with whole genome sequencing (Method #2)	0.10 (95% CI, 0.05-0.19)	8% (95% CI, 3-14%)
Sensitivity analysis #1 (attributed transmission to a smear-negative case even if the secondary case had also been exposed to a smear-positive individual)	0.13 (95% CI, 0.07-0.24)	11% (95% CI, 5-17%)
Sensitivity analysis #2 (excluded contacts who had only been in Canada less than 180 days)	0.08 (95% CI, 0.04-0.17)	7% (95% CI, 2-12%)
Behr et al. 1999(4)	0.22	17%
Tostmann et al. 2008(5)	0.24	13%
Hernandez-Garduno et al. 2004(3)	n/a	17-41%

[a] Test comparing Method #1 vs Method #2, p=0.048. Global test comparing Method #1 vs Method #2 vs Sensitivity analysis #1 vs Sensitivity analysis #2, p=0.038.

4.3.2 Method #2: combined conventional and molecular epidemiology supplemented with genome sequencing

We then looked for secondary cases amongst 23,131 contacts of 1176 adult pulmonary TB cases diagnosed from January 2004 to December 2016. The characteristics of smear-negative vs smear-positive patients are described in **Table 4.2**. Smear-negative patients were more likely to be foreign-born (84 vs 76%), less likely to present with cavitation (7% vs 44%) and had fewer

total contacts (6 ± 9 vs 32 ± 64) but the same number of paediatric close contacts under 5 years of age (0.5 ± 1.5 vs 0.7 ± 1.7).

Table 4.2. Characteristics of the 1,176 patients 14 years of age or older with culture-positive pulmonary TB

	Smear-Negative N=563 (%)	Smear-Positive N=613 (%)
Female	264 (47)	251 (41)
Age (years)	49 (SD 21)	48 (SD 20)
Cavitation (missing n=1 from smear-negative; n=1 from smear-positive)	41 (7)	270 (44)
HIV (missing n=4)	35 (6)	35 (6)
Relapse/re-treatment	47 (8)	59 (10)
Resistance to any first-line agents	71 (13)	69 (11)
Ethnicity[a] **CB, non-Indigenous** **Indigenous** **Foreign-Born**	 32 (6) 62 (11) 469 (84)	 48 (8) 105 (17) 460 (76)
Total number of contacts	3,409	19,376
Total number of close contacts	2,670	6,153
Close contacts/case	4.7 (SD 6.7) Median=4 (IQR 4)	10 (SD 15) Median=5 (IQR 8)
All contacts/case **(Close contacts + casual contacts)/case**	6 (SD 9) Median=4 (IQR 5)	32 (64) Median=12 (IQR 25)
Number of close pediatric contacts (<5 years of age)	0.5 (1.5)	0.7 (1.7)

[a] CB=Canadian-born; Indigenous refers to First Nations, Métis, or Inuit peoples according to the Constitution Act of 1982

There were 64 Type-1 secondary cases (4 from smear-negative and 60 from smear-positive patients) and 33 Type-2 secondary cases (4 from smear-negative and 29 from smear-positive patients). See Table 3. In addition to Type-1 and Type-2 secondary cases there were 23 putative sources with potentially 28 Type-3 secondary cases. Using core SNV analysis on these isolates, we were able to determine that 13 were consistent with transmission (see Fig. 2). From these 13 Type-3 secondary cases, one transmission was attributed to a smear-negative and 12 to a smear-positive source. The characteristics of all secondary cases are described in table 3.

Thus, once we included geographically and temporally linked DNA fingerprint clusters that were confirmed to be linked by core SNVs, the proportion of cases attributable to smear-negative cases remained at 8% (9/110), 95% CI, 3–14%, $p = 0.05$, and the relative transmission rate at 0.10 (9/563)/(101/613), 95% CI, 0.05–0.19 (see **Table 4.1**).

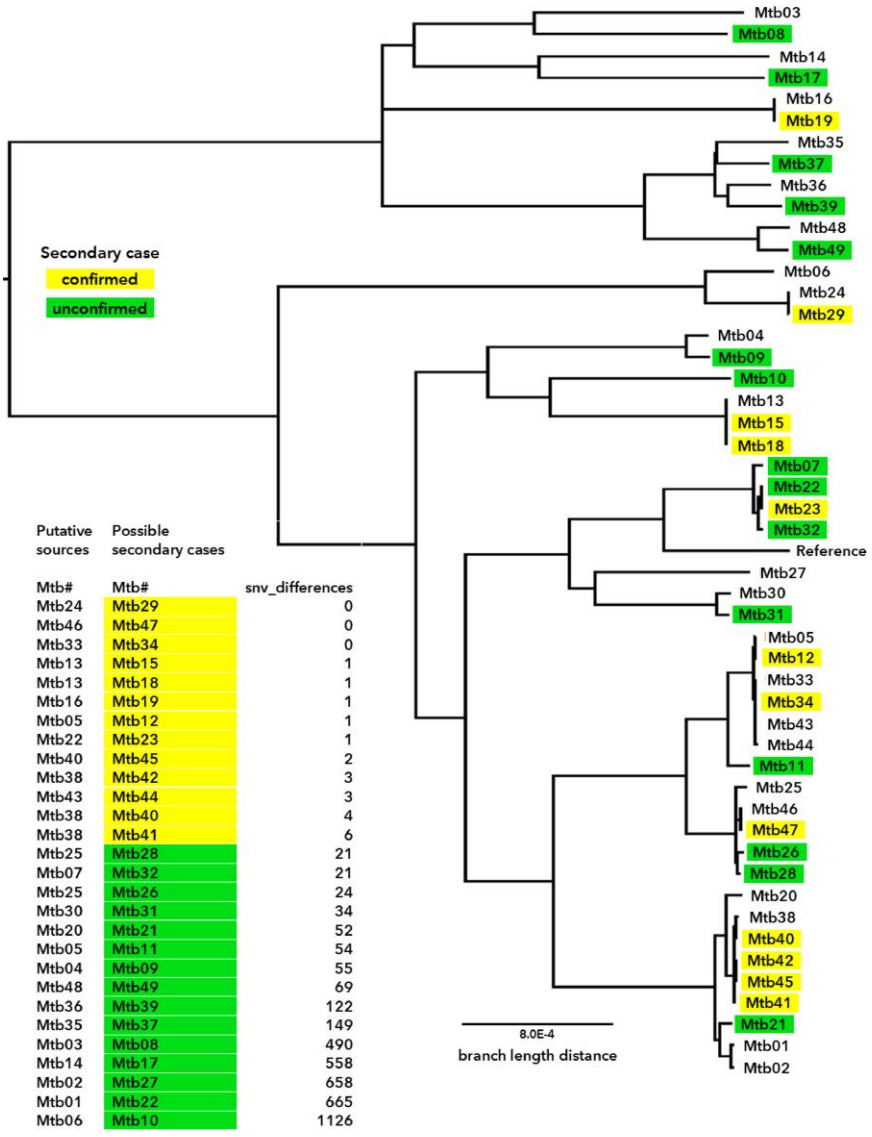
Table 4.3. Characteristics of secondary cases, according to source case smear-status

	Smear-Negative	**Smear-Positive**
Type-1 (N=64)	**N=4 (%)**	**N=60 (%)**
Days to diagnosis of contact (mean, SD)	243 (185)	119 (143)
Close contact	3 (75)	52 (87)
Household contact	2 (50)	35 (58)
Average age (SD) Median age (IQR)	33 (10) 31 (12)	33 (19) 30 (11)
<5 years old (all contacts)	0	2 (3)
Between 5-14 years old	0	8 (13)
HIV (+)	1 (25)	3 (5)
Foreign-born	2 (50)	18 (30)
Positive airway secretion smear-status	1 (25)	16 (27)
Diagnosed within 6 months of arrival to Canada	0	0
Type-2 (N=33)	**N=4 (%)**	**N=29 (%)**
Days to diagnosis of contact (mean, SD)	33 (30)	47 (49)
Close contact	4 (100)	36 (100)
Household contact	4 (100)	23 (79)

Average age (SD) Median age (IQR)	20 (26.4) 7 (22)	8 (9.5) 4 (9)
<5 years (all contacts)	2 (50)	16 (55)
Between 5-14 years old	1 (20)	9 (31)
HIV (+)	0	0
Foreign-born contact	4 (100)	9 (31)
Diagnosed within 6 months of arrival to Canada	2 (50)	6 (21)
Type-3 (N=13)	**N=1 (%)**	**N=12 (%)**
Days to diagnosis of contact (mean, SD)	279	293 (200)
Average age (SD) Median age (IQR)	55	40 (10) 39 (11)
<5 years	0	0
Between 5-14 years old	0	0
HIV (+)	0	0
Foreign-born contact	0	3 (25)
Diagnosed within 6 months of arrival to Canada	0	0
Positive airway secretion smear-status	1 (100)	6 (50)

In the first sensitivity analysis, out of the 110 transmissions which occurred, only three more cases would be re-classified to be attributable to smear-negative patients. The proportion of cases attributable to smear-negative patients would be 11% (12/110), 95% CI, 5–17%, and the relative transmission rate would be 0.13 (12/563)/(98/613), 95% CI, 7–24%. In the second sensitivity analysis, we excluded eight contacts who were diagnosed with TB within 6 months of arrival to Canada. All excluded cases would have constituted a Type-2 transmission event. Therefore, when considering transmission to known contacts, the proportion of cases attributable to smear-negative cases was further reduced to 7% (7/102), 95% CI, 2–12%, and the relative transmission rate decreased to 0.08 (7/563)/(95/613), 95% CI, 0.04–0.17. There was a statistically significant difference in the global analysis when comparing proportions of transmissions attributable to smear-negative sources as determined by the various methods and sensitivity analyses (Method #1, Method #2, and the two sensitivity analyses) (see **Table 4.1**). When looking only at

transmissions to close contacts, the relative transmission rate was [8/563]/[75/613]=0.12 (95% CI 0.06–0.25).



Putative sources	Possible secondary cases	
Mtb#	Mtb#	snv_differences
Mtb24	Mtb29	0
Mtb46	Mtb47	0
Mtb33	Mtb34	0
Mtb13	Mtb15	1
Mtb13	Mtb18	1
Mtb16	Mtb19	1
Mtb05	Mtb12	1
Mtb22	Mtb23	1
Mtb40	Mtb45	2
Mtb38	Mtb42	3
Mtb43	Mtb44	3
Mtb38	Mtb40	4
Mtb38	Mtb41	6
Mtb25	Mtb28	21
Mtb07	Mtb32	21
Mtb25	Mtb26	24
Mtb30	Mtb31	34
Mtb20	Mtb21	52
Mtb05	Mtb11	54
Mtb04	Mtb09	55
Mtb48	Mtb49	69
Mtb36	Mtb39	122
Mtb35	Mtb37	149
Mtb03	Mtb08	490
Mtb14	Mtb17	558
Mtb02	Mtb27	658
Mtb01	Mtb22	665
Mtb06	Mtb10	1126

Figure 4.2 Phylogenetic tree from core SNVs of 49 *M. tuberculosis* isolates

4.4 Discussion

Using the DNA fingerprint clustering technique reported in three separate studies (3-5), we found that the proportion of TB transmitted from smear-negative patients was 16% and the relative transmission rate was 0.19, a finding in line with these previous studies. However, when we combined both molecular and conventional epidemiology and supplemented it with core

SNV analysis, we found that smear-negative cases were ~50% less infectious than previously thought.

When Tostmann et al. looked at transmission based on epidemiologic data alone, only 6% (26/417) of source cases were smear-negative(5). They attributed the discrepancy in their findings to epidemiologic links failing to reveal all contacts. While relying only on epidemiologic data may miss certain transmission events, it is also possible that DNA fingerprint clustering techniques in the absence of epidemiologic data techniques overestimate transmission events (7, 24).

In most settings, accounting for TB transmission is very challenging (25). However, our resource-rich, low-incidence setting provides an ideal real-world condition for studying transmission biology. First, there is a high proportion of foreign-born patients. These individuals are often re-activating imported strains of *M. tb* rather than acquiring new strains. This means there is a higher diversity of strains, fewer large clusters, and an enhanced ability to discern transmissions. In our cohort, only 35% of cases had clustered DNA fingerprints and 85% of the clusters included five or fewer cases. Furthermore, we routinely implement rigorous contact tracing and incorporate social network analysis as necessary. The comprehensiveness of our routine contact tracing enhances our confidence in the assessment of transmissions.

Our study also includes Type-2 secondary cases (culture-negative active TB diagnoses). These patients were diagnosed based on a clinical case definition that included radiographic findings or rarely, sputum smear-positive but culture-negative instances. Only 5/33 of the Type-2 secondary cases were adult-type cases (over 14 years old). To our knowledge, no other study comparing transmissions from smear-negative to smear-positive patients has included such secondary cases. Because young children are highly vulnerable and therefore may serve as sensitive sentinels of

transmission even when the bacillary burden of the source case is low, we believe their inclusion is crucial. In fact, the tendency to rely on only molecular or even genomic techniques to determine transmission may lead to a neglect of paediatric TB in transmission analyses.

Despite the strengths of our study, there are also several limitations. First, smear-negative source cases have fewer close and casual contacts. Based on the Canadian TB standards(2), our protocols dictate that contact tracing of a smear-negative source can begin with only household contacts whereas for smear-positive sources, all close contacts (including non-household) are included in the first round of contact tracing. This likely accounts for the large difference in close-contacts for smear-negatives (4.7 ± 6.7) versus smear-positives (10 ± 15). While this can introduce bias, it is mitigated by the fact that if we identify tuberculin skin test (TST) conversions or secondary cases in the household, contact tracing is expanded to non-household close contacts and beyond. Furthermore, if it is assumed that contacts under the age of 5-years-old are close household contacts (which unfortunately are not captured separately from other close contacts), then close household contacts are similar for smear-negatives (0.5 ± 1.5) vs (0.7 ± 1.7). Also, given that 91% of the transmissions within the whole cohort occurred amongst close contacts and 63% amongst household contacts, the first "concentric circle" of household contacts should be identifying most cases and triggering further contact tracing, as necessary. Finally, the inclusion of the temporally and geographically linked cases that were confirmed by core SNV analysis should help to identify transmissions amongst non-identified contacts. When we conducted a sensitivity analysis which only considered transmission arising in close contacts, the relative transmission rate was 0.12 (95% CI 0.06–0.25). However, we think that considering only close contacts is a less accurate representation of transmission risk posed by the two groups because an important feature of smear-positivity is precisely the fact that there is the

potential for “superspreading” to casual (or distant) contacts. Given the outcome measure we chose to examine, which was selected out of a desire to easily compare our findings to previous studies, we could not adjust for number of contacts or clustering. Future studies should look at the odds of transmission arising from a patient with smear-negative disease as their main outcome, and consider accounting for household clustering, symptoms, and other clinical parameters. Importantly, though, even in such a study, the outcome (number of transmissions) would still rely on the contact-tracing methodology.

Finally, while we are the first group to use core SNV analysis in ascertaining transmissions attributable to smear-negative patients, given the known higher resolution of genome sequencing over DNA fingerprinting, we would ideally, financial constraints aside, have sequenced all clusters. Nevertheless, there remains a high degree of certainty around the validity of the transmission when there is both a clear epidemiologic link and 100% matched DNA fingerprint by conventional methods(24).

We recognize the growing body of literature exploring the role of subclinical (asymptomatic) disease in transmission of tuberculosis(26, 27) and the evolving understanding of the dynamic nature of TB disease progression (26). This could mean that we may be systematically underestimating the contribution of subclinical cases (27), the vast majority of which are smear-negative (28). Mechanistically, if you consider the significant aerosol production arising from routine activities like speaking or singing, cough would not be required for transmission (29, 30). While we believe our findings are an accurate reflection of transmission ascertainment reliant on routine contact tracing practice and while we attempt to account for unidentified secondary cases, the evolving literature around the role of transmission from subclinical cases shows the challenge in attributing precise transmission rates based on smear-status. Developing

the most accurate picture of transmission and attack rates requires the use of a variety of methodologies ranging from mathematical modelling to conventional epidemiology reliant on contact tracing.

The aim of this study was to replicate previous techniques while enhancing the previously used molecular clustering technique with conventional epidemiology and genomics. Our findings, therefore, are an update of these previous techniques. Based on this update, we believe that smear-negative cases result in a lower burden of transmissions than previously estimated. And our exploration of different methodologies of ascertaining transmission highlights the continued importance of conventional field epidemiology and the importance of revisiting questions about TB biology with the assistance of new tools. By showing that smear-negative cases contribute to fewer transmissions than previously thought, our findings can update estimates used in TB disease modelling and may assist in triaging of resources in increasingly resource constrained public health programs.

4.5 Chapter 4 References

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Chapter 5: Conclusion

Preventing transmission is a cornerstone of communicable disease public health, yet evidence of programmatic success to fulfill this critical function remains sparse. The study presented in Chapter 2, **"Effectiveness of Canada's TB surveillance strategy in identifying immigrants at risk of developing and transmitting TB: a population-based retrospective cohort study**," attempts to fill this knowledge gap. Compatible with the existing literature, we demonstrated that referrals were more likely to be diagnosed with pulmonary TB than non-referrals, even when compared to those from endemic (high-risk) countries. A novel contribution, however, was the demonstration that referrals were far less likely (80%) to transmit. In fact, no close contact of a referral case developed active TB disease. These findings may be interpreted as evidence of the program's success, suggesting that early identification of referrals may prevent progression to a more infectious disease state and may also reduce both the duration of exposure and the number of contacts. However, an alternative hypothesis posits that unlike referrals, who have a history of TB disease treatment or radiographic evidence of old, healed TB, non-referrals with TB infection may not have developed an immune response as capable of controlling *M. tuberculosis* dissemination. This hypothesis is supported by the understanding that the formation of granulomas can signal a transition in the immune response, from attempting to eliminate the mycobacterium to a phase of tolerance aimed at limiting tissue damage (1, 2). Within granulomas, pro-inflammatory cytokines in the central core inhibit *M. tuberculosis* while anti-inflammatory cytokines in the periphery simultaneously limit tissue damage caused by the immune response. Such compartmentalization is thought to be crucial for preventing bacterial dissemination (2, 3).

Kurt Toman's work from the 1970s also offers important insights into the timing of the development of infectious, smear-positive disease (4). Using the findings of two large

longitudinal studies from Kolin, Czechoslovakia and Niigata, Japan, which employed periodic mass radiography screening, Toman found no difference in the time to development of smear-negative versus smear-positive disease. He noted that smear-positive disease could manifest in patients with normal radiographs from just 12 months prior. Even in a study explicitly designed to detect chest radiographic abnormalities at the earliest possible stage and employing a 4-month interval for chest radiograph assessment in contacts, 21% of cases were already at moderate or advanced stages of the disease. Toman emphasized that smear-positive disease can develop rapidly and without any perceptible radiographic lesions, countering the notion that the development of highly infectious disease is necessarily the result of a linear, chronic process. The lack of a linear progression to severe TB disease is in line with our evolving understanding of the dynamic natural history of TB (5).

These ideas motivated the study in Chapter 3, "**Is there a fundamental flaw in Canada's post-arrival immigrant surveillance system for tuberculosis?".** Here, we examined the progression of chest radiographs from pre-immigration to post-arrival TB diagnosis in a cohort of newly arrived migrants who had undergone pre-immigration chest radiographs as a component of the immigration medical examination. Our study indicated that non-referrals moved from TB infection to pulmonary TB disease more quickly and more aggressively than referrals. These findings not only suggest a potential difference in the biology between the two groups, but also highlight a subgroup at risk of the kind of active TB disease that should concern public health programmes. Worryingly, this subgroup often remains unnoticed until after they become symptomatic. This then brings us to challenging questions of how Canada's foreign-born TB surveillance program can be improved.

5.1 Challenges of latent TB infection screening programs in migrants

As of May 2019, the national TB surveillance program in Canada underwent a minor change. It now requires pre-arrival TB infection screening, preferably with IGRA, for a specific subset of migrants. The high-risk group includes individuals with known close contact with an active TB case, HIV-positive serology, history of certain head and neck cancers, dialysis or advanced CKD, or organ or bone marrow transplant recipients on immunosuppressive therapy (6). Although this expanded screening should detect more cases, its impact on the domestic TB burden is expected to be limited.

A study conducted in British Columbia, Canada examined all active TB cases and underlying risk factors (which were diagnosed at least 6 months prior to their active TB diagnosis) in one million foreign-born migrants to Canada with permanent residency immigration status. They found that if they were to have undertaken TB infection screening on individuals living with HIV infection, those requiring dialysis, those with a history of solid organ/bone marrow transplant/silicosis, or those using TNF alpha inhibitors, only 4.2% of total active TB cases would have been prevented. Adding steroid use, disease modifying anti-rheumatic drugs (DMARDs) use, or certain head and neck cancers increased this number to 8.3% of cases but required the testing of 176 people to prevent one case (7). Given the small incremental benefit of testing only those at the very highest risk of TB reactivation, the most recent Canadian TB standards recommend testing migrants whose risk of TB disease within 5 years of a positive IGRA or TST is 1% or higher (6). This threshold is achieved within specific subsets of migrants, including those with certain high-risk medical conditions and specific refugee groups. Additionally, it is met when considering various combinations of three key factors: age, time since arrival, and the incidence of TB in the country of origin.

While the potential benefits of addressing latent TB infection among migrants are well recognized (8-10), several challenges complicate the scale up of migrant screening efforts (11, 12). The current widespread practice of symptom inquiry and chest radiograph, designed for active TB detection, is not suitable for diagnosing latent TB infection. Furthermore, tests for latent TB infection, which rely on detecting immunologic responses rather than viable bacteria, have a poor positive predictive value for progression to TB disease. This limitation complicates testing and treatment algorithms and raises ethical concerns due to the need to treat many migrants who may not personally benefit from treatment but may experience side effects. Extensive examination of cost-effectiveness has yielded conflicting results depending on factors such as the receiving country, whether to choose pre-arrival or post-arrival LTBI screening, which risk groups are targeted, choice of TB preventative therapy, and consideration of post-TB sequelae (13-16). In one Canadian study, the most cost-effective strategy involved pre-arrival IGRA screening for migrants arriving from countries with a TB incidence rate exceeding 30 cases per 100,000, followed by a 4-month rifampin regimen (13). This approach could reduce TB incidence by more than 45% at costs of less than $50,000 per quality-adjusted life year gained. However, it would require in-country follow-up of 18% of new arrivals, which is much higher than the current rate of 2 to 2.5% undergoing screening. Furthermore, concerns arise regarding retention in the cascade of LTBI care. Systematic reviews examining the LTBI cascade of care have shown low overall completion rates of LTBI therapy. For instance, Alsdurf et al. found a completion rate of 18.8% with isoniazid (17) while Sandgren et al. noted the completion rate to be highly variable (18). In a modelling study of various post-arrival migrant LTBI strategies in Australia, even when 90% follow-up at each step of the care cascade was assumed,

cost-effectiveness was unlikely to be achieved at willingness-to-pay thresholds of approximately $45,000 to $75,000 Australian dollars (approximately 30,000 to 65,000 USD) (19).

Most individuals diagnosed with LTBI do not and will not pose a health risk to the population nor will they be a financial burden on receiving countries. Consequently, the argument for mandating LTBI screening and treatment becomes less compelling. If screening occurs pre-arrival, not only are the costs passed on to the individual migrant, but their use of healthcare resources adds further pressure to health systems with other urgent priorities. It is not enough to focus solely on the cost-effectiveness of screening strategies. Mechanisms for enforcing screening and potentially transferring the costs must also be carefully considered to minimize harm and clarify the responsibilities for immigration authorities and receiving countries (20).

In the Canadian context, the structure of the federal health system present additional challenges. The Immigration and Refugee Protection Act (IRPA) provides the legal framework for controlling the entry of foreign nationals into the country(21). Under the IRPA, a foreign national may be deemed inadmissible to Canada on health grounds if their health condition might:

1. Be a danger to public health (section 38(1)(a)).
2. Be a danger to public safety (section 38(1)(b)).
3. Cause excessive demand on health or social services (section 38(1)(c)).

Active TB is considered a public health danger. Consequently, the federal government, specifically, Immigration, Refugee, and Citizenship Canada, is tasked with identifying prevalent active TB cases pre-arrival and ensuring that those at highest risk of re-activation are followed up on arrival. On the other hand, under the Canada Health Act of 1984 (22), the provision of healthcare is the responsibility of provincial and territorial governments. While latent TB

infection is not an imminent public health and safety threat, it may be crucial for the individual migrants' health, making it a healthcare matter falling under provincial jurisdiction. This distinction complicates the coordination of foreign-born TB surveillance between federal and provincial authorities.

Some jurisdictions in Canada have reported favorable outcomes in migrant LTBI screening. For instance, a refugee clinic in Estrie, Quebec demonstrated an overall completion rate of 69%. And the cost to avert a single case of TB was estimated at $16,056, compared to the expenditure of $32,631 associated with managing a TB case (23). Nevertheless, experiences from the United Kingdom serve as a reminder of the challenges in constructing a migrant LTBI screening program.

In 2015, England implemented a non-obligatory, nationwide migrant LTBI screening strategy. This initiative involved post-arrival screening at the time of primary care registration. Migrants who had arrived in England within the preceding 5 years from countries with a high TB incidence (≥150 cases per 100,000 people per year or any country in sub-Saharan Africa) were eligible for IGRA screening and subsequent follow up and treatment (10). Upon evaluating the program's effectiveness, researchers observed a reduction in the risk of TB incidence attributable to LTBI testing and treatment. Specifically, the hazard ratio for incident TB disease was 0.76 (95% CI, 0.63-0.91) when comparing those who had LTBI testing compared with those with no testing (24). However, it was also noted that out of 368,097 eligible participants, only 10% underwent testing, and among the 6,640 individuals who tested positive with IGRA, merely 26% initiated TB preventative therapy. A commentary on the study emphasized the need to reimagine the approach to addressing migrant health disparities, calling for collaboration between stakeholders to redesign comprehensive, integrated, and culturally sensitive infectious disease

screening programs that prioritize patient-centered care (10). Canada, like other low TB incidence settings, must establish thresholds for migrant TB screening that consider ethical obligations, cost-effectiveness, and feasibility within the constraints of a burdened healthcare system. Overcoming these challenges will not be easy, but given the federal government's plans to significantly increase immigration, it should remain a top priority (25).

By examining Canada's TB surveillance program in Chapters 2 and 3, we observed that patients with active pulmonary TB who were smear-negative on sputum smear microscopy seldom had close contacts who subsequently developed TB disease. This prompted a review of the existing data on relative transmission from sputum smear-negative versus smear-positive individuals, and we found that the previous studies relied heavily on molecular clustering (26-28). In other words, they lacked complementary epidemiologic and genotypic data that could better discern chains of transmission. Consequently, in Chapter 4, we attempted to identify transmissions from culture-positive pulmonary TB cases in the province of Alberta using these additional data and methodologies. For our study, we included clinically diagnosed secondary cases and spatiotemporally linked cases (who were not identified as named contacts), and then further interrogated transmission chains with whole genome sequencing. Using both detailed conventional epidemiologic data and whole-genome sequencing techniques, we discovered that the risk of transmission from people with sputum smear-negative disease was 50% less than previously described. These findings may not be generalizable to settings outside those with low TB- incidence, high-income, and universal access to health care, but they can inform local prioritization of contact-tracing and de-isolation policies.

5.2 Whole genome sequencing: applications and issues

Determining chains of transmission can be a complex endeavor, complicated by numerous limitations and assumptions. Within this context, whole genome sequencing has emerged as a crucial tool for shedding light on transmission dynamics. In Chapters 3 and 4, as well as in studies previously published by our group (29), we have examined "type 3 transmission events," defined as secondary cases that are spatiotemporally linked and possess identical molecular fingerprints (DNA) to a presumed source but who are not named as contacts in epidemiologic investigations. In Chapter 4, we further investigated these putative transmission chains using genome sequencing. We found that of the 28 type-3 transmissions, less than half (n=13) were consistent with recent transmission when investigated with whole genome sequencing (WGS). This suggests that DNA fingerprint clustering, even when constrained by spatiotemporal criteria, can overestimate transmission events.

Similar findings have been reported in other studies, particularly in lower-incidence settings like the United Kingdom (30), Australia (31) and the Czech Republic (32, 33). One Swedish study retrospectively paired MIRU-VNTR and WGS data on 93 isolates from patients with documented epidemiological connections, finding WGS to be more highly correlated with the conventional epidemiology than did the MIRU-VNTR. When MIRU-VNTR was compared to WGS, the sensitivity was 96%, and the specificity was 80% (34). In a systematic review that used conventional epidemiology as the gold standard and set thresholds of 6-10 SNPs to establish recent transmission, 8 of the 12 included studies showed perfect correlation between epidemiology and WGS (100% sensitivity), while specificity ranged from 17-95% (35).

5.2.1 The advantages and limitations of whole genome sequencing in studying TB outbreaks in Canadian Inuit communities

Numerous published studies, including several in the Canadian context, have now used WGS to break up larger molecular genotyped clusters (36, 37). TB endemic Inuit communities in Northern Canada have demonstrated both the insights gained from WGS as well its limitations. The TB incidence among Inuit peoples in Canada is over 350 times higher than among non-Indigenous Canadian-born persons, attributable to sporadic and ongoing outbreaks (38). Studies in these communities have found that while WGS breaks up large DNA fingerprint clusters, the low diversity (small number of SNVs separating isolates) complicates analyses, making it difficult to determine whether incident TB reflects transmission, reinfection or reactivation (39, 40).

In one Nunavik village of 933 persons, in an outbreak that consisted of 50 TB cases, 49 isolates shared an identical DNA fingerprint (40). WGS separated the cluster into three groups and integration of epidemiologic data further separated them into five distinct chains of transmission and one reactivation. Then, to address the issue of low genetic diversity, deep sequencing was employed on the same outbreak isolates in a subsequent study (41). Employing depths of 500-1000X instead of the ~50X used in the preceding study, they identified a previously undetected super-spreader who was the likely source of 17 cases, approximately one-third of the cases in the outbreak. Acknowledging the issues with relying solely on WGS in Northern Canada, Alvarez et al. combined social network analysis and WGS (with a conservative threshold of three or fewer SNP differences) to assess an outbreak in Iqaluit. They also found that superspreading played a crucial role in propagating local outbreaks (42).

While WGS offers greater discriminatory power than DNA fingerprinting while also providing insights into lineage and broader transmission dynamics, its use in areas of low genetic

diversity—often locations with high incidence rates—remains complex, occasionally ambiguous, and still reliant on conventional epidemiologic data. The insufficiency of WGS alone to resolve chains of transmission has been corroborated in studies conducted outside Canada's Inuit communities, including those in the Canadian Prairie First Nations (36) , London, England (43) as well as Valencia, Spain (44).

5.2.2 Limitations and challenges of WGS

One of the challenges in interpreting WGS data is in determining the relatedness of Mycobacterium tuberculosis complex (MTBC) strains. In low-incidence settings, a cut-off of six single nucleotide polymorphisms (SNPs) or fewer is commonly used as the threshold for recent transmissions (within the prior three years) (45). However, this threshold has been questioned. A recent study of epidemiologically linked case-pairs from Los Angeles County, California, from 2015-2018 (46) examined the "case-pair interval" to inform these criteria. The "case-pair interval" was defined as the time between the collection of the source case specimen and that of the secondary case specimen and was used as a proxy for the estimated rate of mutation. Contrary to the underlying assumptions of SNP differences to establish timing of infection, some secondary active cases resulting from reactivation of remote infection had few SNP differences when compared to the source. This study did not indicate a linear relationship between SNP distance and the duration of infection period. In their review of the existing literature, the authors found four epidemiologic studies estimating the rate of change between latent and disease periods. Of those, two studies found a constant rate of mutation (47, 48), while two others found that the mutation rate was lower during latent infection (49, 50). As a result, like Tyler et al. (2017) (39), the researchers argued that a universal threshold for establishing recent transmission is not possible, nor can a universal rate of change be assumed. Instead, these values are likely

highly dependent on the location of the outbreak and unique epidemiologic factors, such as the structure of the outbreak, host, contact, and bacterial factors.

Several critical questions persist regarding whole-genome sequencing (WGS) techniques and methodologies, such as the significance of within-host diversity and standardization practices. In certain cases, individuals may be infected with multiple strains, or a single strain may undergo microevolution, giving rise to clonal variants (51). Failing to account for within-host diversity can lead to errors in transmission ascertainment. Although this potential source of error is acknowledged (43), no consensus exists on the acceptable threshold for the proportion of minority alleles (i.e., a wide range of 75-90% is observed across studies) (35, 45).

Not only is there a lack of consensus around the acceptable threshold for allele frequency, but also with regards to the excluded genome regions, software used, and applied parameters (52, 53). The bioinformatic tools and algorithms used to process the data generated from the sequence of an entire genome of *M. tuberculosis* is referred to as the "pipeline". While the use of a well-defined pipeline ensures efficiency and reproducibility, these pipelines are custom-built by each institution and given the lack of international standards, the inputs may vary considerably.

Jajou et al. (2019) compared four different SNP-based pipelines by having each of the four institutes examine the same set of WGS data from the Netherlands using their own pipeline. Overall, they found high concordance between the different pipelines in determining epidemiologic links (52). By contrast, Walter et al. (2020) reported varying epidemiologic conclusions when four genomic epidemiology groups employed their own WGS pipelines on identical real-life outbreak sequence data (53). For example, using one pipeline, 81% of sample comparisons had fewer than a 5 SNP difference (indicating recent transmission), whereas for two other pipelines, only 0.5% of comparisons met the 5 SNP threshold. In the Jajou et al. (2019)

study, the pipelines had employed similar genomic filters and 4 of the pipelines had used the "Samtools" SNP calling software; this was not the case in in the Walter et al. (2020) study. Given the variability in pipelines and the subsequent consequences for genetic distance measurement and phylogenetic structure, the authors advocate for further research and caution against over-reliance on WGS for interpreting chains of transmission.

5.2.2 Whole genome sequencing for predicting drug resistance

While WGS for transmission analyses is an important tool, its key use at the present time—particularly globally—may be in predicting drug resistance profiles (54-58). CRyPTIC, the "Comprehensive Resistance Predication for Tuberculosis: an International Consortium" has published on the high accuracy of genotypic predictions of first-line drug resistance (59) and continues to analyze thousands of genomes to determine resistance profiles (56, 57). Even if WGS cannot be widely adopted, clarifying gene mutations associated with resistance to newer drugs could help develop new genotypic arrays, aiding clinical labs in identifying mutations of clinical and public health importance. In a UK economic analysis, routine WGS use proved cost-effective (55), with savings from reduced laboratory costs of phenotypic/molecular drug susceptibility testing and care improvements due to expedited drug susceptibility results, rather than from reduced transmission via genomic epidemiology.

WGS's programmatic potential could be maximized with direct, culture-free, real-time WGS on clinical samples. Direct WGS yields rapid results and detects more within-host diversity (51). However, technical challenges persist, including the presence of human and other microbe DNA (particularly non-tuberculous mycobacteria) (60, 61). If direct sputum sequencing techniques can be refined (51, 60, 62-64), the potential is considerable. For instance, Goig et al. conducted direct WGS on 37 isolates from 27 TB patients (65). They were not only able to accurately

predict drug-susceptibility but also to make accurate transmission inferences. While this group provided a cost estimate of the process at approximately 280 USD per sample, this did not include the highly relevant cost of personnel hours. Nevertheless, Soundararaj et al. emphasize that if WGS could function as a "standalone replacement" for all tests, including culture-based DST, it has the potential to be highly informative and cost-effective, even in low- and middle-income countries (60).

5.3 TB Transmission and Super-spreading

"Transmissibility is the defining characteristics of infectious disease." – Mark Woolhouse (66)

It may seem axiomatic that infectious diseases are defined by their transmissibility, but quantifying transmission, particularly for an infection like TB with its complex natural history, presents significant challenges. WGS has paved the way for new insights and improved quantification of *M. tuberculosis* transmission. A critical insight from WGS studies has been the identification of the role of super-spreaders, or superspreading events (SSE), in TB transmission dynamics (41, 67, 68). The subsequent section delves into the heterogeneity of transmission in infectious diseases, the role of superspreading events in TB, and how innovative diagnostic tools that exploit the airborne mode of transmission of TB may aid in pinpointing potential sources at the epicenter of "super-spreader" events.

5.3.1 Heterogeneity in transmission of infectious diseases

Transmissions in various infectious diseases were recognized to occur in clusters (69). For vector-borne parasites and sexually transmitted infections, it was noted that 20% of the host population accounted for at least 80% of transmissions (69). This 80/20 rule, also known as the Pareto principle, applies to a range of disciplines, including economics and nutrition, and appeared similarly explanatory for numerous infectious diseases. Super-spreader events had also

been documented in influenza (70), rubella (71), and measles outbreaks (72, 73). However, due to the challenges of modeling these respiratory diseases—where there is no vector and spread is not strictly limited to a finite number of sexual contacts—heterogeneity in their transmission was less well recognized. This perspective changed with the onset of the SARS pandemic in 2002. Given that SARS-CoV-1 was a novel pathogen with a high morbidity and high symptomatic rate, its emergence could be tracked through a series of well-documented super-spreader events (74) including a hotel in Hong Kong (75), an outbreak in Singapore (76), a flight to Beijing (77), and a hospital in Toronto (78). In an early study of these superspreading events, defined as outbreaks involving 8 or more individuals, the authors posited that the presence of superspreading events was a key determinant of whether epidemic spread would occur after the virus was introduced into a country (74).

Mathematically, the basic reproductive number, R0, is defined as the average number of secondary cases of infection resulting from a single case introduced into a susceptible population (69, 79-81). However, this population-average does not capture the fact that there can be considerable variation in the number of transmissions that arise from one infected case (82). Because the early modelling of SARS did not take this variance into account, many of the early models did not conform with observed outbreak dynamics (83). This prompted a closer look at the transmission of respiratory infections. In a landmark publication in Nature in 2005, Lloyd-Smith et al. compared the fit of 3 different distributions (Poisson, geometric, and negative binomial) generating 3 candidate models for secondary case distribution (79). They used the empirical contact tracing data from 8 directly transmitted diseases (non-STI and non-vector infectious diseases) to challenge these candidate models. They found that the models that assumed homogeneity did not fit with the surveillance data and that “the distribution of

individual infectiousness around R0 is often highly skewed". It was the negative binomial model that best fit the contact tracing data for most of the infections and allowed for comparison of the propensity for superspreading (of different pathogens) through the "dispersion parameter". The variance of the negative binomial model is R0(1+R0/k), where *k*=the dispersion parameter. This statistical parameter reflects the degree of superspreading, such that a the lower the *k* (the dispersion parameter), the higher the heterogeneity. Using this approach, the authors were able to show that SARS and measles exceeded the aforementioned 80/20 rule, such that fewer than 20% of cases accounted for 80% of cases.

5.3.2 Understanding the role of heterogeneity in TB transmission

While it was not yet mathematically quantified, knowledge of the marked variability among TB patients in their ability to transmit dates back to the foundational work of Richard Riley and colleagues (84). In a series of experiments carried out in the 1950-60s, air was directly exhausted from a TB ward into a distant chamber housing guinea pigs. Guinea pigs inhaling *M. tuberculosis* infected aerosols developed TB infection. However, in one study, only 4% of patients produced 73% of infections in guinea pigs (84) and in another study, merely 13% of patients accounted for 100% of guinea pig infections (84). This finding of heterogeneity has also been corroborated in more recent animal studies with MDR and XDR TB (85, 86).

Beyond animal studies, in 2013, Ypma et al. appear to have been the first group to estimate the dispersion parameter of TB using epidemiologic data (87). They used DNA fingerprint (RFLP) clustering in the Netherlands to estimate the dispersion parameter as *k*=0.10 (95% CI, 0.09-0.12). In 2019, Melsew examined all TB infections (including latent infection diagnoses via TST positivity of 10mm or greater) in Victoria, Australia (88). They determined k=0.16, with 75% of

all secondary infections arising due to a super-spreading event (using the definition proposed by Lloyd-Smith et al., 2005), and 20% of index cases resulting in 90% of secondary infections. Analysis of a network of XDR-TB in KwaZulu-Natal, South Africa concluded that missing cases in their transmission chains were likely to be partly due to unrecognized superspreading events (89). And a modelling study of transmissions in South Africa suggested that non-repeated contacts, which are less likely to be identified via routine contact tracing practices, contribute to 79% of TB disease development (90). Their estimated *k* ranged between *k*=0.12 (0.06-0.27) to 0.18 (0.094-0.37), assuming 44% and 99% case detection, respectively. The limitation of these studies was that they used only outbreak data, had missing cases, or employed non-WGS genotypic methods known to overestimate cluster size.

A 2023 systematic review of heterogeneity in TB transmission focused solely on studies that had undertaken whole-genome sequencing on population-based or surveillance data (67). To allow for specific assessment of population-level dynamics, they explicitly excluded outbreaks. From the nine included studies, they estimated that k= 0.02-0.48. In other words, only 2-31% of source cases accounted for 80% of TB transmissions. Taken together, this body of evidence clearly suggests the highly over-dispersed nature of TB transmission.

5.3.3 Implications of heterogeneity of TB transmission and risk factors for superspreading

An important implication from the understanding that most transmissions arise from a small proportion of source cases is that targeted control measures could have a marked impact on reducing TB incidence. The challenge for over-burdened TB programmes, particularly in medium and high TB incidence settings, is to identify this small proportion of cases who are likely to account for most transmissions.

When assessing the contagiousness of an infectious disease, four factors are traditionally considered: the organism, the susceptible contact, the environment, and the infectious host (91). Any one of them alone or any combination of these factors, can increase likelihood of transmission and result in superspreading. While there is considerable interest in whether certain *M. tuberculosis* strains are less transmissible (drug-resistant strains) or more transmissible (the Beijing/W strains), to date, they have not been proven to have intrinsically variable transmission potential (1, 92). Focusing on the susceptible contact, there can be variability in susceptibility to both infection and progression to disease. For instance, BCG vaccination during infancy appears to provide protection against infection (93, 94). There is some evidence that the risk of disease after re-infection (which occurs at least 18 months after the first infection) may be much lower than with the initial infection (1, 95). And higher risk of progression from infection to disease among immunocompromised individuals is well established. Vulnerable susceptible contacts include children under 5 years of age (96), people living with HIV, and individuals with a history of silicosis or diabetes or those receiving immunosuppressive drugs (97). With respect to environmental factors, a source case living in a poorly-ventilated home with little sunlight or an individual who is incarcerated in a crowded correctional facility occupy environments that are more conducive to superspreading (1).

While TB transmission is multifactorial and requires the interplay of the pathogen, the contact, the environment, and the host, due to its immediate availability and importance in guiding therapy, TB control programmes typically begin prioritization of contact tracing by considering host (source case) factors. Host level predictors of infectiousness include: sputum-smear microscopy status, typical vs atypical radiographic findings, cavitary vs non-cavitary radiographs, the presence of laryngeal TB, pulmonary vs extrapulmonary diseases, cough and

cough duration, as well as delays in diagnosis and appropriate therapy(1). In a meta-analysis of risk factors for infectiousness, the authors reviewed 37 studies and found that treatment delays of >28 days, cavitary disease, and sputum-smear positivity were associated with increased infectiousness (98).

5.3.4 Use of bioaerosols to investigate infectiousness and superspreading potential

Among the host-level characteristics associated with infectiousness, sputum smear microscopy stands out as the most widely used marker. As demonstrated in Chapter 4, and as other studies have found (26, 28, 98, 99), sputum smear-positivity (versus sputum smear-negativity) correlates with infectiousness. However, the association between increasing sputum smear-grade and increasing infectiousness is not clear. How then should overburdened TB control programmes in high TB incidence settings prioritize contact tracing to maximize the identification of cases with superspreading potential?

Given the WHO's guidance for use of WHO-recommended rapid diagnostic such as the Xpert MTB/Rif Ultra assay, it would be reasonable to consider the cycle threshold value as a surrogate of infectiousness. However, its strong correlation with sputum smear-positivity (100) makes it an unlikely source of new information about infectiousness. A more promising direction may come from our understanding that *M. tuberculosis* transmission occurs via inhalation of *M. tuberculosis*-containing aerosols. Assessing the infectiousness of theses bioaerosols directly represents an exciting avenue for future research.

Two technologies have attempted to capture and quantify the infectiousness of exhaled air: the cough-aerosol sampling system (CASS) and facemask sampling. Using the CASS, Kevin Fennelly and colleagues have closely scrutinized cough aerosols as indicators of infectiousness (101-105).

The CASS samples the air using two Andersen cascade impactors filled with solid media plates. Andersen cascade impactors, first used by Riley et al. in the 1950s, operate on the principle of inertial impaction, where particles with different aerodynamic diameters possess varying inertia, causing them to impact onto different stages of the impactor (106). Since its initial application in 2004 (107), the CASS has been used by two groups on over 700 patients to culture viable *M. tuberculosis*. It has been used in the United States, Uganda, and Brazil, on a total of 262 TB patients across five cohorts. A separate team applied the technology on a cohort of 452 TB patients in South Africa (108). Both groups have shown that cough aerosols exhibit a dose-response relationship with markers of TB infection, such as TST induration size (102, 105) and IGRA levels (102, 105). They have also noted a similar pattern with the development of active disease in exposed contacts(102, 104). Importantly, this dose-response relationship was not observed with sputum smear microscopy grade. Furthermore, the relationship between sputum smear microscopy grade and cough aerosol production is not necessarily linear. Specifically, the proportion of patients whose coughs produced high-level aerosols (defined as >10 CFU) was 21%, 7% and 32% for 1+, 2+ and 3+ sputum-smear grade, respectively (105). Most high-grade smears were in fact aerosol negative, suggesting that triaging cases based on sputum smear grade alone could result in inefficient prioritization. In terms of prediction of aerosol positivity, Theron et al. found that the areas under the curve (AUCs) for the receiver operating characteristic (ROC) analysis of smear grade and GeneXpert cycle threshold values were similar, at 0.72 (0.66, 0.77) and 0.71 (0.66,0.77), respectively. Sputum smear grade's specificity at rule-out thresholds (95% sensitivity) was suboptimal at 42%, while its sensitivity at rule-in thresholds (95% specificity) was poor at 14% High peak cough flow and low symptom score were clinical correlates of aerosol-positivity.

Taken together, Fennelly et al. argue that that they have satisfied the Bradford Hill criteria for causation linking aerosols and the risk of infection and have partially fulfilled the Bradford Hill criteria for risk of progression to active disease (103). They propose that cough aerosols are a superior marker of infectiousness than sputum smear-microscopy (102, 104, 105) and suggest that we should prioritize the contact tracing of individuals with an "aerosol-positive phenotype" (101).

Aerosol-sampling devices that can directly measure bioaerosols may be useful tools in identifying patients who are more likely to be involved in superspreading events. However, the implementation of aerosol sampling devices is hindered by prohibitive costs and technical complexities associated with their operation. To overcome these concerns, other groups have examined exhaled air in facemasks.

Although facemask sampling does not provide direct measurement of bioaerosols, it attempts to quantify the presence of bacilli in exhaled breath. Williams CM et al. have conducted investigations into facemask sampling techniques for *M. tuberculosis* (109, 110) and COVID-19. In these studies, participants were asked to wear facemasks containing either a gelatine or polyvinyl alcohol (PVA) matrix for varying durations (109) (110). The researchers observed that *M. tuberculosis* was detected four times more frequently through facemask sampling when compared with contemporaneous sputum samples. In a cohort comprising 20 patients, Xpert MTB/RIF analysis on facemask samples identified *M. tuberculosis* infection six weeks earlier than sputum sampling in four of the six patients who were diagnosed with pulmonary TB. This finding suggests that facemask samples may constitute a more sensitive specimen compared to sputum (110). These same researchers were unable to identify any clinical or radiologic correlates to facemask positivity.

In a subsequent study to look at facemask sampling for determining infectiousness, Williams et al. (2023) examined 46 smear-positive pulmonary TB patients and their 217 household contacts(109). The primary outcome was incident infection in household contacts as defined by a QFT conversion from negative to positive. Among the source cases, 91% had a PCR-positive facemask sample, and 41% were classified as highly positive (defined as an IS6100 copy number ≥20 000 copies). There was no statistically significant difference in sputum smear grade between individuals with high vs low-level positive (<20,000 or negative) facemask samples. The adjusted odds ratio for QFT conversion in household contacts of high vs low positivity source cases was 3.20 (1.26-8.12), p=0.01, while there was no statistically significant association between AFB smear status of the source case (1+ vs >1+) and risk of infection in household contacts. That is, facemask positivity was a superior predictor of incident TB infection in household contacts than sputum bacillary load [75].

Interestingly, parallel findings were observed in a small facemask sampling study of 34 patients with SARS-CoV-2 and their household contacts. Specifically, facemask sample SARS-CoV-2 RNA viral load was associated with an increased risk of household transmission whereas upper respiratory tract viral load was not (111).

5.4 Concluding thoughts

Around the globe, TB inflicts a heavy toll, causing significant morbidity and mortality. In Canada, the burden falls disproportionately on Canadian-born Indigenous populations and among foreign-born individuals, who constitute the largest proportion of incident TB cases. The original research presented in this thesis began with an investigation of Canada's TB surveillance system among the foreign-born. Our findings revealed that most incident cases and the majority of the most infectious cases occurred in individuals who had not been considered high-risk for

reactivation prior to immigration. That is, pre-arrival radiographic screening was not identifying the cases that were ultimately the most infectious.

One hypothesis, supported by historical literature, suggests that individuals previously treated for TB disease or who have radiographic evidence of old, healed TB, may have achieved a more immunotolerant state with *M. tuberculosis* whereby the host immune system is better able to prevent dissemination. We examined this hypothesis in Chapter 3, and we found that recently arrived migrants who developed TB, but who had normal chest radiographs prior to immigration, had more rapid progression of their disease than those who had abnormal chest radiographs pre-immigration.

Taken together, our studies highlighted the existence of a subgroup of individuals at risk of developing the type of infectious TB disease that should be prioritized by public health programs but who remain unidentified by Canada's current foreign-born TB surveillance system. In Chapter 5, I discussed the potential benefits and challenges of alternative foreign-born screening strategies that move beyond radiographic screening, aiming to test and treat TB infection with the goal of preventing TB disease.

Another observation from the study in Chapter 2 was that many individuals who were diagnosed via active in-country surveillance had smear-negative pulmonary disease and did not pose a significant transmission risk. This observation prompted the study in Chapter 4, which reassessed the infectiousness of smear-negative cases by combining multiple epidemiological tools to describe transmission in a large cohort. By pairing detailed contact information gleaned from conventional epidemiology with the additional granularity of WGS supplementing molecular epidemiology, the findings support that, at least in a low TB-incidence high-income setting,

people with smear-negative pulmonary disease were considerably less infectious than previously reported.

Our use of whole genome sequencing in Chapter 4 led to a detailed examination of its benefits and limitations in Chapter 5. Despite challenges in determining strain relatedness and standardization practices, when paired with conventional epidemiology, WGS increases confidence in transmission chain determination. This enhanced resolution has led to the identification of more superspreading events as well as a more reliable estimate of the dispersion parameter of *M. tuberculosis*. Our ability to better quantify the role of superspreading provides evidence in support of the benefits of targeted prevention efforts. Unfortunately, the current surrogate marker of infectiousness, sputum smear microscopy, is suboptimal for pinpointing the most infectious cases. Therefore, I concluded this work by exploring the possibility of using bioaerosols—the actual infectious moiety for TB—to determine infectiousness and to identify individuals most likely to be involved in superspreading events.

We stand at an exciting juncture in TB history, where the following scenario can be imagined in the near future: A patient presenting with a cough is given a facemask from which a breath sample is collected. From there, a WHO-recommended rapid diagnostic performed directly on the facemask sample identifies both *M. tuberculosis* and provides a real-time drug susceptibility panel for all first-line agents. Effective treatment begins without delay. Simultaneously, based on pre-established cut-offs, the patient is identified as being in the top 5th percentile of infectious bioaerosol producers. Given the potential for superspreading, contact tracing starts urgently, involving not just close and casual contacts but also including transit routes and social gathering places. Within a few days, when direct WGS from the facemask sample is complete, its results

are entered into a national database where a match is made with an identical genome in an adjacent province, uncovering a linked index case.

Along with established epidemiological practices, the incorporation of tools like whole genome sequencing and bioaerosol analysis can reshape our approach to contact investigations. This offers hope that improved scientific understanding of transmission may aid in reducing and then ultimately eliminating the global burden of *Mycobacerium tuberculosis*.

5.5 Chapter 5 References

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