University of Alberta

Novel Perspectives on Foreign-born Tuberculosis: Trends, Targets, and Transmission

by

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Dedication

This thesis is dedicated first and foremost to my wife, Cherie. Your commitment to my academic pursuits was steadfast, as was your support and encouragement. Quite simply, this research would not have been possible without you. A thank you cannot capture the depth of my gratitude to you but luckily I have a lifetime to show you. This thesis is also dedicated to my children, Tate and Brynn, who demonstrated a level of understanding and patience for my studies that was well beyond their years. Thank you for your insatiable curiosity and desire for learning -- you two are such a tremendous source of inspiration to me. I also thank my parents, Edmund and Darlene, for being extraordinary role models. Your love and support fostered my curiosity, confidence and independence. I credit you with my belief that dreams can be achieved through determination, dedication and an honest effort.

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Abstract

Immigrant-receiving countries like Canada must meet the challenge of tuberculosis (TB) in their foreign-born populations. One puzzling question is why, despite fluctuating levels of immigration from high incidence source countries, only modest variations in foreign-born TB incidence have been reported for decades. An additional concern is the disproportionate reactivation of latent TB infection (LTBI) in certain groups within this population. Despite these factors, routine screening for LTBI has not been widely implemented, primarily due to concern regarding cost-effectiveness. Another issue related to foreign-born TB is the potential importation of hypervirulent strains and/or strains with increased transmissibility. It is frequently speculated that the Beijing family of strains is included in this category. If true, TB resulting from Beijing strains could impact national TB control efforts.

This thesis aimed to (i) explain the relative constancy of TB incidence in the foreign-born in Canada by examining the trends in TB incidence among immigrant groups; (ii) identify high-yield target groups for routine screening for LTBI; and (iii) determine whether Beijing strains constitute an increased public health threat relative to other genotypes in Alberta, Canada in terms of infectiousness, drug-resistance and transmission.

These investigations found that relative constancy in foreign-born TB incidence is explained by a complex convergence of factors, including annual immigration levels, age at arrival, country of birth, and time since arrival. Consequently, immigrants ≤2 years post-arrival who were aged 15-35 years at arrival and born within countries with TB incidence rates >50/100,000 population were recommended as high-yield targets for the screening for LTBI. In Alberta, Beijing strains had similar disease presentations as non-Beijing strains apart from the former having significant associations with polyresistant-TB and multidrug-resistant TB as well as an association of borderline significance with respiratory TB. Beijing strains also had no greater of an association with recent transmission than non-Beijing strains in Alberta.

The high global prevalence of TB, the emergence of extensively and totally drug-resistant TB, and unprecedented levels of human migration indicate that immigrant-receiving countries will be challenged by TB in their foreign-born populations for the foreseeable future. Serious consideration must be given to the implementation of effective screening strategies.

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List of Abbreviations

AFB	Acid-fast bacteria (bacilli)
AIDS	Acquired immune deficiency disorder
aOR	Adjusted odds ratio
BCG	Bacille Calmette-Guérin
CB	Canadian-born
CIC	Citizenship and Immigration Canada
CNS	Central nervous system
CTBRS	Canadian Tuberculosis Reporting System
DNA	Deoxyribonucleic acid
DOTS	Directly observed therapy – short course
EMB	Ethambutol
FB	Foreign-born
FB-O	Foreign-born 'other'
FB-WP	Foreign-born Western Pacific
HIV	Human immunodeficiency virus
ICD-9	International Classification of Diseases, 9th edition
IME	Immigration medical examination
INAC	Indian and Northern Affairs Canada
INH	Isoniazid
IRR	Incidence rate ratio
LSP	Large sequence polymorphism

List of Abbreviations (Con't)

LTBI	Latent tuberculosis infection
MDR-TB	Multidrug-resistant tuberculosis
MIRU-VNTR	Mycobacterium interspersed repetitive unit variable number tandem repeats
MTBC	*Mycobacterium tuberculosis complex*
NTF	Noise transfer function
OR	Odds ratio
PCR	Polymerase chain reaction
PYRs	Person-years of observation
PZA	Pyrazinamide
RD	Region of difference
RFLP	Restriction fragment length polymorphism
RMP	Rifampin
RR	Relative risk or risk ratio
SNPs	Single-nucleotide polymorphisms
STM	Streptomycin
TB	Tuberculosis disease
TDR-TB	Totally drug-resistant tuberculosis
TNF	Tumor necrosis factor
VNTR	Variable number tandem repeats
WHO	World Health Organization
XDR-TB	Extensively drug-resistant tuberculosis

CHAPTER 1: INTRODUCTION

"If the importance of a disease for mankind is measured by the number of fatalities it causes, then tuberculosis must be considered much more important than those most feared infectious diseases, plague, cholera and the like."
Dr. Robert Koch, 1882[1]

1.1 Statement of the Problem

The expression "tuberculosis anywhere is tuberculosis everywhere" should resonate strongly with immigrant-receiving countries that have a low incidence of active tuberculosis disease (TB).* Despite remarkable reductions in TB incidence since the mid-20th century, immigrant-receiving countries continue to be challenged by TB in their foreign-born populations.[2-5] Foreign-born individuals account for 65% or more of national TB case counts in several immigrant-receiving countries[6-9] and recent trends suggest that a decrease in these proportions is unlikely.[7, 10, 11] An ecological study conducted by the World Health Organization (WHO) also suggests that when foreign-born cases constitute 70% or more national TB cases, the use of regular TB control programs cannot be expected to achieve more than a 2% decrease in annual national TB rates.[12] Under these circumstances, it will be increasingly difficult for immigrant-receiving countries to continue to progress towards TB elimination (1 case per million persons).

There is a general consensus in immigrant-receiving countries that TB elimination is contingent on the successful prevention of TB in the foreign-born. Presumably, this would be achieved through the main priorities of TB control programs in immigrant-receiving countries, namely (i) to minimize *Mycobacterium tuberculosis* transmission through the early identification and curative treatment of infectious cases; and (ii) to prevent TB in individuals with

*For this program of study, low TB incidence immigrant-receiving countries were defined as countries with very high levels of human development and an estimated incidence of smear positive TB of less than 15 per 100,000 population (Appendix A).

latent TB infections (LTBI) through screening and preventive therapy.[13] It has also been emphasized that recently arrived immigrants and refugees from high incidence countries are high risk groups for the reactivation of LTBI (the infections presumably having been acquired prior to immigration).[8, 13-20] Nevertheless, the screening for LTBI among foreign-born individuals has not been systematically or widely implemented due to patient, provider, and logistical barriers as well as a general lack of political will.[14, 21] Consequently, routine screening for LTBI remains primarily within the context of contact investigations.

In the absence of intensified prevention efforts, increased immigration from higher incidence source countries would be expected to increase the incidence of foreign-born TB given that country of birth (or origin) and time since arrival are critical determinants of TB in the foreign-born.[3, 4, 9, 16, 22] This has been demonstrated in the Netherlands where an increased incidence of pulmonary TB index cases coincided with an increased number of immigrants from very high incidence source countries (rate ≥200 per 100,000 population) in the same year.[23] Surprisingly however, only modest variations in annual foreign-born TB incidence have been reported for decades in Canada and other immigrant-receiving countries despite fluctuating levels of immigration from high incidence source countries.[3, 4, 16, 21, 24] A comprehensive understanding of trends in foreign-born TB incidence and factors associated with its relative constancy is essential if effective national TB prevention strategies are to be designed and implemented.

In addition to being an important determinant of TB epidemiology in low incidence settings,[12] large-scale immigration can influence the *M. tuberculosis* population structure within host countries through the importation of non-stereotypical strains.[19] In particular, molecular epidemiological studies have revealed stable associations between the patient's region of birth and the *M. tuberculosis* lineage of the infecting pathogen ('phylogeographical lineages'), an association that persists even when transmission occurs outside of the region of origin.[25-27] In urban centers within a host country, individuals who are infected with phylogeographical TB lineages from outside of the their respective regions of origin disproportionately involve high-risk individuals with impaired

immunity.[26] Currently, it remains to be seen if these patterns reflect host-pathogen associations or sociological and epidemiological factors or a combination thereof.[26]

Given the epidemiological significance of TB in the foreign-born, the importation of hypervirulent and multidrug-resistant strains may have considerable implications for TB control efforts within immigrant-receiving countries. An example of this type of threat may be posed by the Beijing family of *M. tuberculosis* strains. Beijing strains are frequently associated with TB outbreaks,[28-33] multidrug-resistant TB (MDR-TB),[34-41] and treatment failure and relapse.[42, 43] It has also been suggested that Beijing strains have gained a selective advantage or accelerated dominance as a result of bacille Calmette-Guérin (BCG) vaccinations.[29, 44] These factors, combined with the rapid global dissemination of Beijing strains, has led to speculation that Beijing strains have a selective advantage over other *M. tuberculosis* genotypes as conferred through increased virulence and transmissibility.[32, 35, 41, 45, 46]

The importation of Beijing strains is inevitable in Canada, as it is a country with one of the highest levels of immigration per capita internationally.[47] Asia, the world region with the highest reported prevalence of Beijing strains, was the source region for nearly 60% of new immigrants to Canada in 2001.[41, 48] Moreover, approximately 20% of new immigrants originate in the Beijing 'hotspot' countries of the Western Pacific (namely China, Vietnam, and Korea) where 54-92% of TB cases result from infections with Beijing strains.[33, 49-52] Between 2001 and 2004, 40% of foreign-born TB cases and 26% of total cases in Canada were found in individuals from the Western Pacific.[7]

Transmission from foreign-born individuals to native-born individuals is relatively uncommon in immigrant-receiving countries.[18] Nevertheless, the rapid dissemination of Beijing strains in some settings has been attributed to transmission from foreign-born to high risk native-born individuals.[32] A native-born group in Canada that is at significant risk for TB is Aboriginal peoples as its rate of TB is 2 times and 26 times higher than that of foreign-born and Canadian-born non-Aboriginal individuals, respectively.[7] In part, the prevention and control

of TB among Aboriginal peoples has been hindered by numerous socioeconomic and biologic risk factors, including: poverty; inadequate and overcrowded housing; malnutrition; alcohol and drug abuse; diabetes mellitus; and end-stage renal disease.[13] Aboriginal peoples may also have genetic factors which increase their susceptibility to symptomatic TB.[53] Consequently, preliminary evidence of the presence of Beijing strains among Aboriginal peoples (R. Long, unpublished) raises substantial concern about the potential for a rapid epidemic spread of this pathogen with a propensity for multidrug-resistance.

The potential public health threat imposed by the importation and emergence of Beijing strains in Canada is currently unknown. Without an understanding of the epidemiology and transmission patterns of TB cases resulting from infections with Beijing strains relative to non-Beijing strains, it will be impossible to determine if current TB control strategies need to be adapted.

1.2 Study Objectives

The two primary aims of this thesis research were to comprehensively examine the profile of TB in the foreign-born in relation to general trends in TB incidence in Canada, as well as to determine the public health implications associated with the importation of a potentially hypervirulent *M. tuberculosis* strain family, the Beijing family of strains. To achieve the first of these goals, the objective was to deconstruct foreign-born TB incidence in Canada in order to identify the relative contributions and trends in TB incidence among immigrant groups. In doing so, it was anticipated that the factors related to the relative constancy in foreign-born TB incidence could be better understood and, as a consequence, high-yield targets for screening and treatment of LTBI could then be identified.

The second major objective was to determine if Beijing strains pose an increased public threat relative to other strains in the low incidence immigrant-receiving province of Alberta in terms of infectiousness, drug resistance and transmission. This objective stems from repeated hypotheses within the literature

that Beijing strains are inherently more virulent and more transmissible compared to other *M. tuberculosis* strain families.[32, 35, 41, 45, 46]

Secondary objectives were: (i) to determine if Beijing strains were associated with more high risk presentations of active TB compared to non-Beijing strains; (ii) to determine if the disease presentation of Beijing strains varied in relation to age or population group; (iii) to identify the risk factors for, and trends in, the transmission of Beijing and non-Beijing strains; and (iv) to determine if Beijing strains were associated with an increased number of recent transmission events relative to non-Beijing strains.

1.3 Thesis Submitted for Partial Fulfillment of PhD

This thesis consisted of a comprehensive literature review and three studies designed to address the primary and secondary objectives. The literature review (Chapter 2) synthesized previous research about foreign-born TB trends in immigrant-receiving countries and in Canada in particular. It also compiled previous evidence related to trends in the incidence and disease presentations of Beijing strains in immigrant-receiving countries and elsewhere. An important component of the literature review was the identification of the various genotyping methodologies used to discriminate Beijing strains from other *M. tuberculosis* strains.

The first study (Chapter 3) dissected foreign-born TB incidence in Canada between 1986 and 2002 using data from national TB and immigration databases. This study was made possible by collaborations between Citizenship and Immigration Canada; the Public Health Agency of Canada, Health Canada; and the Tuberculosis Program Evaluation and Research Unit, University of Alberta.

The second study (Chapter 4) examined the incidence, demographic characteristics and clinical presentations of culture-confirmed TB cases infected with *M. tuberculosis* Beijing strains and those infected with non-Beijing strains in the province of Alberta, Canada between 1991 and mid-2007. This molecular epidemiologic study used a population-based retrospective cohort study design

and combined demographic and clinical data from the provincial TB registry with data from the Provincial Laboratory for Public Health.

The final study (Chapter 5) examined the risk factors for, and transmission of, culture-confirmed respiratory TB cases in Alberta between 1991 and mid-2007 that were attributed to Beijing and non-Beijing strain infections. This molecular epidemiologic investigation also used a population-based retrospective cohort study design. Additionally, a transmission index was used to quantify the recent transmission of Beijing and non-Beijing strains.

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CHAPTER 2: LITERATURE REVIEW

2.1 Epidemiology of Tuberculosis

2.1.1 Global Tuberculosis Epidemiology

Mycobacterium tuberculosis is a major but yet still largely neglected human pathogen, causing more deaths annually than any other pathogenic bacterium.[1] Estimated to infect one-third of the global human population,[2] tuberculosis disease (TB) claimed the lives of 1.7 million people in 2009, equivalent to 4700 deaths per day.[3] In the same year, there were an estimated 14 million prevalent cases and 9.4 million incidence cases of TB.[3] With molecular analysis confirming a high frequency of TB as early as 3500 to 500 BC,[4] the success of *M. tuberculosis* has clearly not been hindered by its existence as an obligate human pathogen.

The first major step toward the elimination of TB was achieved in 1882 when Dr. Robert Koch discovered the tubercle bacillus to be the etiologic agent of TB.[5] Another major milestone was the introduction of effective chemotherapeutic agents for the treatment of TB beginning in the mid-1940s and an ensuing recognition of the benefits of multidrug therapy in TB.[6] Despite these achievements, global TB incidence rates and TB-related deaths continued to increase for decades. In 1991, the World Health Assembly resolved to achieve unprecedented levels of TB control.[7] This resolve, and the declaration of TB as a global emergency by the World Health Organization (WHO) in 1993,[8] led to the emergence of several global initiatives, including: implementation of *Directly Observed Therapy, Short Course (DOTS)* as a core component of the World Health Organization (WHO) TB control strategy in 1995;[9] establishment of the *Global Fund to Fight AIDS, Tuberculosis and Malaria* in 2002;[10] and the launch of the *Stop TB Strategy* by the WHO in 2006.[11] In 2005, the global TB incidence rate declined for what may have been the first time in history and progressive albeit slow improvements have followed since. Specifically, the global TB incidence rate declined from a peak rate of 142 cases per 100,000 population in 2004 to 137 cases per 100,000 population in 2009.[3] Significant achievements also

include a global case detection rate of all forms of TB of 63% and the successful treatment of 86% of new smear-positive cases.[3] Since its launch in 1995, the DOTS program and Stop TB Strategy have been credited with saving 6 million lives.[3]

Demonstrable achievements in global TB control, while encouraging, are undermined by the emergence of multidrug-resistant TB (MDR-TB), extensively drug-resistant TB (XDR-TB) and most recently totally drug-resistant TB (TDR-TB).[12-15] Drug-resistant TB is a significant threat to TB control programs as it makes treatment more complex, more costly and less effective than that of pan-susceptible TB.[12, 15] The emergence of TDR-TB may also be predictive of a time in which progressively fewer cases will benefit from effective chemotherapeutics for TB.[16] The most recent estimates indicate that MDR-TB cases account for 3.6% of global incident cases and that 5% to 10% MDR-TB cases were XDR-TB (although it is suspected that XDR-TB case numbers are significantly underestimated due to limited laboratory capacities in resource-limited areas).[17, 18] It is also noteworthy that 85% of global MDR-TB cases occurred within 27 high MDR-TB burden countries -- China and India account for 50% of global MDR-TB cases alone.[17] Despite MDR-TB having a case mortality rate of 26% in HIV-negative individuals[17] and the mortality rate of XDR-TB cases being generally higher than that of MDR-TB cases,[15] less than 3% of MDR-TB and XDR-TB cases receive treatment in accordance with WHO recommended standards.[17]

Global TB control efforts have also been significantly impacted by the human immunodeficiency virus (HIV) epidemic. In 2009, one-third of the 33.3 million people who were living with HIV were also co-infected with TB and 11% to 13% of global TB incident cases had TB-HIV co-infection.[3, 19, 20] HIV significantly impairs the immunologic containment of *M. tuberculosis* by inducing cell-mediated immune deficiency.[21] Consequently, HIV is the most important risk factor for the development of active TB[22] and results in a risk of TB that is 20 to 50 times greater than in those without HIV infections.[20, 22] In turn, TB is leading cause of death among people living with HIV, accounting for more than 25% of deaths among those with HIV infection.[23, 24]

2.1.2 Tuberculosis Epidemiology in Canada

There has been a remarkable improvement in TB incidence in Canada since the middle of the 20th century, the rate decreasing from nearly 100 per 100,000 population in 1951 to 4.7 per 100,000 population in 2007.[25] This achievement reflects not only the general success of TB control practices but also broad improvements in living conditions.[21]

The most substantive improvements in TB incidence have been observed among Canadian-born non-Aboriginal individuals. Tuberculosis case counts in Canadian-born non-Aboriginal individuals decreased by 95% between 1970 and 2007 (from 3100 cases to 170 cases, respectively) and resulted in an incidence rate of 0.7 per 100,000 population in 2007.[21, 25] Despite accounting for nearly 70% of TB cases in 1970, the burden of TB disease among Canadian-born non-Aboriginal individuals has reduced to 11%.[21, 25]

Progress towards TB elimination in Canadian-born Aboriginal peoples (includes Status and non-Status Indians, Métis, and Inuit) has been markedly slower than that of Canadian-born non-Aboriginal peoples. While case counts have decreased by nearly 53% between 1970 and 2007 (from 650 cases to 307 cases, respectively), the rate of TB remained high at the end of this period at 26 per 100,000 population (up from 20 per 100,000 population in 2000).[21, 25] Consequently, in 2007, the rate of TB among Canadian-born Aboriginal peoples was 37 times higher than that of Canadian-born non-Aboriginal individuals. Furthermore, despite comprising only 3.8% of the Canadian population,[26] 20% of TB cases in Canada were among Aboriginal peoples.[25]

Like other high-income immigrant-receiving countries,[27-29] in Canada there has been a dramatic increase in the proportion of TB cases contributed by individuals not born in Canada ('foreign-born'); from 18% in 1970 to 67% in 2007.[21, 25, 30] This trend, which began in the 1960s following a shift in immigration pattern from low to high TB incidence source countries, was accelerated by a concurrent reduction in TB incidence among Canadian-born non-Aboriginal individuals.[25, 30-32] Additionally, the proportion of the Canadian population that was foreign-born increased from 15% to 20% between 1970 and

2007 due to nearly a 2-fold increase in the foreign-born population.[33] Between 1970 and 2007, there was also a 34% increase in the annual case counts of TB among the foreign-born.[21, 25] With a rate of 14 per 100,000 population in 2007, the rate of TB in the foreign-born was 21 times greater than that of Canadian-born non-Aboriginals and 0.6 times that of Canadian-born Aboriginal peoples.[25] Given that country of birth/origin and time since arrival are key determinants of foreign-born TB incidence,[27, 30, 34-37] it is surprising that only modest variations in annual foreign-born TB case counts and rates have been reported for the past few decades (rate reductions in the last decade being largely attributed to increasing annual immigration levels).[21, 25, 30] This trend remains unexplained despite similar situations in other immigrant-receiving countries.[27, 38, 39]

Geographic variations in the incidence of TB within Canada correlate closely with the distributions of the foreign-born and Canadian-born population groups. Among the Canadian-born, differences in the distribution of TB between the non-Aboriginal and Aboriginal populations, as well as within the Aboriginal population, is a function of time since first contact with Old World explorers and traders.[40] The highest TB rates in Canada are consistently associated with the territories (Yukon, Northwest Territories and Nunavut) and the provinces of Saskatchewan and Manitoba, where Aboriginal peoples account for a higher proportion of the total provincial and territorial populations than elsewhere in Canada.[25, 26, 33] Conversely, foreign-born and Aboriginal peoples comprise a substantially smaller proportion of the total provincial populations in the Atlantic provinces than in the other provinces or territories, which record consistently lower TB rates than the rest of country.[25, 26, 33]

2.2 Molecular Genotyping

2.2.1 Public Health Applications

The *Mycobacterium tuberculosis complex* (MTBC) is a genetically monomorphic bacterial pathogen due to its low DNA sequence variation.[41, 42] Nevertheless, several sub-species exist within the MTBC including the human pathogen *Mycobacterium tuberculosis.* Initial reports indicated negligible

amounts of DNA sequence variation in *M. tuberculosis* compared to other bacteria, suggesting that bacterial factors have minimal influence on the clinical manifestations of TB.[43, 44] An increasing body of evidence, however, indicates that the initial sequence diversity was underestimated[45-47] and that genetic diversity does have significant phenotypic consequences in experimental models.[43, 48-50] It currently remains uncertain if, and to what extent, human disease is influenced by *M. tuberculosis* genetic variation.[50]

Initially, genotyping of *M. tuberculosis* isolates was used to supplement conventional contact tracing investigations.[51] In particular, it was used as a means of differentiating between TB cases resulting from reactivation of LTBI and those resulting from recent transmission.[52] This required the primary assumption that isolates with the same genotype were either (in)directly part of the same transmission cluster and hence epidemiologically linked whereas unique ('unmatched') genotypes developed from the reactivation of LTBI that was presumably acquired outside of the geographical and temporal limitations of the investigation.

The application of genotyping extends significantly beyond the ability to merely differentiate between reactivated and recently transmitted disease. Genotyping has been used to: trace chains of transmission; identify unsuspected transmission; confirm outbreaks or transmission chains as suspected by conventional contact tracing; and identify the risk factors for, and groups at risk of, transmission.[52-54] Genotyping has also been utilized to detect mixed infections and to discriminate between recurrent TB due to reactivation or exogenous reinfection.[55, 56] Molecular techniques have also been used to identify predominant strain types (clonal strains) within specific populations;[57, 58] evaluate the incidence, transmission and broader geographic dissemination of specific *M. tuberculosis* families;[59-61] identify strain-specific differences in virulence and transmissibility;[62-65] and investigate the evolution of MTBC.[66, 67] It has also identified vast differences in TB trends within and between native-born and foreign-born populations in immigrant-receiving countries.[68-70] The application of genotyping has also highlighted the need for improved quality control practices

within laboratories through the detection of laboratory cross-contamination[71, 72] and assisted in the evaluation and enhancement of TB control programs (e.g. level of clustering, number of outbreaks, etc).[53, 73]

Genotyping has become a requisite tool for TB control in higher-income settings. However, due to the high levels of transmission and lack of capacity to conduct routine cultures of isolates, genotyping in high-burden settings has largely been limited to research programs.[52]

2.2.2 Molecular Epidemiological Studies

The first commonly-used and standardized genotyping method for molecular epidemiologic studies of *M. tuberculosis* was IS*6110* restriction fragment length polymorphism (IS*6110* RFLP) analysis.[74, 75] IS*6110* RFLP provides for a high degree of differentiation between individual strains of *M. tuberculosis* on account of the high positional and numerical polymorphism of the transposable element IS*6110*.[75-78] Importantly, however, secondary genotyping with an alternate method is necessary in isolates with less than six copies of IS*6110* due to the limited discriminatory power of IS*6110* RFLP in such isolates.[79-84] IS*6110* RFLP is also a gel-based method that does not involve nucleic acid amplification and hence relatively large quantities of DNA are required (i.e. biological amplification via bacterial culture).[74] As bacterial cultures require several weeks, IS*6110* RFLP is incapable of providing real-time results. In addition to analysis with sophisticated computer software, the analogue band patterns generated by IS*6110* RFLP frequently require visual interpretation, further impeding the inter-laboratory comparison of fingerprinting patterns.[21, 85-87]

Spoligotyping (or spacer oligotyping) is a commonly used genotyping method that is based on the detection of 43 interspersed spacer sequences of variable length located between the direct repeats (DR) in the genome.[88] Sufficient amounts of DNA can be obtained from clinical samples as polymerase chain reaction (PCR) is used as an amplifying step,[74] thereby providing spoligotyping with a real-time advantage over IS*6110* RFLP.[81, 83, 88] In addition to being highly reproducible, spoligotype patterns are highly amenable to intra-

laboratory comparisons as the patterns can be stored in a simple digital format with basic computer software .[53, 81, 89, 90] This has facilitated the development of an international spoligotyping database SpolDB4.[91] Although spoligotyping can adequately differentiate between strains with few or no copies of IS*6110*,[82, 83, 92] its main limitation is that it has lower discriminatory power than IS*6110* among strains with more than 5 copies of IS*6110*. [81, 88, 89, 93] Consequently, spoligotyping is an unreliable stand-alone method for assessment of transmission chains.[81, 89, 93] At the same time, several *M. tuberculosis* strain families (e.g. Beijing, Manila, Central Asia) can be identified by their respective spoligotype patterns[57, 94-96] and spoligotyping can be used to differentiate between *M. tuberculosis* and *M. bovis.*[83, 88]

The 'gold standard' for the first-line genotyping of *M. tuberculosis* is changing from IS*6110* RFLP to mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing.as[52, 97] The polymorphic nature of MIRU-VNTR is derived from the variable number of copies of the repeat unit at different loci scattered throughout the *M. tuberculosis* genome (41 loci have been identified).[98, 99] The initial 12 loci MIRU-VNTR methodology had less discriminatory power than IS*6110* RFLP in isolates with high copy numbers of IS*6110* and therefore was used conjunction with spoligotyping for first-line genotyping.[79, 92, 98, 100] As the discriminatory power of MIRU-VNTR can exceed that of IS*6110* RFLP with an increased number of loci,[79, 85, 87, 101] it is recommended that a standardized set of 15 highly discriminatory MIRU loci be used for first-line genotyping and an optimized set of 24 MIRU loci (or more) be used for phylogenetic analysis.[101] As another PCR-based method, MIRU-VNTR has many of the same advantages as spoligotyping: it can be applied to small quantities of DNA obtained from clinical samples; the results are highly reproducible; it provides 'real-time' genotyping; and it has a numeric pattern that is unambiguous, easily stored, and easily compared between laboratories.[85, 87, 98] One potential limitation is that the discriminatory power of MIRU loci may differ by *M. tuberculosis* lineage.[102]

Although IS*6110* RFLP, spoligotyping and MIRU-VNTR have been used for evolutionary studies of *M. tuberculosis*, these techniques have a propensity for convergent evolution (homoplasies) that limit their use for strain classification and phylogenetics.[102]

2.2.3 Genotyping for Strain Classification

Following completion of the first whole genome sequence of *M. tuberculosis* laboratory strain H37Rv in 1998,[103] whole-genome sequencing was used to compare a clinical *M. tuberculosis* strain (CDC1551) with H37Rv.[45] In doing so, several genomic deletions in the form of large sequence polymorphisms (LSPs; also known as regions of difference [RD]) were detected as were single-nucleotide polymorphisms (SNPs).[45] LSPs behave as irreversible and often unique events due to the absence of ongoing horizontal gene transfer in the MTBC.[66, 74, 104] LSPs in a progenitor strain will also be detectable in the progenies of that strain because LSPs result from successive deletions and not independent events.[50, 105] Consequently, LSPs are ideal genetic markers for strain classification and for tracking single strains with specific and previously identified LSPs via real-time PCR with probes for LSP detection.[52, 53, 66] At the same time, genomic LSPs evolve too slowly to serve as suitable markers for molecular epidemiological investigations of individual strains within the same strain family but are better suited for phylogenetic investigations of MTBC than IS*6110* RFLP, spoligotyping or MIRU-VNTR as LSP-defined lineages do not suffer from convergent evolution.[53]

2.2.4 Phylogenetic Analyses

Evolutionary analyses of MTBC can be performed through the comparison of the entire genome of multiple clinical strains of MTBC. A single phylogenetic tree with minimal homoplasy has been identified for MTBC with a high degree of congruency in the phylogenetic trees derived through LSPs,[66, 67] SNPs (using multilocus sequence analysis of 89 complete genes in 108 strains),[46] and other DNA sequencing analyses.[44, 47, 102, 106] Of these techniques, however, *de novo*

DNA sequencing provides the most complete MTBC phylogeny (as opposed to phylogenies based on previously known LSPs or SNPs as identified through one-way comparisons with the laboratory strain H37Rv).[50]

Using this highly congruous phylogeny, human MTBC strains have been classified into six discrete strain lineages (Lineage 1 through 6).[46, 47, 67, 102] Within the MTBC literature however, inconsistent nomenclature abounds due to the frequent use of more traditional names for MTBC strain families and lineages. This is especially true for a sublineage within Lineage 2 which is commonly referred to as Beijing family of strains (East Asian Lineage; Beijing strains; strain-W; Beijing/W strains; N-strains).[102]

The unambiguous and robust classification of MTBC strains via DNA sequence data has provided invaluable insights into the evolutionary history and global spread of MTBC.[44, 46] Increasing evidence of strain diversity and phenotypic variations within the *M. tuberculosis* species is also being accrued through the assignment of case isolates to specific lineages.[62, 102, 107] However, the analysis of DNA sequences currently has limited relevance for molecular epidemiologic purposes as a higher degree of strain differentiation is required (as achieved, for example, through IS*6110* RFLP, spoligotyping or MIRU-VNTR genotyping methods).[102]

2.3 The Beijing Family of *M. tuberculosis* Strains

2.3.1 Epidemiology of Beijing Strains

The Beijing family of strains (or Beijing genotype family) accounts for 13% of strains globally[91] and is one of the largest genotype families of *M. tuberculosis*.[94] First described in 1995,[57] Beijing strains have disseminated worldwide, although with notably diverse distribution patterns.[108] Considered to be endemic in East and Southeast Asia (which broadly correlates with the Western Pacific region as defined by the World Health Organization),[108] Beijing strains comprise 54-92% of all *M. tuberculosis* strains in China, South Korea and Vietnam.[57, 109-111] Analyses of stored biopsy specimens in China also suggest that high endemic levels of Beijing strains have been sustained for at least 60 years.[112]

In marked contrast, there is an epidemic spread of drug resistant Beijing strains in parts of South Africa[65, 113] and much of the former Soviet Union[108, 114, 115] while Beijing strains are only emerging in other geographic areas.[108, 116-119]

Tuberculosis resulting from infection with Beijing strains has frequently been associated with TB outbreaks,[61, 120] antituberculosis drug resistance,[108, 111, 121, 122] treatment failure[123] and relapse.[123-125] Of particular concern is that Beijing strains are frequently associated with MDR-TB[122, 126, 127] and, more recently, XDR-TB.[128-130] In contrast, some studies have found no significant associations between Beijing strains and various clinical presentations of TB.[108, 111, 119, 127, 131-134] This inter-study variability may reflect programmatic differences in TB control, inherited and acquired host factors, socioeconomic circumstances, or chance.[65, 135, 136] It may also relate to the intragenotypic variations in virulence that have been described within the Beijing family of strains.[64, 137, 138]

The rapid global expansion of Beijing strains and their frequent association with large TB outbreaks as well as with younger patients has led to speculation that Beijing strains have a selective advantage over other *M. tuberculosis* genotypes as conferred through increased virulence and transmissibility.[61, 108, 113, 128, 139] Beijing strains also appear to have an enhanced ability to circumvent immunity induced through bacille Calmette-Guérin (BCG) vaccination, potentially resulting in a selective advantage of this genotype in populations with high rates of BCG vaccination.[49, 140, 141] While these hypotheses cannot be directly (dis)proven by experimental evidence, they do accord with the increased virulence demonstrated by Beijing strains in animal and *in vitro* studies relative to other *M. tuberculosis* strains.[48, 49, 142-144] In addition, evidence suggests that the fitness of some Beijing strains is retained after the acquisition of drug resistance.[145] Currently, there is insufficient evidence to support or refute the proposed increased transmissibility of Beijing strains.[50]

2.3.2 Molecular Characteristics of Beijing Strains

The Beijing family is monophyletic and the high degree of similarity in its polymorphic markers suggests that it emerged relatively recently.[57, 65, 94] That

Beijing strains are highly conserved genetically also allows for a precise definition of these strains on the basis of genetic markers and enables Beijing strains to be distinguished without difficulty from other *M. tuberculosis* strains. The distinctive spoligotype pattern of Beijing strains has an absence of hybridization to spacers 1 through 34 and hybridization to at least three of spacers 35-43, with hybridization to all of the last nine spacers being most common.[57, 94, 146] Beijing strains have 15 to 26 copies of IS*6110*[109, 139] and their IS*6110* RFLP patterns share more than two-thirds of the IS6110-containing *PvuII* restriction fragments.[57, 90] The molecular profile of Beijing strains also includes a specific A1 insertion of IS*6110* in the origin of replication between the *dnaA-dnaN* genes (region A)[147] and the *katG463/gyrA95* genotype (principal genetic group 1).[42, 147] Although Beijing strains were previously defined as having one or two IS*6110* insertions in the noise transfer function (NTF) region (referred to as the "modern" Beijing subfamily; two IS*6110* insertions indicate W strains), more recent evidence indicates an absence of IS*6110* insertions in the NTF region of the "ancient" subfamily of Beijing strains (previously known as the N family).[60, 148-150] Beijing strains are also characterized by an IS*1081* element on a 3.5-kb *PvuII* fragment[57, 90]and nearly identical VNTR types.[90] The molecular profile of Beijing strains was most recently expanded to include a deletion of the LSP RD105.[67, 151]

In addition to the division of Beijing strains into two major groups based on the specific IS*6110* insertion in the NTF (the ancient and modern subfamilies), Beijing strains have been classified into evolutionary lineages on the basis of LSPs.[151] This refined population structure has four monophyletic groups with a deletion of RD105 in common and invariable deletions of RD207, RD142, RD150 and RD181.[119, 151, 152]

2.3.3 Genotyping to Differentiate Beijing Strains from Non-Beijing Strains

Several molecular markers have been used to differentiate between Beijing strains and non-Beijing strains. In general, however, molecular epidemiological investigations of Beijing strains are challenged by the close genetic relatedness within this family.[153]

Isolates of *M. tuberculosis* that had spoligotype patterns lacking hybridization to spacers 1 through 34 but hybridization to spacers 35 to 43 were initially considered to be Beijing strains. However, isolates with spoligotype patterns that lacked one or more of the last nine spacers were also found to be Beijing strains based on IS*6110* RFLP pattern analysis and the presence of an IS*6110* element in the origin of replication between the *dnaA – dnaN* genes.[57, 94, 139] Subsequently, in an effort to minimize classification inaccuracies and to develop a standardized study approach, it was recommended that *M. tuberculosis* isolates with spoligotype patterns demonstrating an absence of hybridization to spacers 1 through 34 and concurrent hybridization to at least three of the last nine spacers (spacers 35 to 43) be classified as Beijing strains.[94] While this became the 'gold standard' definition for Beijing strains, spoligotyping was unable to discriminate between Beijing strains involved in different chains of transmission or between strains in the Beijing subfamilies.[94, 154]

Another common but more ambiguous method for the identification of Beijing strains involved the comparison of IS*6110* RFLP patterns to those of 19 Beijing reference strains selected from an international database.[94] Fingerprint patterns that shared >80% pattern homology with one of the 19 reference strains was classified as a Beijing strain; secondary testing with an alternate genotype method (i.e. spoligotyping or region A RFLP) was recommended for strains with 75-80% pattern homology. Due to the diversity of IS*6110* RFLP patterns, it was recommended that this methodology was more suitable as a database screening tool rather than a primary identification tool for Beijing strains.

Genomic deletion analysis based on LSPs is increasingly used for the identification of Beijing strains and Beijing sublineages in population-based studies (i.e. PCR-based detection of the presence/absence of RD105).[62, 65, 67, 119, 152, 155] This recently validated genotyping method is especially relevant for investigations aimed at understanding the role of strain variation in TB as genomic deletion analysis enables strains to be classified into unambiguous and robust strain families (lineages).[119] In doing so, the epidemiological characteristics and clinical presentations of Beijing strains can readily be

compared to those of non-Beijing strains within and between studies.[119] Genomic deletion analysis is also an appealing genotyping tool due to its efficiency and technical simplicity and a multiplex PCR approach has been developed for the high-throughput screening of LSPs.[119, 156]

To achieve a level of discrimination between unrelated Beijing strains that is comparable to IS*6110* RFLP, three hypervariable loci have been used to augment standardized 15-loci or 24-loci MIRU-VNTR typing.[153, 157, 158] However, the use of hypervarible loci for Beijing strain discrimination is best for the second-line typing of clustered Beijing strains following standardized 15-loci MIRU-VNTR due to technical concerns that previously resulted in their exclusion from standardized MIRU-VNTR typing.[101, 157] Importantly, there are geographic variations in the level of Beijing strain discrimination afforded by MIRU-VNTR typing, even when augmented with hypervariable loci.[153] In particular, some loci are less polymorphic in geographic settings that have had a more recent clonal expansion of Beijing strains compared to settings in which Beijing strains have circulated for much longer periods.[153]

2.3.4 Beijing Strains in Canada

Beijing strains are of interest in Canada on account of the strong associations noted between *M. tuberculosis* lineage and the country of origin of TB patients.[119, 159] Asia and the Pacific are the world region with the highest reported prevalence of Beijing strains.[108] They are also the source region for an average of 49% of all new permanent residents to Canada between 2000 and 2009.[160] Furthermore, approximately 20% of the 200,000 to 250,000 annual number of new permanent residents originated from the Western Pacific's Beijing 'hotspot' countries of China, Vietnam and South Korea.[160] These factors, combined with the knowledge that immigration is the most important determinant of *M. tuberculosis* population structure and TB epidemiology in low incidence settings,[1, 161] suggest that the inevitable importation of Beijing strains may have considerable ramifications for TB control and elimination efforts in Canada.

In addition to foreign-born individuals, the emergence of Beijing strains may have important implications for Canadian-born Aboriginal peoples. In a preliminary investigation in Alberta, the IS*6110* RFLP patterns of all new active and relapsed respiratory TB case isolates in 1994 through 1998 were compared with the fingerprint patterns of the 19 international Beijing reference strains (unpublished data, R. Long). It was found that Beijing strains accounted for 26% of foreign-born TB cases, 7% of Canadian-born non-Aboriginal cases and 16% of Canadian-born Aboriginal cases. The presence of Beijing/W strains in the Aboriginal subpopulation suggests a larger role for the cross-ethnic transmission of *M. tuberculosis* than previously reported.[69, 162, 163] The penetration of Beijing strains, with their suspected increased virulence and transmissibility, into a vulnerable demographic group already struggling with on-going *M. tuberculosis* transmission also suggests a 'perfect storm' for Beijing strains not unlike that of New York or Gran Canaria.[61, 120]

The results of the preliminary investigation (unpublished data, R. Long), while insightful, are unlikely to reflect the current epidemiology of Beijing strains in Alberta. In particular, the preliminary results will have underestimated the incidence of Beijing strains due to the exclusion of non-respiratory cases and the likelihood of measurement error (that is, the use of IS*6110* RFLP patterns as a primary tool for the identification of Beijing strains). Additionally, there has been ongoing and increasing immigration from the Western Pacific since the end of the study period.

The current epidemiological and potential public health implications of Beijing strains in Canada are essentially unknown. The single published estimate of Beijing strains in Canada came from a study investigating the *M. tuberculosis* population structure among Montreal residents (2001-2007) using different genotyping methodologies; this study found 9% of TB cases were attributed to Beijing strains based on genomic deletion analysis.[119] Neither the epidemiological and clinical presentations nor the transmissibility of Beijing strains relative to other *M. tuberculosis* strains have been reported in Canada.

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CHAPTER 3: PIECING THE PUZZLE TOGETHER: FOREIGN-BORN TUBERCULOSIS IN AN IMMIGRANT-RECEIVING COUNTRY*

3.1 Abstract

Introduction: In immigrant-receiving countries, annual foreign-born tuberculosis case counts and rates are relatively constant. Why this is so, and who might be a high-yield target for screening for latent tuberculosis infection, remain open questions.

Methods: Foreign-born tuberculosis in Canada during 1986-2002 was retrospectively examined using national tuberculosis and immigration data as well as census data. Case counts and rates were analyzed in relation to demographics, immigration period, and time since arrival.

Results: Pre-1986 immigrants (n=3,860,853) and 1986-2002 immigrants (n=3,463,283) contributed 8,662 and 9,613 tuberculosis cases, respectively. Immigrants who were ≤5 years since arrival and those >10 years since arrival contributed almost equally to the annual foreign-born tuberculosis case count despite a 3.5-fold difference in in-country person-years. Remarkably stable and relatively low tuberculosis incidence was observed among immigrants >10 years post-arrival. Conversely, tuberculosis incidence within 5 years of arrival was dynamic, demonstrating a strong inverse association with time since arrival and higher sensitivity to changes in immigration level than shifts toward higher incidence source countries.

Conclusion: Relative constancy in foreign-born TB incidence is explained by a complex convergence of several factors. Immigrants born in high incidence countries (>50/100,000 population) who were ≤2 years since arrival, and who were age 15-35 years upon arrival, constitute high-yield targets for preventive therapy.

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3.2 Introduction

Immigrant-receiving countries with low tuberculosis (TB) incidence are frequently challenged by TB in their foreign-born people.[1-4] The World Health Organization (WHO) suggests that when foreign-born cases constitute 70% or more of national TB cases, one cannot anticipate more than a 2% decrease in annual national TB rates through the use of regular TB control programs.[5] Under such circumstances, further reductions in national TB incidence and TB elimination require a rethinking of prevailing TB prevention and control strategies.

Canada, with one of the highest levels of immigration per capita internationally,[6] is acutely aware of the increasing burden that TB in the foreign-born represents. Following a shift in immigration pattern from low to high TB incidence countries in the 1960s and a substantial reduction in Canadian-born non-Aboriginal TB incidence, the proportion of foreign-born TB cases increased from 18% in 1970 to 67% in 2007.[2, 7-10] In the same period, the foreign-born population nearly doubled, increasing the proportion of the foreign-born Canadian population from 15% in 1970 to 20% in 2007.[11] Reflecting the WHO's projections, Canada's national TB incidence rate declined by an average of 2% annually between 2001 and 2007.[10]

That a shift in immigration pattern from low to high incidence TB countries contributed to an increased proportion of foreign-born cases is a reasonable assumption given that 'country of birth/origin' and 'time since arrival' are key determinants of foreign-born TB incidence.[1, 2, 12-15] Similarly, an increase in both TB case counts and rates would seem logical following a sustained shift. However, only modest variations in annual foreign-born TB case counts and rates have been reported for several decades (with recent rate reductions being primarily attributed to denominator size).[2, 16] This relative stability in foreign-born TB incidence in spite of high levels of immigration from regions of elevated incidence remained unexplained despite similar trends elsewhere.[1, 17, 18]

There is a general consensus that TB elimination in immigrant-receiving countries will be contingent on the successful prevention of TB in the foreign-

born. However, without a comprehensive understanding of how foreign-born TB incidence is constructed, it will be exceedingly difficult, if not impossible, to effectively design and appropriately target any national TB prevention strategies. To bridge this knowledge gap, this study aimed to deconstruct foreign-born TB incidence in Canada in order to identify relative contributions and trends in TB incidence among immigrant groups. In doing so, it was anticipated that the factors related to the relative constancy in foreign-born TB incidence would be elucidated and high-yield targets for screening and treatment of latent TB infection (LTBI) be identified.

3.3 Methods

Study population

The population of foreign-born permanent residents (stock) in Canada from January 1, 1986 through December 31, 2002 ("study period") was divided into two groups:

i. Immigrants who arrived in 1986-2002: Individual level data from Citizenship and Immigration Canada, the federal government department responsible for immigration and settlement, was used to identify immigrants and refugees who were granted permanent residency and arrived in Canada during the study period ("1986-2002 immigrants"). Data abstraction included year of arrival, age-at-arrival, sex and country of birth.

ii. Immigrants who arrived before 1986: Canadian census data was used to estimate the size and age-sex distribution of the foreign-born permanent resident population in 1986.[19, 20]

Temporary foreign-born residents (visitors, students, workers, and refugee claimants [whose status remained to be determined] within the refugee determination process) were excluded from the denominator as this information was not maintained by Citizenship and Immigration Canada.

Tuberculosis cases

Foreign-born TB cases diagnosed during the study period were identified from the Canadian Tuberculosis Reporting System (CTBRS), a prospective national TB registry of all active cases. Cases were included in the study if immigration status at the time of diagnosis was reported as 'permanent resident' or if immigration status was unknown. The diagnosis of active TB is based upon positive bacteriology in 80% of cases; in the remainder, it is based upon a standardized case definition.[21]

WHO country groups

Countries of birth for 1986-2002 immigrants were divided into four groups based on the average country-specific WHO estimated incidence rate of smear-positive TB[22] in 1993 through 1995: <15 (Group 1); 15-50 (Group 2); 51-100 (Group 3); and >100 (Group 4) per 100,000 population (Table 3-1).

Statistical analysis

'Time since arrival' denotes the number of years between the year of arrival and the year of diagnosis. Cases diagnosed in the calendar year of arrival were categorized as 'year 0' cases (i.e. <1 year since arrival), cases occurring in the calendar year following the year of arrival were 'year 1' cases, etc. Thus, cases diagnosed in year 1 could have been in Canada between one day and two years, or one year on the average. For the cohort of immigrants who arrived in Canada prior to 1986, 1986 was considered to be year 0.

Incidence rates per 100,000 person-years were calculated overall as well as for sex, age-at-arrival, country group, and time since arrival strata. The calculation of 95% confidence intervals (CIs) for rates assumed a Poisson distribution for case counts. Standardized incidence rates were calculated using the direct method with the age and/or sex distribution of total 1986-2002 immigrants as the standard population. Rates were compared with incidence rate ratios (RR) and 95% CIs. Summary statistics consist of the mean and standard

deviation ($\bar{x}$ [SD]), and *P* values were two-sided and considered significant if <0.05.[23]

The size of each annual cohort of immigrants for each year between their arrival and the end of 2002 was estimated with the age-sex-calendar year specific survival rates of the total Canadian population.[24-29] Person-years of observation were obtained by adding annual cohort sizes over the total or partial observation period or across cohorts for a specific year. This was also done for each sex, age-at-arrival, and country group. In the year of arrival, each 1986-2002 immigrant was assumed to contribute 0.5 person-years. Person-years for pre-1986 immigrants were estimated with the same procedure except that each immigrant was assumed to contribute a whole year of observation in 1986.

The relative contributions of immigrants who had arrived within 0-5, 6-10 and >10 years to the total burden of TB was assessed for those who arrived in 1986-2002 and for all immigrants. Of necessity, the latter group was limited to data from the 1995-2002 period, as 1995 was the earliest year in which all pre-1986 immigrants resided in Canada for >10 years after the arrival year.

Among 1986-2002 immigrants, the potential impact of prevalent active but unrecognized disease upon arrival on TB incidence rates in year 0-2 was explored with sensitivity analysis. Based on the assumption that a proportion of year 0-1 cases were "actually" active upon arrival (by inference to have developed TB between the date of the immigration medical examination [IME] and date of arrival), the absolute number of cases in year 0-1 were incrementally reduced until statistically insignificant RRs (year 0-2 compared to year 3-5) were observed.

Stata/IC 11.1 for Windows (StataCorp, College Station, TX) was used for data analysis.

Ethics approval was not required as anonymous and routinely collected surveillance data were used.

3.4 Results

The CTBRS received notification of 18,524 foreign-born TB cases during the study period. After excluding 1.3% of cases due to record errors, 18,275 were analyzed; 8,662 (47.4%) among pre-1986 immigrants and 9,613 (52.6%) in 1986-2002 immigrants.

Tuberculosis among pre-1986 immigrants

There were 3,860,853 immigrants who resided in Canada before 1986.[19] During the study period, the TB rate in this group was 14.2/100,000 person-years. Higher rates were associated with males and those aged >64 years (Table 3-2).

Tuberculosis among 1986-2002 immigrants

There were 3,436,283 individuals who immigrated during the study period. Average annual immigration was 202,134 (SD 30,418) individuals, ranging from 113,513 in 1986 to 245,927 in 1990. The majority of immigrants (49.1%) were born in countries with TB incidence rates of 15-50/100,000 population. Nonetheless, a shift in immigration pattern to higher incidence countries of birth was evident and primarily involved a progressive increase in the proportion of immigrants from WHO Group 3 (51-100/100,000 population) (Figure 3-1), from 17.1% in 1986-1990 to 24.9% in 1998-2002.

The overall rate of TB among 1986-2002 immigrants was 34.3/100,000 person-years, i.e. 2.4 (95%CI: 2.4, 2.5) times that of pre-1986 immigrants. Males and those aged >64 years at arrival also had the highest TB rates (Table 3-3).

Rates varied substantially by WHO country group and progressively increased from Group 1 (<15/100,000 population) to Group 4 (>100/100,000 population) (Table 3-4). As a result, countries of birth within Groups 3-4 (>51/100,000 population) were associated with two-thirds of TB cases and only one-third of arrivals.

Tuberculosis rates generally decreased with increased time since arrival (Figure 3-2a) and this association persisted in stratified analyses (sex, Figure 3-2b; age-arrival, Figure 3-2c; and WHO Group, Figure 3-3). Consequently, the

most critical period for TB incidence was between 0-2 years (Table 3-4). Moreover, the rate in year 0-2 was 2.1 (95%CI: 2.0, 2.2), 3.0 (2.8, 3.2) and 4.6 (4.2, 5.1) times higher than that of year 3-5, 6-10, and 11-16, respectively. Sensitivity analysis revealed that TB rates in year 0-2 remained significantly higher than those of year 3-5 until the year 0-1 case count was reduced by >60%.

It is also noteworthy that immigrants aged >64 years at arrival maintained higher rates than younger arrivals throughout the post-arrival period (Figure 3-2c).

The overall TB rate of 1986-2002 immigrants did not fall below 15/100,000 person-years until >10 years after the year of arrival (Table 3-4). Still, immigrants aged ≥35 years at arrival and those from WHO country groups 3-4 (>51/100,000 population) continued to have rates in excess of 15/100,000 person-years.

Deconstruction of total foreign-born TB incidence in Canada

Among combined pre-1986 and 1986-2002 immigrants, those ≤5 years after the year of arrival (year 0-5) contributed an average of 41.8% (SD 4.0%) of total foreign-born TB cases annually in 1995-2002. This proportion was remarkably similar to that of immigrants >10 years post-arrival (mean 41.1% [SD 4.7%]) despite a 3.5-fold difference in the average annual person-years of observation (1,132,351 versus 3,910,130 person-years in year 0-5 and year >10, respectively).

Cases were stratified into four groups (Figure 3-4 and 3-5). Group 'A' (pre-1986 immigrants) exemplifies what would have happened to foreign-born TB incidence if immigration was halted in 1986. As per Figure 3-5, the rate in 'A' would have decreased significantly between the beginning and end of the follow-up period [RR 0.59 (95%CI: 0.52, 0.66); P <0.0001]. Of note, however, is the relative stability in TB incidence in 'A' after 10 years (Figure 3-4 and 3-5).

The three remaining groups, composed of immigrant arrivals in 1986-2002, illustrate that newly arriving groups accounted for an increasing proportion of total cases (Figure 3-4). Specifically, the absolute number of cases contributed by 'B' (1986-1990 immigrants) progressively increased during the 5 years in

which new members entered the cohort ("intake years") and then progressively decreased once 'B' was closed to additional arrivals. As with 'A', the incidence in 'B' was relatively stable once 'B' became composed exclusively of immigrants >10 years since the arrival year (Figures 3-4 and 3-5).

Group 'C' (1991-1995 immigrants) and 'D' (1996-2002 immigrants) had similar incidence patterns as 'B' (Figures 3-4 and 3-5). Relative to 'B' however, the case count during the intake years of 'C' was 45.3% higher than that of 'B' (Figure 3-4). This coincided with an 11.5% increase in immigration levels in 'C'. Similarly, 'D' had a simultaneous 6.4% decrease in immigration level and 17.4% decrease in case count within its intake years compared to 'C'.

The proportion of immigrants from WHO Groups 3-4 (>50/100,000 population) increased by 1.7% and 6.0% between 'B-C' and 'C-D', respectively. While these shifts to higher incidence countries of birth coincided with progressively increased rates in year 1 (Figure 3-5), they appeared to have less of an influence on case counts than changes in immigration level.

3.5 Discussion

In the present analysis of foreign-born TB incidence in Canada, immigrants' greatest risk for active TB was within the first few years of arrival. Despite a 3.5 fold difference in annual person-years of observation, the pool of immigrants who were ≤5 years since arrival and those >10 years since arrival contributed almost equally to the annual foreign-born TB case count (42% and 41%, respectively). Clearly, events preceding and immediately following arrival of the foreign-born are critical for TB prevention and control.

As demonstrated in this study and others, there is a characteristic inverse relationship between foreign-born TB rates and increased time since arrival that persists regardless of demographic or country group.[13-15] The interval between the arrival year and the second year following the year of arrival (year 0-2) is of particular importance given a TB rate that is 2 to 3 times that of year 3-5 despite completion of immigration medical screening for pulmonary disease within a year of departure. Before permission for arrival is granted, all foreign nationals ≥11

years of age applying for permanent residency must undergo radiographic screening for pulmonary disease as part of the Canadian immigration medical screening process. Medical decisions rendered on the basis of the examination are valid for a period of 12 months, after which a repeat examination is required.[21]

Although the existence of prevalent active disease upon arrival (and before departure) may contribute to high TB rates shortly after arrival, sensitivity analysis indicates that the TB rate in year 0-2 would remain significantly higher than that of year 3-5 even if 40% of cases in the year of arrival and the year following the year of arrival were incidence cases. Similarly, it is unlikely that the described incidence trends were significantly altered by referral of high-risk immigrants (i.e. those diagnosed with inactive pulmonary TB during the IME) to medical surveillance programs due to the limited effectiveness of such programs in preventing future TB cases.[30, 31]

In contrast to TB incidence among immigrants who were ≤5 years since arrival, TB incidence among those >10 years since arrival is characterized by remarkably stable and relatively low TB case counts and rates (14/100,000 person-years). Nevertheless, these immigrants have a rate of TB that is 20 times that of Canadian-born non-Aboriginal persons.[10]

Immigration is a dynamic process, with host countries frequently adjusting annual immigration targets and source countries of new immigrants in response to political, social and international factors.[7] In the current study, shifts in immigration pattern had a more subtle influence on TB incidence than that of changing immigration levels. That shifts in immigration pattern only had a minimal impact on TB rates after the arrival year presumably relates to the majority (>60%) of immigrants in Canada being born in countries with relatively low TB incidence (≤50/100,000 population). With continued shifts to higher incidence source countries and immigration projected to represent an increasing proportion of population growth for the foreseeable future,[6, 32] it is speculated that more marked increases in foreign-born TB case counts and rates are imminent.

That immigrants aged >64 years at arrival maintained substantially higher TB rates than younger arrivals throughout the post-arrival period is reasonably explained by aging. In sub-group analyses, TB rates of pre-1986 immigrants aged >64 years in 1986 and 1986-2002 immigrants aged >64 years at arrival were found to progressively increase with each consecutive 5-year increase in age (data not shown). The increasing likelihood of TB with the aging of older arrivals emphasizes that there is an increased role for primary care providers in the screening for LTBI among older immigrants, especially when immigrants originate from high incidence countries and/or have other high risk factors for the development of active disease.

The relative constancy seen in foreign-born TB incidence in Canada is the result of the convergence of all the factors discussed above. Clearly, strategies designed to reduce LTBI prevalence in the foreign-born will be critical to address the burden of TB within this vulnerable population given this complex interplay of factors and previous findings suggesting that the majority of foreign-born TB cases in low incidence countries result from reactivation of LTBI.[33-35] Although routine screening and treatment for LTBI in the foreign-born was previously discouraged due to poor cost-effectiveness,[36] this strategy may emerge as a high priority and cost-effective reality in the near future due to technological advances. In particular, interferon-gamma release assays, which add specificity to the tuberculin skin test,[37, 38] have recently received approval in national guidelines and promising short-course LTBI treatment regimens on the horizon offer to improve acceptance and completion of treatment of LTBI.[39-42]

Presumably, the cost-effectiveness of routine screening for LTBI is increased by targeting those at highest risk for TB. This study identified the highest-yield targets as being permanent residents ≤2 years post-arrival who were aged 15-35 years at arrival and born within countries with TB incidence rates >50/100,000 population. Although arrivals >64 years old are at higher risk, they are not ideal targets for systematic screening due to higher rates of serious adverse effects of standard treatment for LTBI (9 months of daily isoniazid).[21, 43, 44] Nevertheless, immigrants >64 years of age at arrival would be an important high-

yield target for systematic screening if an alternate preventive therapy was provided that conferred similar or better efficacy as 9 months of daily isoniazid without the age-associated increase in serious adverse effects. Current guidelines for systematic screening of LTBI in the foreign-born should also be maintained to ensure that other high-risk, albeit lower-yielding, groups are appropriately managed. In Canada, this includes arrivals referred for medical surveillance by immigration authorities, children <15 years who are ≤2 years post-arrival from high incidence countries (>15/100,000 population), and foreign-born with high risk medical conditions.[21]

Should expanded LTBI screening and local public health responsibility emerge as a critical component of foreign-born TB control, it should not be undertaken to the exclusion of strategies aimed at reducing TB incidence in source countries. Enhanced national TB control programs not only constitute the most cost-effective health intervention in resource limited settings,[45] but modeling also demonstrates that high-income countries can achieve cost-effective reductions in foreign-born TB morbidity and mortality by funding efforts to expand TB control in selected high-incidence countries.[46]

The methodological strength of this study was the use of data from national TB and immigration databases. This methodology provided precise denominator and demographic information on a large study population over a prolonged period. Additionally, it eliminated the impact of post-immigration (secondary) migration within Canada and other jurisdictional limitations encountered with province-specific studies,[14, 33, 47, 48] making it the most comprehensive report of foreign-born TB incidence in Canada to date.

This study had some limitations. Availability of year of arrival only, without day and month, for 59% of 1986-2002 immigrants necessitated an assumption about the person-years contributed. The resulting assumption of each immigrant contributing 0.5 person-years in the year of arrival reflects the distribution of arrivals with complete date of arrival information. Rate calculations may have been limited by mortality estimates and the assumption of no out-migration from Canada. An evaluation of TB among temporary foreign-

born residents was beyond the scope of this study as the study population was limited to foreign-born permanent residents. This notwithstanding, incomplete data on immigration status within the CTBRS may have resulted in the inclusion of TB cases among temporary foreign-born residents. The overestimation in rates from such inclusions would be negligible, however, as temporary foreign-born residents account for only 5-9% of total foreign-born TB cases as per national[10] and province-specific data (Database Manager, Alberta Health Services, personal communication, 2010).

An area for future study is the potential impact of migrant type (economic, family reunification, refugee, skilled worker, etc.) on the distribution of TB in foreign-born populations. Immigration to highly developed economies is a dynamic process and migrants may not equally reflect the TB incidence rates of their place of origin.

With immigration being the single most important determinant of TB dynamics within high-income countries[5] and a demonstrated inability to make substantive reductions in foreign-born TB incidence using current guidelines,[2, 9, 10, 16] the status quo is no longer acceptable if progress toward TB elimination is to be made in immigrant-receiving countries.

Table 3-1: Countries of Birth within each WHO Country Risk Group by TB Incidence Rate

Group 1 (<15/100,000 population)			
Albania	Czech Republic	Malta	Switzerland
American Samoa	Denmark	Mauritius	Tonga
Andorra	Dominica	Monaco	Trinidad & Tobago
Anguilla	Finland	Montserrat	Turks & Caicos Islands
Antigua & Barbuda	France	Netherlands	United Arab Emirates
Australia	Germany	Netherlands Antilles	United Kingdom
Austria	Greece	New Caledonia	United States of America
Barbados	Grenada	New Zealand	United States Virgin Islands
Belgium	Iceland	Norway	Uruguay
Bermuda	Ireland	Oman	West Bank and Gaza Strip
British Virgin Islands	Israel	Puerto Rico	
Canada	Italy	Saint Kitts & Nevis	
Cayman Islands	Jamaica	Saint Lucia	
Costa Rica	Jordan	Samoa	
Cuba	Libyan Arab Jamahiriya	San Marino	
Cyprus	Luxembourg	Sweden	

Table 3-1 (Con't): Countries of Birth within each WHO Country Risk Group by TB Incidence Rate

Group 2 (15-50/100,000 population)			
Algeria	Egypt	Lithuania	Singapore
Argentina	El Salvador	Maldives	Slovakia
Armenia	Eritrea	Mexico	Slovenia
Azerbaijan	Fiji	Nauru	Spain
Bahamas	French Polynesia	Nicaragua	Sri Lanka
Bahrain	Georgia	Niue	Suriname
Belarus	Guam	Northern Mariana Islands	Syrian Arab Republic
Belize	Guatemala	Panama	Tajikistan
Benin	Guyana	Paraguay	The former Yugoslav (Macedonia)
Bosnia & Herzegovina	Honduras	Poland	Tokelau
Brazil	Hungary	Portugal	Tunisia
Brunei Darussalam	Iran, Islamic Republic of	Qatar	Turkey
Bulgaria	Iraq	Republic of Korea	Turkmenistan
Cameroon	Japan	Republic of Moldova	Ukraine
Chile	Kazakhstan	Romania	Uzbekistan
China	Kuwait	Russian Federation	Venezuela
Colombia	Kyrgyzstan	Saint Vincent & The Grenadines	Wallis & Futuna Islands
Comoros	Latvia	Saudi Arabia	
Cook Islands	Lebanon	Seychelles	

Table 3-1 (Con't): Countries of Birth within each WHO Country Risk Group by TB Incidence Rate

Group 3 (51-100/100,000 population)			
Afghanistan	Ecuador	Liberia	Rwanda
Angola	Equatorial Guinea	Madagascar	Sao Tome & Principe
Burkina Faso	Ethiopia	Malaysia	Senegal
Burundi	Gabon	Micronesia	Sudan
Cape Verde	Gambia	Mongolia	Thailand
Central African Republic	Ghana	Morocco	United Republic of Tanzania
Chad	Guinea	Myanmar	Vanuatu
Congo	Guinea-Bissau	Niger	Viet Nam
Côte d'Ivoire	India	Nigeria	Yemen
Democratic Republic of the Congo	Kenya	Pakistan	
Dominican Republic	Laos	Palau	
Group 4 (>100/100,000 population)			
Bangladesh	Indonesia	Nepal	Swaziland
Bhutan	Kiribati	Papua New Guinea	Timor-Leste
Bolivia	Lesotho	Peru	Togo
Botswana	Malawi	Philippines	Tuvalu
Cambodia	Mali	Sierra Leone	Uganda
Democratic People's Republic of Korea	Mauritania	Solomon Islands	Zambia
Djibouti	Mozambique	Somalia	Zimbabwe
Haiti	Namibia	South Africa	

Table 3-2: Tuberculosis Incidence Among Pre-1986 Immigrant Arrivals in Canada, 1986 to 2002

Characteristic	Cases (%)	Immigrants in 1000s	Pyrs	Rate*	95% CI	Adjusted Rate†	95% CI	RR‡	95% CI	*P* value§
Sex										
Female	4088 (47.2)	1972.5	314.2	13.0	12.6, 13.4	12.8	12.4, 13.2	1.0		
Male	4574 (52.8)	1888.4	297.8	15.4	14.9, 15.8	13.9	13.5, 14.3	1.1	1.0, 1.1	0.0004
Age in 1986 (years)										
<15	316 (3.6)	180.5	30.6	10.3	9.2, 11.5	10.4	9.3, 11.6	1.0		
15-34	2560 (29.6)	1000.4	169.1	15.1	14.6, 15.7	15.1	14.6, 15.7	1.5	1.3, 1.6	<0.0001
35-64	3714 (42.9)	2018.5	331.9	11.2	10.8, 11.6	11.2	10.8, 11.5	1.1	1.0, 1.2	0.194
>64	2072 (23.9)	661.5	80.5	25.7	24.6, 26.9	26.9	25.8, 28.0	2.6	2.3, 2.9	<0.0001
Total	8662 (100.0)	3860.9	612.1	14.2	13.9, 14.5	13.3	13.0, 13.6			

Abbreviations: Pyrs, person-years of observation (100,000s); CI, confidence interval; RR, rate ratio

* Incidence rate per 100,000 person-years

† Rates per 100,000 person-years were standardized to the age and/or sex distribution of the 1986-2002 immigrant population.

‡ Adjusted rates were used for incidence rate ratios (RR) calculations.

§ *P* values are two-sided

Table 3-3: Tuberculosis Incidence Among 1986-2002 Immigrant Arrivals in Canada, 1986 to 2002

Characteristic	Cases (%)	Immigrants in 1000s	Pyrs	Rate*	95% CI	Adjusted Rate†	95% CI	RR‡	95% CI	*P* value§
Sex										
Female	4651 (48.4)	1750.3	142.8	32.6	31.7, 33.5	31.9	31.0, 32.9	1.00		
Male	4962 (51.6)	1686.0	137.2	36.2	35.2, 37.2	37.5	36.5, 38.6	1.2	1.1, 1.2	<0.0001
Age-at-arrival (years)										
<15	616 (6.4)	738.2	59.5	10.4	9.6, 11.2	10.4	9.6, 11.3	1.00		
15-34	5300 (55.1)	1578.7	134.6	39.4	38.3, 40.5	39.4	38.4, 40.5	3.8	3.5, 4.1	<0.0001
35-64	2723 (28.3)	993.7	77.0	35.4	34.1, 36.7	35.4	34.1, 36.8	3.4	3.1, 3.7	<0.0001
>64	974 (10.1)	125.6	8.9	109.2	102.5, 116.3	114.8	107.9, 122.1	11.0	10.0, 12.2	<0.0001
Total	9613 (100.0)	3436.3	279.9	34.3	33.7, 35.0	34.3	33.7, 35.0			

Abbreviations: Pyrs, person-years of observation (100,000s); CI, confidence interval; RR, rate ratio

* Incidence rate per 100,000 person-years

† Rates per 100,000 person-years were standardized to the age and/or sex distribution of the 1986-2002 immigrant population.

‡ Adjusted rates were used for incidence rate ratios (RR) calculations.

§ *P* values are two-sided

Table 3-4: Association of Tuberculosis Incidence and WHO Country Group Among Permanent Residents Who Arrived in Canada During the Study Period, 1986-2002

WHO Country Group*	Cases (%)	Immigrants in 1000s (%)	Person-Years in 100,000s	Crude Rate†	95% CI	Adjusted Rate‡	95% CI
1 (<15)	304 (3.2)	600.9 (17.5)	52.6	5.8	5.2, 6.5	6.1	5.5, 6.8
2 (15-50)	2898 (30.1)	1685.8 (49.1)	139.7	20.7	20.0, 21.5	20.8	20.1, 21.6
3 (51-100)	3494 (36.3)	5711.2 (20.7)	51.9	67.3	65.1, 69.6	63.6	61.4, 65.8
4 (>100)	2917(30.3)	438.4 (12.8)	35.7	81.8	78.9, 84.8	84.2	81.2, 87.2
Total	9613 (100.0)	3436.3 (100.0)	279.9	34.3	33.7, 35.0		

Abbreviations: WHO, World Health Organization; CI, confidence interval.

* Country of birth groupings based on the WHO estimated incidence of smear positive TB per 100,000 population at mid-study period: <15 (Group 1); 15-50 (Group 2); 51-100 (Group 3); and >100 (Group 4).

† Crude incidence rate per 100,000 person-years

‡ Rates were standardized to the sex and 5-year age group distribution of total permanent resident arrivals in 1986 through 2002 as included in this study.

Table 3-5: Association Between Tuberculosis Incidence Rates and Time Since Arrival Among Permanent Residents Who Arrived in Canada in 1986-2002

Group	Interval Between Year of Arrival and Year of Diagnosis*								≤ 2 yrs : 3-5 yrs	
	≤ 2 yrs		3-5 yrs		6-10 yrs		11-16 yrs			
	Cases	Rate†	Cases	Rate†	Cases	Rate†	Cases	Rate†	RR	95% CI
Sex										
Female	2291	56.6	1227	30.9	908	20.2	225	12.7	1.83	1.71, 1.97
Male	2666	68.6	1125	29.7	919	21.4	252	14.3	2.31	2.15, 2.48
Age-at-arrival (years)										
<15	305	17.9	119	7.2	151	8.2	41	5.5	2.50	2.01, 3.11
15-34	2755	75.0	1381	38.0	944	22.0	220	11.9	1.97	1.85, 2.11
35-64	1357	59.6	662	30.1	532	22.5	172	20.3	1.98	1.80, 2.18
>64	540	192.7	190	71.6	200	73.6	44	58.7	2.69	2.28, 3.19
WHO Country Group‡										
1 (<15)	178	12.5	63	4.4	43	2.6	20	2.7	2.87	2.14, 3.89
2 (15-50)	1530	39.0	689	17.7	532	12.0	147	8.5	2.20	2.01, 2.41
3 (51-100)	1761	111.1	834	57.8	725	46.8	174	28.4	1.92	1.77, 2.09
4 (>100)	1488	147.5	766	77.7	527	46.8	136	30.6	1.90	1.74, 2.07
Total	4957	62.5	2352	30.3	1,827	20.8	477	13.5	2.06	1.96, 2.17

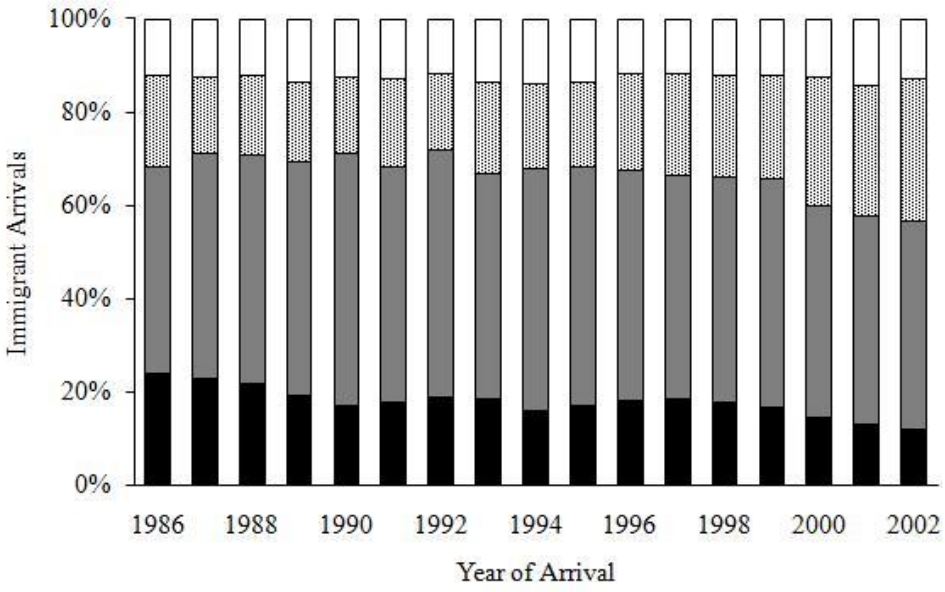
Abbreviations: yrs, years; RR, incidence rate ratio; CI, 95% confidence interval; WHO, World Health Organization.

* Time since arrival represents the maximum number of years between an immigrant's year of arrival and year of diagnosis.

†Rates are per 100,000 person-years.

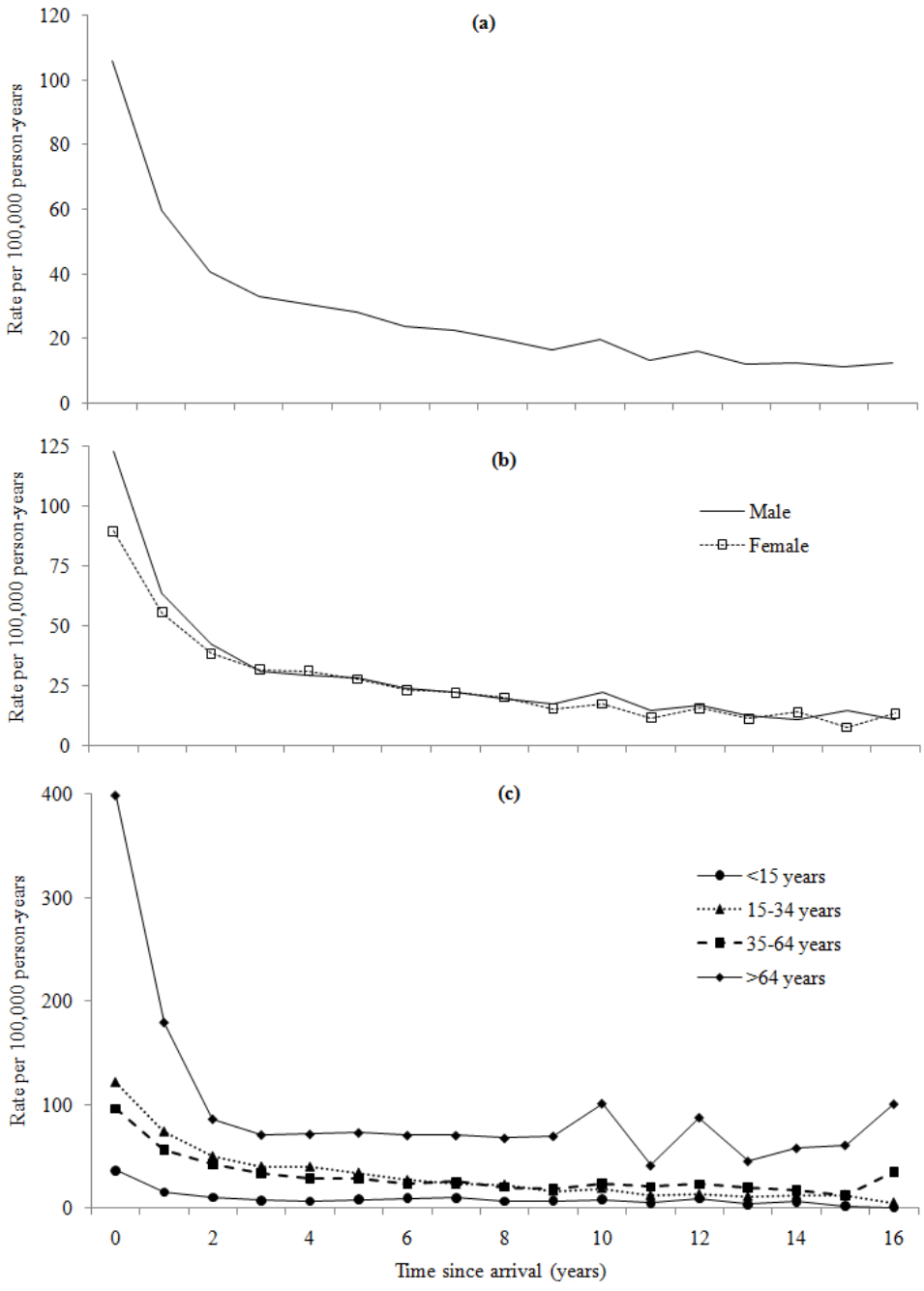
‡Based on the WHO estimated incidence of smear positive TB per 100,000 population at mid-study period (3-year average).

Figure 3-1: **Percentage of Foreign-Born Permanent Residents Arriving Annually in Canada by the World Health Organization Estimated TB Incidence Rate in the Country of Birth, 1986-2002**



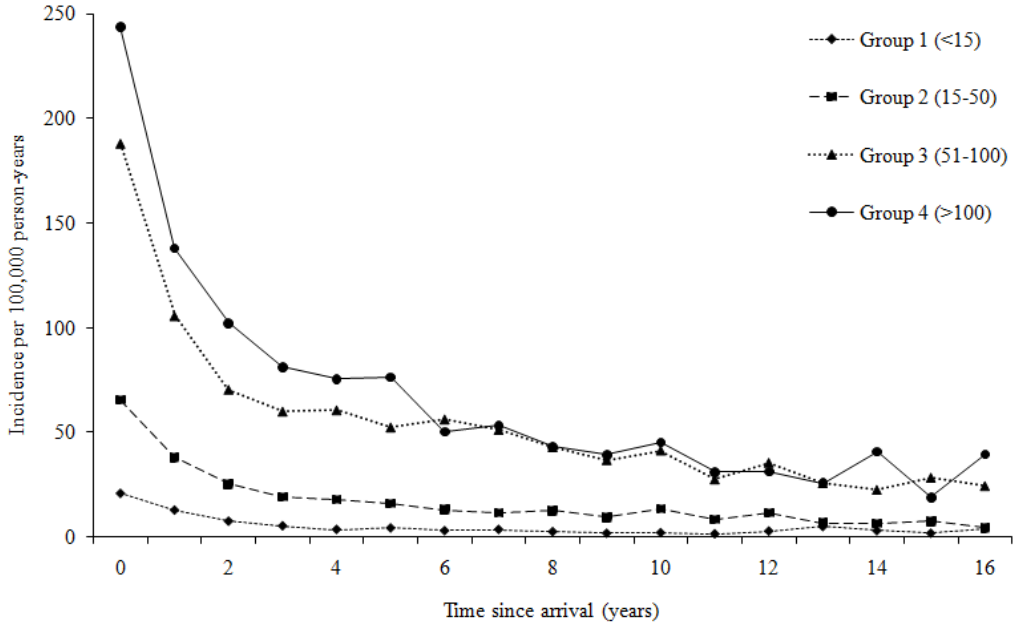
Countries of birth were grouped according to the country-specific WHO estimated incidence rates of smear positive TB per 100,000 population at mid-study period (3-year average): Group 1, <15/100,000 population (*black bars*); Group 2, 15-50/100,000 population (*gray bars*); Group 3, 51-100/100,000 population (*dotted bars*); and Group 4, >100/100,000 population (*white bars*).

Figure 3-2: Tuberculosis Incidence Rates by Time Since Arrival Among Foreign-born Permanent Residents That Both Arrived in Canada and Were Diagnosed with TB in 1986-2002



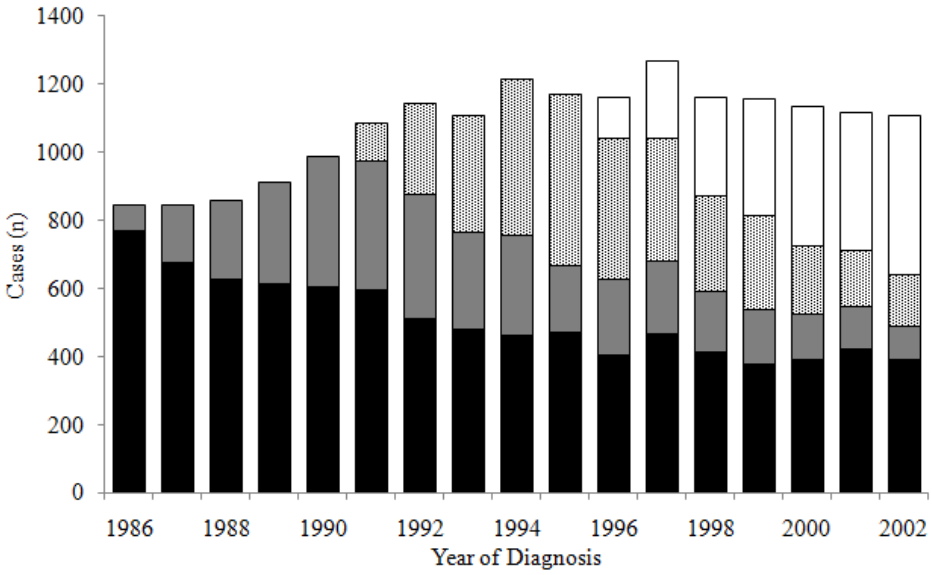
(a) Crude TB incidence rates; *(b)* Sex-specific TB incidence rates; *(c)* Rates stratified by age-at-arrival (years).

Figure 3-3: Tuberculosis Incidence Rates Among Foreign-born Permanent Residents Who Both Arrived in Canada and Were Diagnosed with TB in 1986-2002 by Time Since Arrival and Country of Birth Group



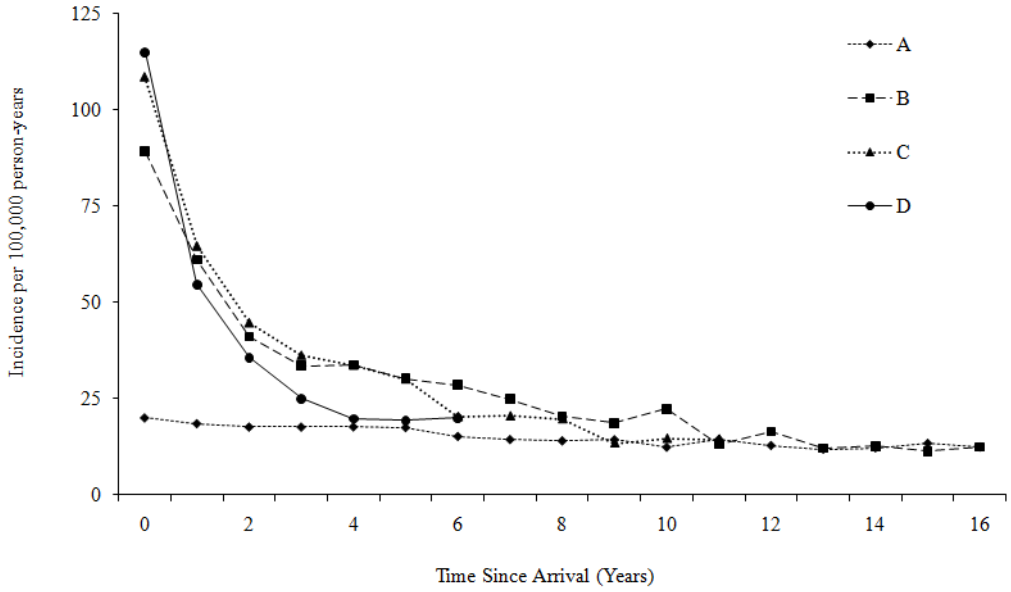
Countries of birth were grouped according to the country-specific WHO estimated incidence rates of smear positive TB per 100,000 population at mid-study period (3-year average): Group 1, <15/100,000 population; Group 2, 15-50/100,000 population; Group 3, 51-100/100,000 population; and Group 4, >100/100,000 population.

Figure 3-4: Annual TB Case Counts Among Foreign-born Permanent Residents in Canada by Period of Arrival, 1986-2002



Foreign-born permanent residents arrived prior to 1986 (*black bars);* those arrived in 1986-1990 (*gray bars)*; those arrived in 1991-1995 (*dotted bars)*; and those arrived in 1996-2002 (*white bars)*.

Figure 3-5: Annual TB Incidence Rates Among Foreign-born Permanent Residents in Canada by Period of Arrival and Time Since Arrival, 1986-2002



Foreign-born permanent residents arrived prior to 1986 (line *A);* those arrived in 1986-1990 (line *B)*; those arrived in 1991-1995 (line *C)*; and those arrived in 1996-2002 (line *D)*. For the cohort of immigrants who arrived in Canada prior to 1986, 1986 was considered to be year 0.

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CHAPTER 4: *MYCOBACTERIUM TUBERCULOSIS* BEIJING STRAINS IN AN IMMIGRANT-RECEIVING COUNTRY: AN EMERGING PUBLIC HEALTH THREAT?*

4.1 Abstract

Introduction: *Mycobacterium tuberculosis* Beijing strains are frequently associated with tuberculosis outbreaks and drug resistance. However, contradictory evidence and limited study generalizability make it difficult to foresee if the emergence of Beijing strains in high-income immigrant-receiving countries poses an increased public health threat. The purpose of this study was to determine if Beijing strains are associated with high risk disease presentations relative to other strains within Canada. Secondarily, this study sought to determine if Beijing disease presentation varied by patients' age or population group.

Methods: This was a retrospective population-based study of culture-confirmed active TB cases in a major immigrant-receiving province of Canada in 1991 through 2007. Of 1852 eligible cases, 1826 (99%) were successfully genotyped. Demographic, clinical, and mycobacteriologic surveillance data were combined with molecular diagnostic data. The main outcome measures were site of disease, lung cavitation, sputum smear positivity, bacillary load, and first-line antituberculosis drug resistance.

Results: A total of 350 (19%) patients were infected with Beijing strains; 298 (85%) of these were born in the Western Pacific. Compared to non-Beijing strains, Beijing strains were significantly more likely to be associated with polyresistance (aOR 1.8; 95% CI 1.0-3.3; p=0.046) and multidrug-resistance (aOR 3.4; CI 1.0-11.3; p=0.049). Conversely, Beijing strains were no more likely than non-Beijing strains to be associated with respiratory disease (aOR 1.3; 1.0-1.8; p=0.053), high bacillary load (aOR 1.2; 0.6-2.7), lung cavitation (aOR 1.0; 0.7-1.5), immediately life-threatening forms of TB (aOR 0.8; 0.5-1.6), and monoresistance (aOR 0.9; 0.6-1.3). In subgroup analyses, Beijing strains were

associated with an increased likelihood of multidrug-resistant TB (aOR 6.1; 01.2-30.4) among individuals born in the Western Pacific as well as with polyresistant TB (aOR 3.1; 1.3-7.5) and sputum-smear positive disease (aOR 1.9; 1.0-3.4; p=0.042) among those aged <35 years at diagnosis. ***Conclusion*:** Other than an increased risk of polyresistant TB or multidrug-resistant TB, Beijing strains appeared to pose no more of a public health threat than non-Beijing strains within a high-income immigrant-receiving country.

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Langlois-Klassen D, Kunimoto D, Saunders LD, Chui L, Boffa J, Menzies, D, Long R. *Mycobacterium tuberculosis* Beijing strains in an immigrant-receiving country: an emerging public health threat? *(submitted)*

4.2 Introduction

Since first being reported in 1995 (Beijing isolates) and 1996 (strain W),[1,2] the Beijing family of *Mycobacterium tuberculosis* strains has garnered much attention in international tuberculosis literature. The largest genotype family of *M. tuberculosis*,[3] Beijing strains account for 13% of strains globally and dominate the *M. tuberculosis* epidemiology in some geographic areas.[4,5] In the Western Pacific countries of China, South Korea and Vietnam 54-92% of *M. tuberculosis* case isolates are Beijing strains.[1,6-9] While other countries are only now experiencing an emergence of the Beijing genotype,[4,10-14] China has had high endemic levels of this genotype for at least 60 years.[15]

Active tuberculosis disease (TB) resulting from infection with Beijing strains has frequently been associated with TB outbreaks,[2,10] antituberculosis drug resistance,[4,8,16,17] treatment failure[18] and relapse.[18-20] Of particular concern is the association between Beijing strains and multidrug-resistant TB (MDR-TB).[17,21,22] Beijing strains also appear to have an enhanced ability to circumvent immunity induced through bacille Calmette-Guérin (BCG) vaccination, potentially resulting in a selective advantage of this genotype in populations with high rates of BCG vaccination.[23-25]

In contrast, other studies have found no significant associations between Beijing strains and either BCG vaccination status[26] or various presentations of TB.[4,8,22,27-31] This inter-study variability may result from the heterogeneous distribution of Beijing sublineages; programmatic differences in TB control; inherited and acquired host factors; socioeconomic circumstances; chance; and other factors.[32-34] Furthermore, little clarity is afforded by evidence of genotypic diversity within the *M. tuberculosis* species because it remains inconclusive as to whether or not genotypic diversity meaningfully influences the outcome of infection *in vivo*.[35] The bottom line – the epidemiologic significance of Beijing genotype strains in the human population remains largely ambiguous.

Contradictory evidence within the Beijing literature and the often limited generalizability of studies make it difficult to foresee whether the emergence of the Beijing genotype in high-income immigrant-receiving countries with low TB

incidence (hereafter referred to as immigrant-receiving countries for brevity) poses an increased public health threat. This study sought to determine if the Beijing family of *M. tuberculosis* strains was associated with more high risk presentations of active TB than other strains in Canada, a country with one of the highest levels of immigration per capita internationally and in which a quarter of the foreign-born population has originated from the Western Pacific.[36,37] A secondary objective was to determine if Beijing disease presentation varied in relation to patients' age or population group.

4.3 Methods

Study Population

This retrospective cohort study investigated cases of active TB diagnosed among residents of the immigrant-receiving province of Alberta, Canada (population of 3,290,355 in 2006) from January 1, 1991 through June 30, 2007. Canadian-born individuals were born in Canada or born in a foreign country to Canadian parents; all others were foreign-born. Aboriginals (defined in this study as First Nations peoples registered with Indian and Northern Affairs Canada [INAC]) were distinguished from Canadian-born 'others' (non-Status Indian, Métis, Inuit, and non-Aboriginal individuals) due to a marked disparity in the TB rates of these groups. Foreign-born individuals were grouped into those born in the Western Pacific region (Table 4-1)[38] and those born elsewhere (foreign-born 'other') given the high prevalence of Beijing strains in the Western Pacific.

Foreign and Canadian-born population estimates were obtained from Canadian censuses (1991, 1996, 2001 and 2006) using customized reports from Statistics Canada. To calculate person-years, these census estimates were combined with estimates between census years as calculated with linear interpolation as well as the population estimates for 2007 which were obtained through linear extrapolation. Estimates for Canadian-born 'others' were those derived from the censuses minus annual Aboriginal population estimates as obtained directly from INAC.

Cases

All culture-confirmed active TB cases diagnosed during the study period as per the Alberta Tuberculosis Registry were eligible for study inclusion. Demographic and clinical data from the TB registry were combined with data from the Provincial Laboratory for Public Health ('Provincial Laboratory') where all of the mycobacteriology in the province is performed. Ethics approval was obtained from the University of Alberta Health Research Ethics Board and analyses of routine surveillance data did not necessitate informed consent as there was no direct patient contact.

Cases were grouped by site (respiratory versus non-respiratory disease) and severity (immediately life-threatening forms of TB versus others). Respiratory cases consisted of: primary, pleural, pulmonary or 'other respiratory' TB (ICD-9 codes 010-012);[39] miliary TB (ICD-9 code 018) with culture-positive respiratory specimen(s); and cases with concurrent respiratory and non-respiratory TB. Miliary TB and TB involving the central nervous system (CNS) (ICD-9 code 013) comprised immediately life-threatening forms of TB.

The infectiousness of respiratory cases was evaluated in relation to sputum smear positivity and the presence of lung cavitation on chest radiograph. Semi-quantitative scores for acid-fast bacilli (AFB) load on baseline sputum smears were also analyzed for respiratory cases diagnosed after 1992 that had positive sputum smears collected on or before the date of diagnosis (the start date of treatment); this data was unavailable for cases in 1991-1992.

Monoresistant-TB refers to resistance to a single first-line antituberculosis drug, namely isoniazid (INH), rifampin (RMP), pyrazinamide (PZA), ethambutol (EMB), or streptomycin (STM). Polyresistance was defined as resistance to two or more first-line antituberculosis drugs but not to both INH and RMP; resistance to at least INH and RMP constituted MDR-TB. Any first-line drug resistance includes monoresistant TB, polyresistant-TB, and MDR-TB.

Cases were also dichotomized as being a new active case (first episode of TB) or retreatment TB case (history of a previous episode of TB).

Laboratory Methods

The Provincial Laboratory conducted all routine mycobacteriologic studies as per the Canadian Tuberculosis Standards[39] and completed routine DNA fingerprinting as previously described.[40] Isolates were assigned to an *M. tuberculosis* genotype group according to the PCR-based detection of large sequence polymorphisms (LSPs).[30,41,42] The Provincial Laboratory analyzed large sequence polymorphisms with an ABI 7000 Real-Time PCR machine (Azco Biotech, Inc., San Diego, CA) using standard conditions and published TaqMan™ primers and probes.[41,42] Isolates with a deletion of RD105 (East Asian lineage) were categorized as Beijing genotype strains and all others as non-Beijing strains.

The genotype assignment of a convenience sample of isolates was confirmed through spoligotyping at the Provincial Laboratory or through large sequence polymorphism analyses at extra-provincial laboratories (M. Behr, McGill University; C. Pepperell, Stanford University). Spoligotype patterns with an absence of spacers 1-34 and a presence of ≥ 3 spacers among spacers 35-43 constituted Beijing strains.[3] An isolate with discordance in *M. tuberculosis* genotype assignment was removed from the study.

Statistical Analysis

Agreement in the genotype assignment of isolates between the initial large sequence polymorphism analysis and confirmatory testing was assessed with the Kappa co-efficient. The incidence rate ratio (RR) was used to compare TB rates between Beijing and non-Beijing strains overall as well as between groups defined on the basis of sex, age at diagnosis, and population group within each lineage. Associations between genotype and various demographic and disease variables were evaluated with binary or multinominal logistic regression; p-values correspond to the likelihood ratio chi-square test in bivariate models.[43] Sex, age at diagnosis, and population group frequently confounded the associations between *M. tuberculosis* genotype and disease presentation (>15% change in the estimated coefficient) and were therefore included in multivariate analyses.[44] Additional adjustment was completed to ensure that associations with disease

presentation were independent of HIV co-infection and associations with drug resistance were not confounded by previous TB or clustering (≥2 isolates with the same DNA fingerprint). Evidence for effect modification within multivariate analyses was based on the likelihood ratio test.[43] Subgroup analysis was planned *a priori* to enhance the transparency of potential differences in Beijing disease presentation across population group (Western Pacific versus others) and age strata.[44] All statistical tests used a 5% level of significance and 95% confidence intervals (CI) were calculated where appropriate; p-values that were $p>0.05$ but $p<0.07$ were considered to be of borderline statistical significance. Statistical analyses were conducted with Stata/IC 11 (StataCorp. 2009. *Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP).

4.4 Results

The Alberta TB Registry was notified of 1852 culture-confirmed *M. tuberculosis* cases from 1991-2007. The genotype of 1826 (99%) isolates was successfully determined and very high agreement in genotype assignment was observed among the 535 (29%) isolates submitted for confirmatory testing (K=0.995) (Figure 4-1). The demographic attributes of included and excluded cases were similar (Table 4-2).

Beijing strains infected 350 (19%) patients and the incidence rate of Beijing strains was 0.2 (CI 0.2-0.3) times that of other strains (rates of 0.7 and 3.1 per 100,000 person-years, respectively). The genotype groups also had marked differences in the distribution of cases by age ($p=0.004$) and population group ($p<0.0001$) but not sex ($p=0.398$) (Table 4-3).

Overall, 3% of Canadian-born patients and 28% of foreign-born patients had Beijing strains, the global dispersion of Beijing strains being evident in the countries of birth of cases (Figure 4-2). Foreign-born individuals accounted for 330 (94%) Beijing isolates and 298 (90%) of these were born in the Western Pacific (Table 4-3). More specifically, 81% (n=282) were born in the Western Pacific's Beijing 'hotspots' of China (including Hong Kong, Macau, and Taiwan), South Korea, and Vietnam. Consequently, the incidence rate of Beijing strains

among individuals born in the 'hotspots' was 10 (CI 6.1-18.0) times that of persons born elsewhere in the Western Pacific, 38 (26.3-56.6) times that of foreign-born 'others', and 430 (273.5-715.6) times that of Canadian-born persons. It is also noteworthy that only 20 Canadian-born cases had Beijing strains (five being Aboriginals) and that the proportion of Beijing strains in the Canadian-born non-Aboriginal group was significantly higher than that of Aboriginal peoples (OR 0.4; CI 0.1-1.0; p=0.046).

An overall trend of declining annual incidence rates of Beijing strains was primarily due to reduced rates among the foreign-born Western Pacific as rates among foreign-born 'others' increased (Figure 4-3). Similar trends were observed for non-Beijing strains with the addition of declining rates in the Canadian-born.

Respiratory TB, sputum smear positivity, high bacillary load or lung cavitation were no more likely among Beijing cases than non-Beijing cases in unadjusted analysis (Table 4-4). However, after controlling for the demographic variables of sex, age at diagnosis and population group, an increased likelihood of respiratory TB among Beijing cases was of borderline significance (aOR 1.3; CI 1.0-1.8; p=0.053) (Table 4-4).

Compared to non-Beijing cases, Beijing cases were not associated with immediately life-threatening forms of TB in unadjusted or adjusted analysis (Table 4-4).

All isolates were tested for INH, RMP, and EMB susceptibility and all but one isolate for STM susceptibility (n=1825). As well, 88% (n=1609) of isolates were tested for PZA susceptibility (50% in 1991-1993 and 99% in 1994-2007). Overall, 87% (n=1592) of isolates were pan-sensitive to the antituberculosis drugs for which they were screened. Beijing strains were not associated with an increased likelihood of any first-line antituberculosis drug resistance or monoresistance in adjusted analysis (Table 4-4). Rather, polyresistance and MDR-TB were 2 to 3 times more likely in Beijing isolates than other isolates independent of demographic factors, albeit with a wider CI around the estimate for MDR-TB. (Table 4-4). These associations between Beijing strains and drug resistance were not confounded by previous TB or clustering (data not shown).

Of potential clinical importance was the finding that 86% (n=6/7) of Beijing isolates with MDR were also resistant to at least two other first-line drugs (EMB, PZA and/or STM)and all but one of these were diagnosed among individuals born in the Beijing 'hotspots'.

A total of 903 (49%) TB patients had consented to HIV antibody testing. However, as all co-infected patients were aged 15-64 years when diagnosed with TB, analysis in relation to HIV status was subsequently limited to the 705 TB cases in this age group who had undergone HIV testing. Beijing strains were associated with 103 (15%) of these TB cases, five (5%) of which were HIV co-infected. Amongst TB cases with non-Beijing strains, 46 of 556 (8%) were co-infected with HIV. Relative to non-Beijing strains, Beijing strains were not associated with TB-HIV co-infection in unadjusted or adjusted analysis (Table 4-5). After adjusting for HIV status and demographic variables, there was also no association between Beijing strains and disease presentation apart from that of polyresistance (Table 4-5).

The associations between Beijing strains and disease presentation were not consistent in all subgroups (Table 4-6 and Table 4-7). Beijing strains were associated with sputum smear-positive respiratory TB among those aged <35 years at diagnosis but not in those aged ≥35 years at diagnosis. In relation to drug resistance, Beijing strains were associated with polyresistance in individuals aged <35 years at diagnosis. Although Beijing strains had an association with polyresistance that was of borderline significance among people born in the Western Pacific, there was a significant association with MDR-TB in this population group.

4.5 Discussion

Beijing genotype strains, representing 19% of TB cases in the major-immigrant receiving province of Alberta, have a definite presence within Canada. This presence is largely limited to persons born in the Western Pacific and especially its Beijing 'hotspots' of China, South Korea and Vietnam. Individuals born in the 'hotspots' accounted for 81% of Beijing isolates, the rate being 10 to

430 times that of the other groups. In contrast, Canadian-born persons remain relatively isolated from infection with Beijing strains (incidence of 0.05/100,000 person-years). These findings generally follow that of other low TB incidence immigrant-receiving countries[14,31,45-47] and further support the well-established correlation between *M. tuberculosis* lineage and the host's country of origin/birth.[30,41,48]

Notwithstanding the emergence of the Beijing genotype in immigrant-receiving countries, there is minimal evidence overall from these countries or this study to indicate that Beijing strains significantly and independently influence the presentation of TB apart from drug resistance.[30,31,45,47,49-51] Beijing strains are significantly more likely than non-Beijing strains in the current study to have polyresistance to first-line antituberculosis drugs independent of demographic factors, clustering, a previous history of TB, or HIV status. A type of polyresistance, MDR-TB, , was also three times more likely among Beijing isolates in adjusted analyses. The association between Beijing strains and MDR-TB in immigrant-receiving countries[31,45,47] is of epidemiologic and clinical significance given that MDR-TB is one of the greatest threats to global TB control. This is exemplified in Germany where the importation of Beijing strains appears responsible for an increasing occurrence of MDR-TB.[21] The United States' experience with the MDR strain W also highlights the importance of sustaining effective TB control programs in low incidence settings given the potential for the rapid dissemination of drug-resistant strains of *M. tuberculosis* in healthcare settings, correctional facilities and elsewhere, especially among persons at high risk for TB (e.g. HIV infected persons).[2,52]

The finding of an association of borderline significance between Beijing strains and respiratory TB in this study accords with associations with pulmonary TB in other immigrant-receiving countries.[49,51] Post hoc analysis found that this similarity persists when site of disease is changed from 'respiratory' (aOR 1.3; CI 1.0-1.8; p=0.053) to 'pulmonary' (aOR 1.3; CI 1.0-1.7; p=0.055). The association of Beijing strains with extrathoracic TB in the United States[50] appears unique compared to other high-income immigrant-receiving countries[14,30,51] and

presumably results from variability in the distribution of Beijing sublineages or sample size limitations.[53]

Although the relatively consistent association between Beijing strains and respiratory/pulmonary TB has potentially important public health consequences, it is equally important that this genotype has not been associated with increased infectiousness in terms of lung cavitation, sputum smear positivity or bacillary load in this and similar studies in immigrant-receiving countries.[49,50,54] There was also no association with immediately life-threatening forms of TB. The lack of an association between Beijing strains and pathogen load (as measured through smear semi-quantification) also suggests that Beijing strains are not intrinsically more transmissible than non-Beijing strains. Together, these findings suggest that the commonly hypothesized hypervirulence and increased transmissibility of Beijing strains is largely unfounded and/or of minimal public health consequence in Alberta, and presumably in other immigrant-receiving areas, when effective TB control programs are in place. By extension, it may merely be coincidental that Beijing strains led to MDR-TB outbreaks in immigrant-receiving countries as MDR variants in other lineages could have been equally successful, all other factors being equal.

Host-pathogen interactions or other population-specific factors plausibly account for the varied disease presentations highlighted in subgroup analyses. Of interest is that Beijing strains were associated with MDR-TB, polyresistant TB and/or sputum smear-positive disease among individuals born in the Western Pacific as well as those aged less than 35 years at diagnosis given that similar groups were identified as high-yield targets for routine screening for latent TB infection (LTBI) in Canada.[55] It may therefore be judicious to also take phylogeographical lineages of *M. tuberculosis* into consideration when defining targets for systematic LTBI screening.

Aboriginal peoples are a highly vulnerable population for TB in Canada and are a significant source of TB transmission.[39,40,56] The minimal incidence of Beijing strains in Aboriginal peoples further substantiates the growing body of

evidence that indicates that immigration has minimal impact on the epidemiology of TB in the Canadian-born population.[40,57,58]

Nevertheless, immigration constitutes a decisive factor in the prevalence of Beijing strains within immigrant-receiving countries. For example, the smaller proportion of Beijing strains in the city of Montreal, Canada compared to this study (9% and 19%, respectively) correlates to a substantially smaller proportion of immigrants from the Western Pacific (<15% in Montreal and 25-30% in Alberta).[30,59] Geographic areas with a considerable proportion of immigrants from high Beijing prevalence countries will therefore predictably experience an increased emergence of Beijing strains with epidemiologic patterns not unlike the region of origin.

This is one of the most comprehensive epidemiologic studies of Beijing strains in a high-income immigrant-receiving country to-date and is the foremost study of its kind in Canada. The inclusion of a measure of pathogen load was a distinguishing feature of this study as it can serve as a proxy for transmissibility. The accuracy of strain classification in this study was enhanced through the use of an unambiguous and validated genotyping methodology[30,41,42] as well as secondary genotyping on a substantial sample of isolates at external laboratories. The amalgamation of data from the provincial TB registry and Provincial Laboratory also minimized selection and information bias.

Study limitations include the relatively small number of polyresistant isolates and Beijing cases with TB-HIV co-infection, the associated estimates having limited precision. Generalizability of the study findings to other immigrant-receiving countries may be limited by differences in immigration patterns, immigration screening practices, and TB control programs. Within Canada, the study findings are anticipated to be especially relevant to the other major immigrant-receiving provinces of Ontario and British Columbia where approximately 20% and 40%, respectively, of immigrants were born in the Western Pacific.[37,59]

In conclusion, there is little evidence apart from an increased risk of polyresistance or multidrug-resistance to indicate that Beijing strains pose any

more of a public health threat than other *M. tuberculosis* strains within a low TB incidence immigrant-receiving country with effective TB control practices in place.

Table 4-1: Countries in the Western Pacific Region of the World Health Organization, with Beijing 'Hotspot' Countries Highlighted

American Samoa	New Caledonia
Australia	New Zealand
Brunei Darussalam	Niue
Cambodia	Northern Mariana Islands
China*	Palau
Cook Islands	Papua New Guinea
Fiji	Philippines
French Polynesia	Pitcairn Islands
Guam	Samoa
Japan	Singapore
Kiribati	Solomon Islands
Korea, Republic of	Tokelau
Lao People's Democratic Republic	Tonga
Malaysia	Tuvalu
Marshall Islands	Vanuatu
Micronesia, Federated States of	Vietnam
Mongolia	Wallis and Futuna
Nauru	

* Includes the Special Administrative Regions of Hong Kong and Macau as well as the Republic of China (Taiwan)

Table 4-2: Demographic Characteristics of Included and Excluded *Mycobacterium Tuberculosis* Cases in Alberta, 1991-2007

Characteristic	Included		Excluded		p*
	n	%	n	%	
Sex					0.077
Female	877	48.0%	17	65.4%	
Male	949	52.0%	9	34.6%	
Age at Diagnosis (years)					0.106
<15	42	2.3%	0	0.0%	
15-34	499	27.3%	6	23.1%	
35-64	693	38.0%	6	23.1%	
>64	592	32.4%	14	53.9%	
Population Group					0.623
Canadian-born Aboriginal	294	16.1%	5	19.2%	
Canadian-born Other	339	18.6%	7	26.9%	
Foreign-born Western	693	38.0%	9	34.6%	
Foreign-born Other	500	27.4%	5	19.2%	
Total	1826		26		

* Based on bivariate logistic regression

Table 4-3: Incidence of Beijing and Non-Beijing Strains of *M. Tuberculosis* in Alberta, 1991-2007

Demographics	PYRs* 100,000s	Beijing Strains			Non-Beijing Strains		
		Cases (%)	Rate† (95% CI)	RR (95%CI)	Cases (%)	Rate† (95% CI)	RR (95% CI)
Sex							
Female	235.4	161 (46.0)	0.7 (0.6, 0.8)	1.0	716 (48.5)	3.0 (2.8, 3.3)	1.0
Male	235.7	189 (54.0)	0.8 (0.7, 0.9)	1.2 (0.9, 1.5)	760 (51.5)	3.2 (3.0, 3.5)	1.1 (1.0, 1.2)
Age at Diagnosis							
<35 years	243.9	104 (29.7)	0.4 (0.3, 0.5)	1.0	438 (29.7)	1.8 (1.6, 2.0)	1.0
35-64years	180.9	109 (31.1)	0.6 (0.5, 0.7)	1.4 (1.1, 1.9)	583 (39.5)	3.2 (3.0, 3.5)	1.8 (1.6, 2.0)
>64 years	46.3	137 (39.1)	3.0 (2.5, 3.5)	6.9 (5.3, 9.1)	455 (30.8)	9.8 (9.0, 10.8)	5.5 (4.8, 6.3)
Population group							
CB Other	381.4	15 (4.3)	0.04 (0.02, 0.06)	1.0	324 (22.0)	0.8 (0.8, 0.9)	1.0
CB Aboriginal	13.6	5 (1.4)	0.4 (0.1, 0.9)	9.4 (2.7, 27.1)	288 (19.5)	21.2 (18.9, 23.8)	25.0 (21.2, 29.4)
FB Other	56.5	32 (9.1)	0.6 (0.4, 0.8)	14.6 (7.7, 29.0)	469 (31.8)	8.4 (7.7, 9.2)	9.9 (8.6, 11.4)
FB-WP	19.6	298 (85.1)	14.6 (13.0, 16.4)	371. 7 (221.7, 672.5)	395 (26.8)	19.4 (17.5, 21.4)	22.8 (19.6, 26.5)
Total	471.1	350 (100.0)	0.7 (0.7, 0.8)		1476 (100.0)	3.1 (3.0, 3.3)	

Abbreviations: PYRs, person-years of observation; RR, incidence rate ratio; CI, confidence interval; CB, Canadian-born; FB, foreign-born; WP, Western Pacific

* Estimates of the foreign-born and Canadian-born populations were derived from customized Statistics Canada census reports. Estimates for Canadian-born 'others' were those derived from the censuses minus the annual population of Canadian-born Aboriginal peoples as obtained directly from Indian and Northern Affairs Canada (see Methodology for additional information).

† Crude incidence rate per 100,000 person-years

Table 4-4: Association Between *M. Tuberculosis* Genotype and Disease Presentation in Alberta, 1991 to mid-2007

Disease Presentation	Beijing (N=350) n (%)	Non-Beijing (N=1476) n (%)	OR (95% CI)*	aOR (95% CI)†
Respiratory TB	259 (74.0)	1122 (76.0)	0.9 (0.7, 1.2)	1.3 (1.0, 1.8)‖
Sputum smear positive‡	112 (45.9)	538 (50.9)	0.8 (0.6, 1.1)	1.1 (0.8, 1.5)
High bacillary load§	16 (29.6)	89(33.3)	0.8 (0.4, 1.6)	1.2 (0.6, 2.7)
Lung cavitation	52 (14.9)	268 (18.2)	0.8 (0.6, 1.1)	1.0 (0.7, 1.5)
Immediately life-threatening TB	16 (4.6)	99 (6.7)	0.7 (0.4, 1.1)	0.8 (0.5, 1.6)
Any first-line drug resistance	73 (20.9)	161 (10.9)	2.2 (1.6, 2.9)	1.2 (0.8, 1.6)
Monoresistance	41 (11.7)	120 (8.1)	1.6 (1.1, 2.4)	0.9 (0.6, 1.3)
INH	11 (3.1)	48 (3.3)	1.0 (0.5, 2.0)	0.4 (0.2, 0.9)
RMP	0	0	---	---
PZA	1 (0.3)	8 (0.6)	0.4 (0.2, 0.6)	0.7 (0.4, 1.2)
EMB	0	2 (0.1)	---	---
STM	29 (8.3)	62 (4.2)	2.2 (1.4, 3.5)	1.4 (0.8, 2.3)
Polyresistance	25 (7.1)	34 (2.3)	3.5 (2.0, 5.9)	1.8 (1.0, 3.3)**
MDR-TB	7 (2.0)	7 (0.5)	4.7 (1.7, 13.6)	3.4 (1.0, 11.3)††

Abbreviations: OR, odds ratio; aOR, adjusted odds ratio; CI, confidence interval; CB, Canadian-born; FBO, foreign-born 'other'; FBWP, foreign-born Western Pacific; INH, isoniazid; RMP, rifampin; PZA, pyrazinamide; EMB, ethambutol; STM, streptomycin; MDR-TB, multidrug-resistant TB.

* Non-Beijing lineage is the reference category; ---, logistic regression could not be completed due to cell count(s) of zero.

† Association between Beijing lineage and each disease presentation after adjusting for sex, age, and population group; non-Beijing lineage is the reference category.

‡ There were 1301 respiratory TB cases with data related to airway secretions, 1057 (81.2%) and 244 (18.8%) being attributed to non-Beijing and Beijing lineages, respectively.

§ There were 321 respiratory TB cases diagnosed after 1992 that had sputum smear-positive specimens collected on or before the date of diagnosis.

‖ p=0.053

** p=0.046

†† p=0.049

Table 4-5: Associations Between *M. Tuberculosis* Genotype and the Disease Presentations of Active TB Among Patients Aged 15-64 Years at Diagnosis with Known HIV Status

Disease Presentation	Beijing (N=103)	Non-Beijing (N=602)	OR (95% CI)*	aOR (95% CI)†
Respiratory TB	74 (71.8)	454 (75.4)	0.8 (0.5, 1.3)	1.5 (0.9, 2.5)
Sputum smear positive‡	40 (58.0)	239 (56.4)	1.1 (0.6, 1.8)	1.5 (0.8, 2.6)
High bacillary load§	11 (36.7)	57 (37.3)	1.0 (0.4, 2.2)	1.4 (0.5, 3.5)
Lung cavitation	19 (18.4)	137 (22.8)	0.8 (0.5, 1.3)	1.0 (0.6, 1.9)
Immediately life-threatening TB	5 (4.9)	46 (7.6)	0.6 (0.2, 1.6)	0.7 (0.2, 1.9)
Any first-line drug resistance	29 (28.2)	75 (12.5)	2.8 (1.7, 4.5)	1.5 (0.9, 2.6)
Monoresistance	13(12.6)	53 (8.8)	1.7 (0.9, 3.4)	1.0 (0.5, 2.0)
Polyresistance	13 (12.6)	16 (2.7)	5.8 (2.7, 12.5)	2.8 (1.2, 6.7)
MDR-TB	3 (2.9)	6 (1.0)	3.6 (0.9, 14.5)	2.1(0.4, 10.1)
HIV positive	5 (4.9)	46 (7.6)	0.6 (0.2, 1.6)	1.4 (0.5, 4.2)

Abbreviations: HIV, human immunodeficiency virus; aOR, adjusted odds ratio; TB, tuberculosis.

* Non-Beijing strains were the reference.

† Adjusted for sex, age, population group and HIV status; non-Beijing genotypes were the reference

‡ Sputum smear microscopy was completed on 493 respiratory cases with known HIV status. Of these, 69 (14%) were Beijing strains and 424 (86%) were non-Beijing strains.

§183 respiratory TB cases diagnosed after 1992 had sputum smear positive specimens collected on or before the data of diagnosis. Of these, 30 (16%) were Beijing strains and 153 (84%) were non-Beijing strains.

Table 4-6: Association Between *M. Tuberculosis* Genotype and Disease Presentation Based on the Population Group of TB Cases

Disease Presentation	Foreign-born Western Pacific			All Others*		
	Beijing (N=298)	Non-Beijing (N=395)	aOR (95% CI)†	Beijing (N=52)	Non-Beijing (N=1081)	aOR (95% CI)†
Respiratory TB	214 (71.8)	256 (64.8)	1.2 (0.8, 1.7)	45 (86.5)	866 (80.1)	1.6 (0.7, 3.6)
Sputum smear-positive‡	95 (47.3)	91 (38.1)	1.5 (1.0, 2.1)§	17 (39.5)	447 (54.7)	0.6 (0.3, 1.0)‖
High bacillary load**	12 (28.6)	9 (19.6)	1.7 (0.6, 4.7)	4 (33.3)	80 (36.2)	0.8 (0.2, 2.7)
Lung cavitation	43 (14.4)	54 (13.7)	1.1 (0.7, 1.7)	9 (17.3)	214 (19.8)	0.8 (0.4, 1.6)
Immediately life-threatening TB	13 (4.4)	20 (5.1)	0.8 (0.4, 1.6)	3 (5.8)	79 (7.3)	0.8 (0.2, 2.7)
Any first-line drug resistance	67 (22.5)	87 (22.0)	1.1 (0.8, 1.6)	6 (11.5)	74 (6.8)	1.6 (0.6, 3.9)
Monoresistance	37 (12.4)	66 (16.7)	0.8 (0.5, 1.3)	4 (7.7)	54 (5.0)	1.5 (0.5, 4.3)
Polyresistance	23 (7.7)	19 (4.8)	1.8 (1.0, 3.5)††	2 (3.9)	15 (1.4)	2.5 (0.5, 11.3)
MDR-TB	7 (2.4)	2 (0.5)	6.1 (1.2, 30.4)	0	5 (0.5)	---

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; TB, tuberculosis; MDR-TB, multidrug-resistant tuberculosis; ---not calculated

* Individuals born outside of the Western Pacific, including Canadian-born Aboriginals and Canadian-born non-Aboriginals.

† Independent of sex and age at diagnosis; non-Beijing strains are the reference.

‡ Of respiratory cases, 440 cases among those born in the Western Pacific and 860 cases among those born elsewhere had smear microscopy data.

§ p=0.057

‖ p=0.069

** Sputum smear positive specimens were collected for 88 foreign-born Western Pacific and 233 'Other' respiratory TB cases

†† p=0.062

Table 4-7: Association Between *M. Tuberculosis* Genotype and Disease Presentation Based on the Age of TB Cases at Diagnosis

Disease Presentation	<35 years at diagnosis			≥35 years at diagnosis		
	Beijing (N=104)	Non-Beijing (N=438)	aOR (95% CI)*	Beijing (N=246)	Non-Beijing (N=1038)	aOR (95% CI)*
Respiratory TB	73 (70.2)	319 (72.8)	1.6 (0.9, 2.7)	186 (75.6)	803 (77.4)	1.3 (0.9, 1.9)
Sputum smear-positive†	34 (48.6)	128 (42.5)	1.9 (1.0, 3.4)‡	78 (44.8)	410 (54.3)	0.9 (0.6, 1.4)
High bacillary load§	7 (36.8)	18 (31.6)	8.1 (0.9, 74.6)	9 (25.7)	71 (33.8)	0.9 (0.3, 2.2)
Lung cavitation	23 (22.1)	81 (18.5)	1.7 (1.0, 3.1)‖	29 (11.8)	187 (18.0)	0.7 (0.5, 1.2)
Immediately life-threatening TB	4 (3.8)	15 (3.4)	1.5 (0.4, 5.1)	12 (4.9)	84 (8.1)	0.7 (0.4, 1.5)
Any first-line drug resistance	31 (29.8)	72 (16.4)	1.3 (0.8, 2.3)	42 (17.1)	89 (8.6)	1.0 (0.6, 1.5)
Monoresistance	15 (14.4)	54 (12.3)	0.8 (0.4, 1.6)	26 (10.6)	66 (6.4)	0.8 (0.5, 1.4)
Polyresistance	13 (12.5)	14 (3.2)	3.1 (1.3, 7.5)	12 (4.9)	20 (1.9)	1.1 (0.5, 2.4)
MDR-TB	3 (2.9)	4 (0.9)	2.4 (0.5, 12.2)	4 (1.6)	3 (0.3)	6.1 (0.9, 42.7)

Abbreviations: aOR, adjusted odds ratio, CI, confidence interval; TB, tuberculosis; MDR-TB, multidrug-resistant tuberculosis; ---unable to calculate

* Independent of sex and origin; non-Beijing strains are the reference.

† Of respiratory cases, 371 cases among those aged <35 years and 929 cases among those aged ≤35 years had smear microscopy data

‡ p=0.042

§ There were 76 respiratory TB cases diagnosed after 1992 that had sputum smear positive specimens collected on or before the date of diagnosis among those born in the Western Pacific and 245 cases among those born elsewhere.

‖ p=0.072

Figure 4-1: Inclusion and Exclusion of *M. Tuberculosis* Cases on the Basis of Isolate Availability and Confirmatory Genotyping

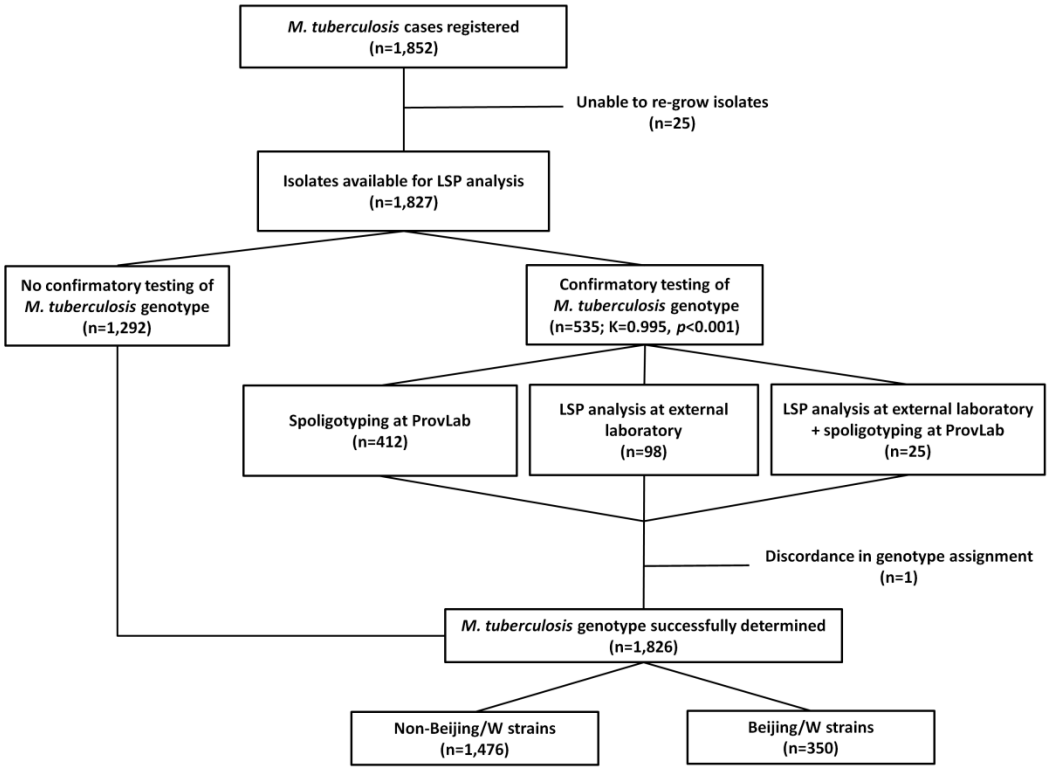
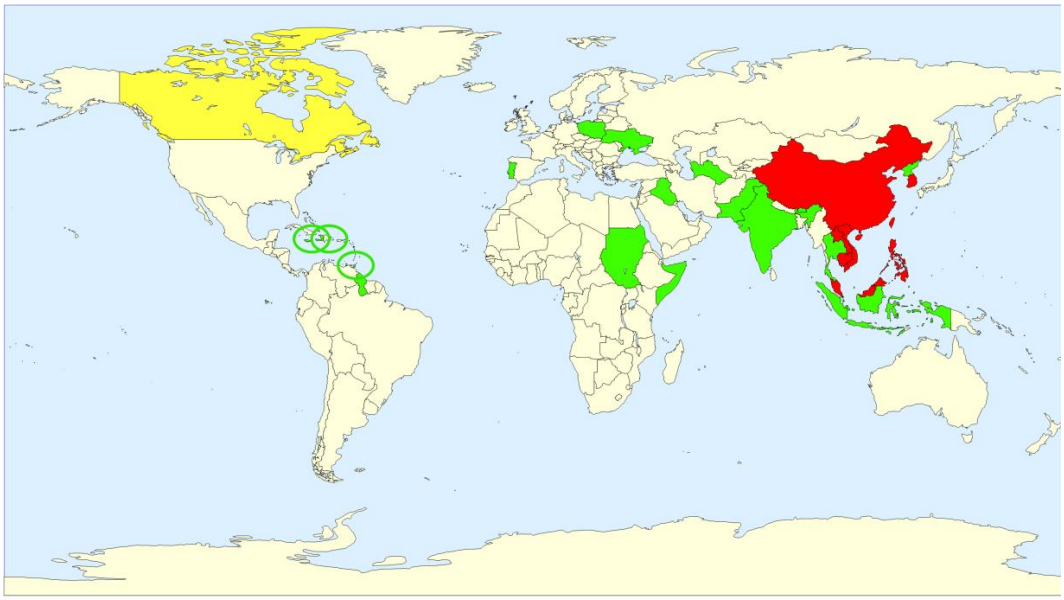
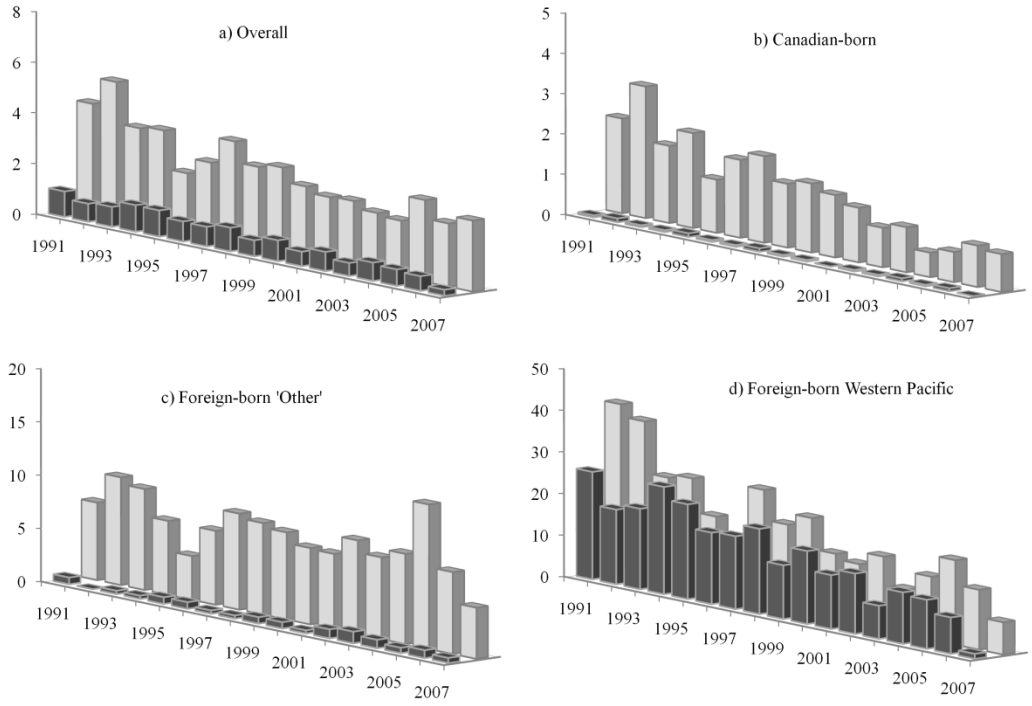


Figure 4-2: Global origins of *M. tuberculosis* Beijing strains according to the countries of birth of TB patients in Alberta, 1991-2007



Countries colored: light gray are cases born in Canada or born in a foreign country to Canadian-born parents; black are foreign-born cases whose country of birth is located within the Western Pacific region of the World Health Organization; and medium gray or circles are other foreign-born cases. The figure was created with Map Maker *Gratis* (www.mapmaker.com) and is used with permission.

Figure 4-3: Annual Incidence Rates of Beijing and Non-Beijing Strains by Population Group



In each figure, incidence rates are per 100,000 person-years (y-axis) and times corresponds to the year of diagnosis (x-axis). Dark gray bars represent Beijing strains and light gray bars are non-Beijing strains.

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CHAPTER 5: TRANSMISSION OF BEIJING STRAINS OF *MYCOBACTERIUM TUBERCULOSIS* IN A LOW INCIDENCE SETTING: TIME TO SILENCE THE ALARMS?*

5.1 Abstract

Introduction: There is repeated speculation that Beijing strains have a selective advantage over other *M. tuberculosis* genotypes as conferred through increased virulence and transmissibility. This is especially concerning given frequent associations between Beijing strains and multidrug-resistant tuberculosis (MDR-TB). This study's purpose was to identify the risk factors for, and trends in, the transmission of Beijing and non-Beijing strains in a low TB incidence immigrant-receiving province of Canada. In particular, it aimed to determine if Beijing strains were potentially a greater public health threat than non-Beijing strains due to higher transmission.

Methods: Culture-confirmed respiratory TB cases in the immigrant-receiving province of Alberta, Canada in 1991 through mid-2007 (n=1399) were eligible for inclusion in this retrospective study. Provincial TB Registry data were combined with mycobacteriologic data; this included DNA fingerprinting (IS*6110* restriction fragment length polymorphism and spoligotyping) and discrimination between Beijing and non-Beijing strains via genomic deletion analysis. Clusters were ≥2 patients with identical DNA fingerprints in 1991-2007. The number of secondary cases that were observed within 2 years per index case was used as a measure of recent transmission, index cases being those whose DNA fingerprint was not observed within the previous 2 years.

Results: Beijing strains were attributed with 258 of the 1379 (19%) included cases. Overall, 55 (21%) Beijing cases and 418 (37%) non-Beijing cases were clustered. Beijing and non-Beijing strains had a similar likelihood of clustering among the foreign-born Western Pacific and the foreign-born 'other' while there was significantly less clustering of Beijing strains in the Canadian-born. On average, Beijing index cases had 0.06 secondary cases within 2 years while non-

Beijing index cases had 0.14 (OR 0.5; CI 0.3-0.8). However, in the adjusted analysis, the number of secondary cases per Beijing and non-Beijing index case was similar (OR0.9; CI 0.4-1.7) whereas it was reduced among index cases aged ≥65 than ≤35 years (0.4; CI 0.3-0.8), born in the Western Pacific (0.2; CI 0.1-0.3) or born elsewhere outside of Canada (0.1; CI 0.1-0.2).

***Conclusion*:** Beijing strains do not result in any more clustering or more frequent recent transmission than non-Beijing strains in a setting with comprehensive and effective TB control practices.

**A version of this paper has been submitted to Emerging Infectious Diseases:*

Langlois-Klassen D, Senthilselvan A, Chui L, Kunimoto D, Saunders LD, Menzies R, Long R. Transmission of Beijing strains of *Mycobacterium tuberculosis* in a low incidence setting: time to silence the alarms? *(submitted)*

5.2 Introduction

The Beijing family of *Mycobacterium tuberculosis* strains is regarded as an emerging public health threat.[1-4] Members of this family account for 13% of *M. tuberculosis* strains globally[5] and up to 27% of *M. tuberculosis* strains within certain low TB incidence immigrant-receiving countries.[6-10] The recent global dissemination of Beijing strains and their increasing prevalence is of concern due to their frequent association with antituberculosis drug resistance and more specifically, multidrug resistance (MDR-TB).[1, 11-14] These concerns are further intensified by the increasing body of evidence that associates Beijing strains with extensively drug-resistant TB (XDR-TB).[12, 15, 16]

The rapid global expansion of Beijing strains and their frequent (albeit inconsistent) association with large TB outbreaks as well as with younger patients has led to speculation that Beijing strains have a selective advantage over other *M. tuberculosis* genotypes as conferred through increased virulence and transmissibility.[1, 2, 12, 17, 18] This hypothesis is supported by experimental evidence in vitro and in animal models of increased virulence demonstrated by Beijing strains relative to other *M. tuberculosis* strains.[19-22] Evidence also suggests that the fitness of some Beijing strains is retained after the acquisition of drug resistance.[23] Nevertheless, intragenotypic variation in virulence has been described in the Beijing family of strains [24-26] and, in a review, Coscolla and Gagneux (2010) concluded that the current body of evidence is insufficient to support the increased transmissibility of these strains.[27]

Immigration is the most important determinant of TB epidemiology in low incidence settings.[28, 29] Consequently, the importation of potentially more pathogenic strains such as those in the Beijing family could have important implications for TB control and elimination efforts within immigrant-receiving countries. Surveillance activities which identify the sources and transmission patterns of emerging and/or expanding *M. tuberculosis* strains will be increasingly vital if TB control programs are to maintain their effectiveness within the context of dynamic immigration policies and highly mobile populations.

The purpose of this study was to identify the risk factors for, and trends in, the transmission of Beijing and non-Beijing strains in a low TB incidence immigrant-receiving province of Canada. In particular, this study sought to determine if Beijing strains are potentially a greater public health threat than non-Beijing strains due to higher transmissibility.

5.3 Methods

Study Setting and Population

Culture-confirmed respiratory TB cases in the province of Alberta, Canada between January 1, 1991 and June 30, 2007 ('study period') as per the provincial TB registry were eligible for inclusion in this population-based retrospective study (see subsection on '*Transmission'* for additional criteria). Ethics approval was received from the University of Alberta Health Research Ethics Board and analysis of surveillance data did not require informed consent as there was no direct patient contact.

People born in Canada or born outside of Canada to Canadian-born parents were Canadian-born; all others were foreign-born. Due to the high prevalence of Beijing strains in parts of Southeast and East Asia,[1] country of birth was used to group the foreign-born into those born in the Western Pacific region and those born elsewhere.[29]

Population estimates were obtained from customized Statistics Canada census reports (1991, 1996, 2001, and 2006). Inter-censuses estimates were derived through linear interpolation and estimates for 2007 were based on linear extrapolation.

Case Characteristics

Demographic and clinical data from the TB Registry were combined with data from the Provincial Laboratory for Public Health (Provincial Laboratory) where all mycobacteriology in the province is performed. Sputum smear status and the presence/absence of lung cavitation on chest radiograph were used as indicators of infectiousness. Baseline sputum smears collected on or before the

date of diagnosis (the start date of treatment) that had Grade 3+ to 4+ scores for acid-fast bacilli (AFB) were categorized as having high bacillary loads.

Monoresistant-TB refers to resistance to a single first-line antituberculosis drug, namely isoniazid (INH), rifampin (RMP), pyrazinamide (PZA), ethambutol (EMB), or streptomycin (STM). Polyresistance was defined as resistance to two or more first-line antituberculosis drugs but not to both INH and RMP; resistance to at least INH and RMP constituted MDR-TB.[30] Any first-line drug resistance includes monoresistant TB, polyresistant-TB, and MDR-TB.

Laboratory Methods

The Provincial Laboratory completed routine mycobacteriologic studies in accordance with the Canadian Tuberculosis Standards.[30] This included the DNA fingerprinting of prospectively archived isolates with the IS*6110* restriction fragment-length polymorphism (RFLP) method and, for those with ≤5 copies of IS*6110*, spoligotyping as described elsewhere.[31-33]

Isolates were assigned to a *M. tuberculosis* genotype group based on genomic deletion analysis completed at the Provincial Laboratory with an ABI 7000 Real-Time PCR machine (Azco Biotech, Inc., San Diego, CA) using standard conditions and published TaqMan™ primers and probes.[34, 35] Isolates with a deletion of RD105 (East Asian lineage) were classified as Beijing strains and all others as non-Beijing strains. The genotype assignment of a convenience sample of 382 isolates was confirmed via spoligotyping at the Provincial Laboratory or genomic deletion analysis at extra-provincial laboratories; no discordance was reported.

Recent Transmission

Of the 1399 eligible respiratory TB cases in the study period, 20 (1%) cases were excluded as either the DNA fingerprint pattern or *M. tuberculosis* genotype could not be determined. The remaining 1379 cases were included in the analysis of clustering to provide an indication of overall transmission. In this

study, a cluster was defined as 2 or more patients whose case isolates had identical DNA fingerprints at any time in the study period.

The method of Borgdorff et al. (2010) was used to quantify recent transmission within the expansive study period given that potential source cases had dissimilar follow-up periods and the probability of propagated transmission by secondary and later generation source cases within the cluster.[36] To do so, survival analysis using a Kaplan-Meier curve was completed with the 1379 respiratory TB cases to determine the cut-off point for the definition of 'recent' transmission.[36, 37] In the 16.5-year study period, the probability of recurrence of an isolate with an identical fingerprint pattern was 0.36 in the study period and 0.28, 0.24 and 0.22 within 5, 3 and 2 years, respectively, from Kaplan-Meier estimates (Figure 5-1). A 2-year period was determined to be the ideal cut-off point as the associated probability was 78% and 89% of the 5 and 3 year probabilities, respectively. The decision to use a 2-year instead of 3-year cut-off point was also influenced by the conventional high-risk period for the development of active TB following infection (18 to 24 months).[30] The potential impact of defining recent transmission with a 2-year instead of 3-year period was explored in a sensitivity analysis.

Using a 2-year cut-off point, an 'index case' was defined as a respiratory TB case with a DNA fingerprint pattern that had not been assigned to another case within the preceding 2 years. The 'transmission index' was the number of secondary respiratory TB cases within 2 years per index case that were either directly or indirectly related to the index case on the basis of DNA fingerprint patterns.[36] Using these definitions, 425 of the 1379 (31%) respiratory TB cases were excluded from the analysis of recent transmission.[36] Specifically, 162 index cases diagnosed in 1991-1992 and their 50 secondary cases were excluded as it could not be determined if the fingerprint patterns of the index cases matched another case in the preceding 2 years. Follow-up periods of <2 years resulted in the exclusion of an additional 124 index cases diagnosed after June 30, 2005 and their 10 secondary cases. Finally, 79 secondary cases were excluded due to diagnosis >2 years after the index case but <2 years after another cluster member.

After these exclusions, 954 (69%) TB cases diagnosed between January 1, 1993 and June 30, 2007 were included.

Statistical Analysis

Stata/IC 11 (StataCorp. 2009. *Stata Statistical Software: Release 11*. College Station, TX: StataCorp LP) was used for data analyses. The relationship between categorical variables was determined using logistic regression; $p<0.05$ was considered statistically significant. Incidence rates for index cases, which were calculated overall and within each strain group, were compared using incidence rate ratios (IRR). Standardized rates were calculated with the direct method using the age- and sex-specific distribution of the total population in Alberta in 1993-2007 as the reference population. The risk factors of index cases that were associated with recent transmission were assessed with bivariate and multivariate Poisson regression using an offset of one each index case.[36] Multivariate modeling was constructed with purposeful selection as follows:[38] variables with $p<0.20$ in bivariate regression were candidates for multivariate modeling; the variable representing the *M. tuberculosis* strain groups was retained regardless of the *P* value; the confounding effects of removed variables was assessed with the percentage rule using a collapsibility criterion of ≤15%;[39] and the significance of potential interactions was based on the partial likelihood ratio test (5% level of significance).[38] All confidence intervals (CIs) are 95% CIs.

5.4 Results

Beijing strains accounted for 258 of the 1379 (19%) respiratory TB cases that were diagnosed from January 1, 1991 through June 30, 2007. Compared to cases resulting from infections with non-Beijing strains, Beijing cases were more often ≥65 years old and born in the Western Pacific (versus Canadian-born) (Table 5-1). The infectiousness of Beijing and non-Beijing cases was similar (Table 5-1). Additionally, polyresistant-TB and MDR-TB were 13 times (CI 3.3, 47.8) and 5 times (CI 1.5, 14.8), respectively, more likely among Beijing cases

although there was no association with monoresistance (OR 1.6; CI 1.0, 2.7, p=0.061).

Clustering

In total, 906 (66%) cases exhibited unique fingerprinting patterns ('non-clustered cases') and the remaining 473 (34%) isolates were distributed between 119 clusters. Of Beijing strains, 203 (79%) were non-clustered cases and 55 (21%) were clustered cases within 22 clusters. Non-Beijing strains accounted for the remaining 703 (63%) non-clustered cases and 418 (37%) clustered cases within 97 clusters. Consequently, the likelihood of clustering was significantly lower among Beijing than non-Beijing strains overall (OR 0.5; CI 0.3, 0.6). Independent of population group, however, Beijing strains were associated with a lower likelihood of clustering among the Canadian-born (OR 0.4; CI 0.1, 0.9) but not those born in the Western Pacific (OR 0.8; CI 0.5, 1.2) or elsewhere (OR 2.2; CI 0.8, 5.7).

None of the demographic factors was associated with clustering among Beijing cases in unadjusted or adjusted analysis (Table 5-2). However, among non-Beijing strains, the likelihood of clustering was significantly less among those aged ≥65 years (versus ≤34 years) and for foreign-born individuals (Table 5-2).

Among the foreign-born, the number of non-clustered Beijing and non-Beijing cases was inversely associated with increased time since arrival (Figure 5-2), 30-32% of cases occurring within 2 years of arrival. Although clustered cases appeared to follow a similar pattern, including 23-25% of cases occurring within 2 years of arrival, interpretation was limited by the relatively small number of these cases.

Incidence Rates of Index Cases

Beijing index cases had a significantly lower incidence rate than non-Beijing index cases (IRR 0.3; CI 0.2, 0.3), the respective rates being 0.4 and 1.6

per 100,000 person-years. The rates of Beijing and non-Beijing index cases were similar among individuals born in the Western Pacific (Table 5-3).

Increased rates of Beijing and non-Beijing index cases were associated with male cases, persons aged ≥65 years (versus ≤34 years), and foreign-born persons (Table 5-4). Persons aged 35-64 years also had an increased rate of non-Beijing index cases. Of foreign-born persons, the rates of Beijing and non-Beijing index cases were increased 22 times (CI 13.5, 37.3) and 2 times (CI 1.7, 2.5), respectively, among those born in the Western Pacific compared to others.

Trends in Index and Secondary Cases

As per Figure 5-3, there was a trend of decreasing annual rates of Beijing and non-Beijing index cases. Compared to the average annual index case rates in 1993-1995, the rates in 2002-2004 (2004 being the last full calendar from which index cases were included) had decreased by 39% (from 0.6 to 0.4 per 100,000 person-years) among Beijing cases and 30% (from 2.0 to 1.4 per 100,000 person-years) among non-Beijing cases. These trends primarily reflect those of the foreign-born Western Pacific given the trend of increasing rates in the foreign-born 'other' and the minimal contribution of Canadian-born rates to overall rates in Alberta (Figure 5-4).

Risk Factors for Recent Transmission

Table 5-5 outlines the number of cases that were attributed to recent transmission based on the characteristics of the index case. On average, an index case resulted in 0.13 secondary cases within 2 years. In unadjusted analysis, the number of secondary cases was significantly increased if the index case was sputum smear-positive, had a high bacillary load or had lung cavitation (Table 5-5). Conversely, fewer secondary cases were associated with index cases aged ≥65 years (versus ≤ 34 years), persons born outside of Canada, or cases infected with Beijing strains. In adjusted analysis, the number of secondary cases was only significantly associated with the age and population group of the index cases; fewer secondary cases occurred if the index case was aged ≥35 years (versus ≤ 34

years) or foreign-born, the association with age having been confounded by lung cavitation (Table 5-5). The number of secondary cases was not independently associated with the *M. tuberculosis* genotype of the index case after controlling for the confounding effects of age, population group, sputum smear status and lung cavitation.

Co-infection with HIV was not a risk factor for recent transmission. Of the 419 index cases for which HIV status had been reported, 21(5%) were TB-HIV co-infected and only one secondary case was attributed to these TB-HIV co-infected index cases. On average, HIV-negative index cases led to 0.15 secondary cases within 2 years while TB-HIV co-infected index cases had 0.5 secondary cases. Consequently, the number of secondary cases was not associated with the HIV status of index cases (relative transmission index = 0.3; CI 0.04, 2.3).

A subgroup analysis of foreign-born index cases also indicated that the number of secondary cases per index case was unrelated to the length of residency in Canada. For example, compared to index cases that were ≤ 2 years since arrival in Canada, the relative transmission indices of those 3-5 years and those >20 years since arrival were 1.1 (CI 0.3, 4.1) and 1.2 (0.4, 3.6), respectively.

Additional subgroup analysis assessed the risk factors for the number of secondary cases among individuals born in the Western Pacific only (Table 5-6). No significant risk factors for the number of secondary cases within 2 years per index case were identified, including age, *M. tuberculosis* genotype or time since arrival. However, polyresistant-TB was a risk factor that was of borderline significance for an increased number of secondary cases compared to index cases with pan-susceptible isolates (Table 5-6).

Sensitivity Analysis

The use of a 3-year cut-off point for defining recent transmission resulted in fewer index cases but had an inconsistent impact on the number of secondary cases in each strain group (Table 5-7). The transmission index for Beijing and non-Beijing cases was also increased by the one year increase in the cut-off point.

5.5 Discussion

Outbreaks of *M. tuberculosis* Beijing strains in high and low TB incidence settings have had significant public health implications.[12, 18, 40-42] Notwithstanding the effect of these outbreaks, the current study finds the transmission of Beijing strains to be similar to that of non-Beijing strains in Alberta, a low incidence immigrant-receiving province of Canada. Speculation regarding the increased transmissibility of Beijing strains has also been refuted in other low TB incidence immigrant-receiving countries and in The Gambia.[13, 43, 44] At the same time, contradictory findings with respect to Beijing strains and transmission have been reported in the Cape Town region of South Africa.[2, 45, 46] The general absence of evidence to suggest that Beijing strains are inherently more transmissible than other *M. tuberculosis* strains is highly informative for TB control programs given the propensity for MDR-TB among cases infected with Beijing strains in this and other studies.[11, 13, 14, 47]

The transmission of *M. tuberculosis* occurs most frequently when TB patients have positive sputum smear microscopy and lung cavitation.[48, 49] Consequently, findings that Beijing strains are not typically associated with smear positive or cavitary disease in this study and others[50-52] accords with the reported similarities in the transmission of Beijing and non-Beijing strains. The bacillary load of Beijing and non-Beijing cases was also similar in this study.

That Beijing strains have been associated with increased transmission in some settings but not in others may reflect geographic variations in virulence phenotypes. In the *M. tuberculosis* complex, evolutionarily modern lineages (including the East Asian lineage and hence Beijing strains) induce lower immune responses than ancient lineages, potentially providing modern lineages with a selective advantage in terms of more rapid disease progression and transmission in the human population.[53] An array of virulence phenotypes have also been demonstrated in the more evolutionarily recent subfamily of Beijing strains (i.e. the 'modern' subfamily as characterized by the insertion of IS*6110* in the noise transfer function [NTF] chromosomal region[54]), including differences in the pathogenic characteristics (and potential transmissibility) of closely related strains

in the same sublineage.[24-26] For example, strains within the "modern" Beijing subfamily have significant variations in their intracellular growth rates and hence significant differences in tumor necrosis factor-alpha (TNF-α) levels.[25] This may be of particular relevance on account of higher TNF-α levels being observed in the bronchoalveolar lavage fluid of TB patients with large cavities.[55] To facilitate an understanding of the potential implications of virulence phenotypes in relation to TB control efforts, future population-based investigations in high and low incidence settings would benefit from discerning between the disease presentations and transmissibility of different *M. tuberculosis* subfamilies or sublineages.

In agreement with previous studies,[28, 31, 48, 56-58] the risk of recent *M. tuberculosis* transmission in the present study was lower for older as well as foreign-born persons and it was unrelated to drug resistance. A deeper exploration into these common risk factors for transmission demonstrates that these factors were independent of *M. tuberculosis* genotype, at least within the broad categories of Beijing and non-Beijing strains.

Foreign-born TB incidence in immigrant-receiving countries has a characteristic and inverse relationship with increased time since arrival.[59-61] The current findings demonstrate that this characteristic relationship is clearly evident for non-clustered Beijing and non-Beijing cases that presumably result from the reactivation of latent TB infections acquired before immigration. It also appears that clustered cases follow a similar pattern. Despite nearly one-quarter of clustered Beijing and non-Beijing cases occurring ≤2years since arrival, recent transmission was not associated with time since arrival, a finding that accords with a previous study.[62] Nevertheless, time since arrival may still have important implications for the inter-population transmission of *M. tuberculosis*.[62] Collectively, these findings emphasize the need for screening and prevention activities in the foreign-born as a critical means of mitigating the reactivation of latent TB infection as early after arrival as possible. It also reinforces the important need for high-income countries to increase their funding of efforts to expand TB control in high-incidence countries.[63] Enhanced TB control in source

regions may have relatively immediate benefits within host countries given that decreases in per capita TB incidence in the Western Pacific and South-East Asia regions[64] coincided with declining index case rates in this study.

This study reinforces that the foreign-born are not a significant source of *M. tuberculosis* transmission (including Beijing strains) despite having considerably higher index case rates than the native-born population.[28, 36, 65] Rather, the proportion of non-clustered cases suggests that the reactivation of latent TB infection accounts for 82% of foreign-born cases (i.e. 80% and 83% of foreign-born Beijing and non-Beijing cases, respectively). The inevitable importation of emerging pathogens such as Beijing strains therefore should not be viewed so much as a threat as a challenge. The challenge lies in the host country's resolve to prevent the reactivation of latent TB infections in recently arrived immigrants and in a larger population of aging immigrants while contending with constantly evolving immigration patterns.[59]

The maintenance of a comprehensive provincial TB dataset derived through the amalgamation of TB Registry and mycobacteriology data was critical for this study and the general evaluation of TB control in Alberta. The accuracy of strain classification was also enhanced through the use of an unambiguous and validated genotyping methodology[8, 34, 35] as well as the confirmatory genotyping of a convenience sample of isolates. As Alberta is one of four primary immigrant-receiving provinces in Canada, three of which have a very similar immigration pattern (Alberta, Ontario and British Columbia), the study results are anticipated to have national relevance. The generalizability of the study results to other low TB incidence immigrant-receiving countries will be influenced by the degree of similarity in immigration patterns.

Unavoidable sampling limitations will have produced underestimates in clustering[66, 67] and impacted the transmission indices.[68] Nevertheless, sampling bias will have been minimized by identifying cases through a provincial TB Registry; ≥88% of cases in Alberta being culture confirmed;[69, 70] an expansive study period; and the inclusion of 99% of eligible culture-confirmed respiratory TB cases. The transmission index used in this study, while advantageous for

quantifying recent transmission within an expansive study period,[36] is subject to the same limitations as other TB transmission indices.[68] As well, the sensitivity analysis is this study and that of Borgdorff et al. (2010) suggest that the estimates of recent transmission are relatively sensitive to the small changes in the time periods used to define recent transmission.[36] Overestimates in clustering may have resulted from the common molecular epidemiologic assumption that cases with identical DNA fingerprints were part of a transmission cluster.[9, 48] Finally, the relatively small number of secondary and Canadian-born Beijing cases in this study limited the ability to comprehensively assess the cross-population transmission of Beijing strains and the strain-specific transmission patterns in Canadian-born Aboriginal peoples.

To conclude, this study has demonstrated that Beijing strains do not result in any more clustering than non-Beijing strains in a setting with comprehensive and effective TB control practices. Combined with the uncommon transmission of *M. tuberculosis* by foreign-born individuals and the relatively small proportion of Beijing strains within the broad *M. tuberculosis* population structures of host countries,[8, 34, 71] there appears to be little cause for concern about the importation of Beijing strains into low TB incidence immigrant-receiving settings.

Table 5-1: Characteristics of Respiratory Cases by *M. tuberculosis* Genotype, 1991-2007

Characteristics	Beijing (N=258) n (%)	Non-Beijing (N=1121) n (%)	p-value
Sex			0.13
Female	100 (38.8)	492 (43.9)	
Male	158 (61.2)	629 (56.1)	
Age at Diagnosis			<0.001
≤34	72 (27.9)	319 (28.5)	
35-64	69 (26.7)	421 (37.6)	
≥65	117 (45.4)	381 (34.0)	
Population Group			<0.0001
Canadian-born	19 (7.4)	520 (46.4)	
Foreign-born Other	26 (10.1)	346 (30.9)	
Foreign-born Western Pacific	213 (82.6)	255 (22.7)	
Sputum Smear Microscopy*			0.15
Negative	132 (54.1)	517 (49.0)	
Positive	112 (45.9)	538 (51.0)	
Bacillary Load†			0.58
Low	38 (70.4)	177 (66.5)	
High	16 (29.6)	89 (33.5)	
Chest Radiography			0.19
No cavitation	206 (79.8)	853 (76.1)	
Cavitation	52 (20.2)	268 (23.9)	
Drug Resistance			<0.0001
Pan-susceptible	202 (78.3)	1000 (89.2)	
Monoresistance	30 (11.6)	90 (8.0)	
Polyresistance	20 (7.8)	25 (2.2)	
MDR-TB	6 (2.3)	6 (0.5)	

*Sputum smear microscopy was not completed on all cases.

†Semi-quantitative scores for acid-fast bacilli (AFB) load on the baseline sputum smear. Positive smears with semi-quantitative scores of 3+ or 4+ were categorized as having high bacillary load; all remaining positive smears were labeled as having a low bacillary load.

Table 5-2: Characteristics of Clustered and Non-clustered Cases by *M. tuberculosis* Genotype in Alberta, 1991-2007

Characteristics	Non-clustered (N=203) n (%)	Clustered (N=55) n (%)	OR (95% CI) Unadjusted	OR (95% CI) Adjusted*
Beijing Strains				
Sex				
Female	81 (81.0)	19 (9.0)	1.0	1.0
Male	122 (77.2)	36 (22.8)	1.3 (0.7, 2.3)	1.4 (0.7, 2.7)
Age at Diagnosis				
≤34 years	55 (76.4)	17 (23.6)	1.0	1.0
35-64 years	50 (72.5)	19 (27.5)	1.2 (0.6, 2.6)	1.3 (0.6, 2.9)
≥65 years	98 (83.8)	19 (16.2)	0.6 (0.3, 1.3)	0.7 (0.3, 1.4)
Population Group				
Canadian-born	12 (63.2)	7 (36.8)	1.0	1.0
Foreign-born Other	20 (76.9)	6 (23.1)	0.5 (0.1, 1.9)	0.5 (0.1, 2.0)
Foreign-born Western Pacific	171 (80.3)	42 (19.7)	0.4 (0.2, 1.1)	0.5 (0.2, 1.3)
Non-Beijing Strains				
Sex				
Female	324 (65.9)	168 (34.1)	1.0	1.0
Male	379 (60.3)	250 (39.7)	1.3 (1.0, 1.6)†	1.1 (0.8, 1.4)
Age at Diagnosis				
≤34 years	185 (58.0)	134 (42.0)	1.0	1.0
35-64 years	239 (56.8)	182 (43.2)	1.1 (0.8, 1.4)	0.8 (0.6, 1.1)
≥65 years	279 (73.2)	102 (26.8)	0.5 (0.4, 0.7)	0.4 (0.3, 0.6)
Population Group				
Canadian-born	204 (39.2)	316 (60.8)	1.0	1.0
Foreign-born Other	304 (87.9)	42 (12.1)	0. 1 (0.06, 0.13)	0.1 (0.06, 0.13)
Foreign-born Western Pacific	195 (76.5)	60 (23.5)	0.2 (0.1, 0.3)	0.2 (0.1, 0.3)

*Adjusted for sex, age at diagnosis and population group.

† p=0.055

Table 5-3: Comparison of Incidence Rates of Index Cases Attributed to Beijing/W and Non-Beijing/W Strains

Characteristic	Unadjusted Rates*			Adjusted Rates*†		
	Beijing	Non-Beijing	IRR (95% CI)	Beijing	Non-Beijing	IRR (95% CI)
Sex						
Female	0.3	1.4	0.2 (0.2, 0.3)	0.3	1.5	0.2 (0.2, 0.3)
Male	0.6	1.7	0.3 (0.3, 0.4)	0.6	1.9	0.3 (0.3, 0.4)
Age at Diagnosis						
≤34 years	0.2	0.7	0.3 (0.2, 0.4)	0.2	0.7	0.3 (0.2, 0.5)
35-64 years	0.3	1.6	0.2 (0.1, 0.3)	0.3	1.6	0.2 (0.1, 0.3)
≥65 years	2.0	5.9	0.3 (0.3, 0.4)	2.1	6.0	0.4 (0.3, 0.5)
Population Group						
Canadian-born	0.03	0.7	0.04 (0.02, 0.08)	0.03	0.8	0.4 (0.03, 0.06)
Foreign-born Other	0.4	4.7	0.1 (0.05, 0.1)	0.4	4.0	0.1 (0.1, 0.2)
Foreign-born WP	8.4	9.8	0.9 (0.7, 1.1)	7.8	8.9	0.9 (0.7, 1.1)

Abbreviations: IRR, incidence rate ratio

*****Rates are per 100,000 person-years

†Calculated using the direct method using the age- and sex-specific distribution of the total population of Alberta in 1993-2007 as the reference population.

Table 5-4: Incidence of Respiratory TB Index Cases Attributed to Beijing and Non-Beijing Strains in Alberta, 1993-2007

Characteristic	PYRs* 100,000s	Index Cases	Crude		Standardized‡	
			Rate† (95% CI)	IRR (95% CI)	Rate† (95% CI)	IRR (95% CI)
Beijing Strains						
Sex						
Female	210.5	68	0.3 (0.3, 0.4)	1	0.3 (0.3, 0.4)	1
Male	210.6	117	0.6 (0.5, 0.7)	1.7 (1.3, 2.4)	0.6 (0.5, 0.7)	1.7 (1.3, 2.3)
Age at Diagnosis						
≤34 years	215.2	49	0.2 (0.2, 0.3)	1	0.2 (0.1, 0.3)	1
35-64 years	163.8	52	0.3 (0.2, 0.4)	1.4 (0.9, 2.1)	0.3 (0.2, 0.4)	1.4 (0.8, 2.5)
≥65 years	42.0	84	2.0 (1.6, 2.5)	8.8 (6.1, 12.8)	2.1 (1.5, 2.7)	9.3 (4.6, 18.8)
Origin						
CB	353.0	11	0.03 (0.02, 0.06)	1	0.03 (0.01, 0.05)	1
FB Other	49.6	19	0.4 (0.2, 0.6)	12.3 (5.6, 28.6)	0.4 (0.3, 0.6)	13.7 (4.2, 45.3)
FB Western Pacific	18.5	155	8.4 (7.1, 9.8)	269.0 (146.2, 555.0)	7.8 (6.5, 9.1)	244.9 (213.8, 280.5)
Total	421.1	185	0.4 (0.4, 0.5)			

Abbreviations: PYRs, person-years; CI, confidence interval; IRR, incidence rate ratio; CB, Canadian-born; FB, foreign-born

* Customized census reports from Statistics Canada were used to obtain the population estimates in each census year; population estimates between census years were derived through linear interpolation whereas linear extrapolation was used to estimates the populations in 2007.

† Rates are per 100,000 person-years

‡ Calculated using the direct method using the age- and sex-specific distribution of the total population of Alberta in 1993-2007 as the reference population.

Table 5-4 (Con't): Incidence of Respiratory TB Index Cases Attributed to Beijing and Non-Beijing Strains in Alberta, 1993-2007

Characteristic	PYRs* 100,000s	Index Cases	Crude		Standardized‡	
			Rate† (95% CI)	IRR (95% CI)	Rate† (95% CI)	IRR (95% CI)
Non-Beijing Strains						
Sex						
Female	210.5	295	1.4 (1.3, 1.6)	1	1.5 (1.4, 1.7)	1
Male	210.6	368	1.7 (1.6, 1.9)	1.2 (1.1, 1.5)	1.9 (1.7, 2.0)	1.2 (1.1, 1.4)
Age at Diagnosis						
≤34 years	215.2	154	0.7 (0.6, 0.8)	1	0.7 (0.6, 0.9)	1
35-64 years	163.8	263	1.6 (1.4, 1.8)	2.2 (1.8, 2.8)	1.6 (1.3, 1.9)	2.2 (1.7, 3.0)
≥65 years	42.0	246	5.9 (5.1, 6.6)	8.2 (6.7, 10.1)	6.0 (5.0, 7.0)	8.3 (5.6, 12.5)
Origin						
CB	353.0	247	0.7 (0.6, 0.8)	1	0.8 (0.7, 0.8)	1
FB Other	49.6	235	4.7 (4.2, 5.4)	6.8 (5.6, 8.1)	4.3 (3.7, 4.9)	5.3 (3.9, 7.0)
FB Western Pacific	18.5	181	9.8 (8.4, 11.3)	14.0 (11.5, 17.0)	9.4 (7.9, 10.8)	11.5 (7.7, 17.4)
Total	421.1	663	1.6 (1.5, 1.7)			

Abbreviations: PYRs, person-years; CI, confidence interval; IRR, incidence rate ratio; CB, Canadian-born; FB, foreign-born

* Customized census reports from Statistics Canada were used to obtain the population estimates in each census year; population estimates between census years were derived through linear interpolation whereas linear extrapolation was used to estimates the populations in 2007.

† Rates are per 100,000 person-years

‡ Calculated using the direct method using the age- and sex-specific distribution of the total population of Alberta in 1993-2007 as the reference population.

Table 5-5: Risk Factors for the Recent Transmission of *M. tuberculosis* in Alberta, 1993-2007

Characteristics	Index Cases		Secondary Cases	Transmission Index*	Relative Transmission Index†	
	Non-Clustered	Clustered			Unadjusted	Adjusted
Sex						
Female	334	29	38	0.10	1.0	
Male	441	44	68	0.14	1.3 (0.9, 2.0)	
Age at Diagnosis (years)						
≤34	186	17	30	0.15	1.0	1.0
35-64	280	35	47	0.15	1.0 (0.6, 1.6)	0.6 (0.4,1.0)‡
≥65	309	21	29	0.09	0.6 (0.4, 1.0)§	0.4 (0.3, 0.8)
Population Group						
Canadian-born	212	46	77	0.30	1.0	1.0
Foreign-born Other	245	9	9	0.04	0.1 (0.1, 0.2)	0.1 (0.1, 0.2)
Foreign-born Western Pacific	318	18	20	0.06	0.2 (0.1, 0.3)	0.2 (0.1, 0.3)
Sputum Smear Microscopy‖						
Negative	366	25	36	0.09	1.0	1.0
Positive	359	46	68	0.17	1.8 (1.2, 2.7)	1.3 (0.9, 2.1)
Bacillary Load**						
Low	134	15	25	0.17	1.0	
High	56	15	23	0.32	1.9 (1.1, 3.4)	

Table 5-5 (Con't): Risk Factors for the Recent Transmission of *M. tuberculosis* in Alberta, 1993-2007

Characteristics	Index Cases		Secondary Cases	Transmission Index*	Relative Transmission Index†	
	Non-Clustered	Clustered			Unadjusted	Adjusted
Chest Radiography						
No cavitation	600	44	65	0.10	1.0	1.0
Cavitation	175	29	41	0.20	2.0 (1.3, 2.9)	1.4 (0.9, 2.2)
Drug Resistance						
Pan-susceptible	663	66	98	0.13	1.0	
Monoresistance	74	4	4	0.05	0.4 (0.1, 1.0)††	
Polyresistance	28	3	4	0.13	1.0 (0.4, 2.6)	
MDR-TB‡‡	10	0	0	0.00		
M. tuberculosis Genotype						
Non-Beijing	602	61	94	0.14	1.0	1.0
Beijing	173	12	12	0.06	0.5 (0.3, 0.8)	0.9 (0.4, 1.7)
Total	775	73	106	0.13		

* The number of secondary cases divided by the number of index cases

† Bivariate and multivariate Poisson regression using an offset of one each index case. Variables with $p<0.20$ in bivariate analysis were eligible for inclusion in the multivariate model. The multivariate model included the main effects of age, population group and *M. tuberculosis* strain. Sputum smear microscopy was retained in the model as it confounded the association between number of secondary cases and age. Chest radiography was also retained in the model as it confounded the association between number of secondary cases and *M. tuberculosis* genotype.

‡p=0.042

§ p=0.046

‖Sputum smear microscopy was not completed with all cases.

** Bacillary load was not included in multivariate modeling due to multicollinearity with sputum smear microscopy.

†† p=0.059

‡‡MDR-TB was excluded from bivariate and multivariate analyses.

Table 5-6: Risk Factors for the Recent Transmission of *M. tuberculosis* Among Index Cases Born in the Western Pacific, 1993-2007

Characteristics	Index Cases		Secondary Cases	Transmission Index*	Relative Transmission Index†
	Non-Clustered	Clustered			
Sex					
Female	123	9	10	0.08	1.0
Male	195	9	10	0.05	0.6 (0.3, 1.6)
Age at Diagnosis (years)					
≤34	84	5	5	0.06	1.0
35-64	110	4	4	0.04	0.6 (0.2, 2.3)
≥65	124	9	11	0.08	1.5 (0.5, 4.2)
Sputum Smear Microscopy‡					
Negative	175	7	7	0.04	1.0
Positive	126	9	11	0.08	2.1 (0.8, 5.5)
Bacillary Load§					
Low	53	3	5	0.09	1.0
High	17	1	1	0.06	0.6 (0.1, 5.3)
Chest Radiography					
No cavitation	253	14	16	0.06	1.0
Cavitation	65	4	4	0.06	1.0 (0.3, 2.9)

Table 5-6 (Con't): Risk Factors for the Recent Transmission of *M. tuberculosis* Among Index Cases Born in the Western Pacific, 1993-2007

Characteristics	Index Cases		Secondary Cases	Transmission Index*	Relative Transmission Index†
	Non-Clustered	Clustered			
Drug Resistance					
Pan-susceptible	240	13	14	0.05	1.0
Monoresistance	51	2	2	0.10	0.7 (0.2, 3.0)
Polyresistance	216	3	4	0.17	3.0 (1.0, 9.2)‖
MDR-TB**		0	0	0	
M. tuberculosis Genotype					
Non-Beijing	172	9	11	0.06	1.0
Beijing	146	9	9	0.06	1.0 (0.4, 2.3)
Time Since Arrival					
≤2 years	76	3	3	0.04	1.0
3 to 5 years	43	1	1	0.02	0.6 (0.1, 5.8)
6 to 10 years	54	6	6	0.10	2.6 (0.7, 10.5)
11 to 20 years	76	5	6	0.07	2.0 (0.5, 7.8)
>20 years	36	3	4	0.10	2.7 (0.6, 12.1)
Total	318	18	20	0.06	

* The number of secondary cases divided by the number of index cases

† Bivariate Poisson regression using an offset of one each index case. Multivariate analysis was not completed as all independent variables had p>0.20 in bivariate analysis

‡ Sputum smear microscopy was not completed with all cases.

§ Bacillary load was not included in multivariate modeling due to multicollinearity with sputum smear microscopy.

‖p=0.052

** MDR-TB was excluded from bivariate and multivariate analyses.

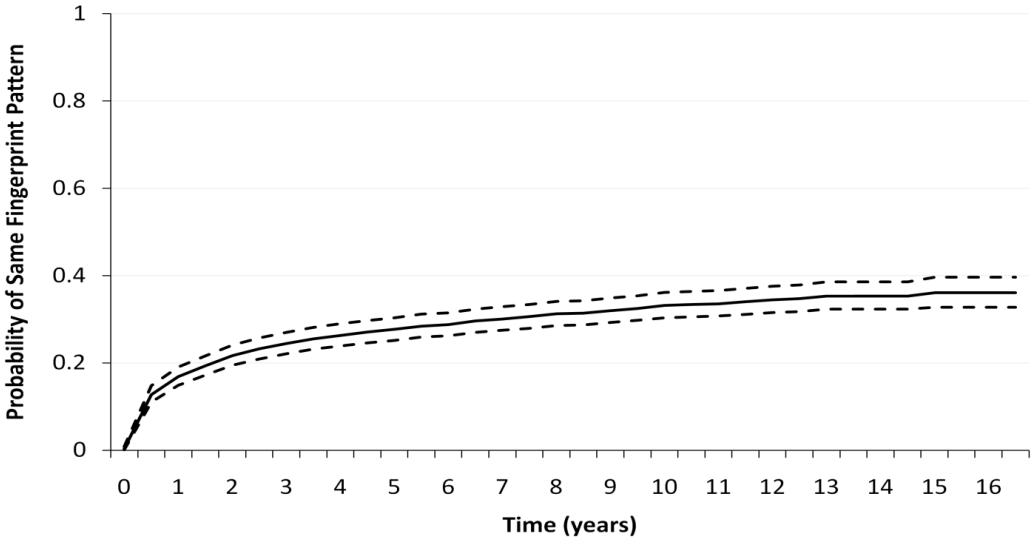
Table 5-7: Sensitivity Analysis Comparing the Use of a 2-year Versus 3-year Cut-Off Period to Define Recent Transmission

	Beijing Strains		Non-Beijing Strains	
	2 year*	**3 year†**	**2 year***	**3year†**
Index Cases (n)	185	157	663	510
Secondary Cases (n)	12	14	94	90
Incidence Rate of Index Cases	0.44	0.40	1.57	1.29
Transmission Index	0.06	0.09	0.14	0.18

* Index cases in 1993 through June 30, 2005 and their associated secondary cases were eligible for inclusion.

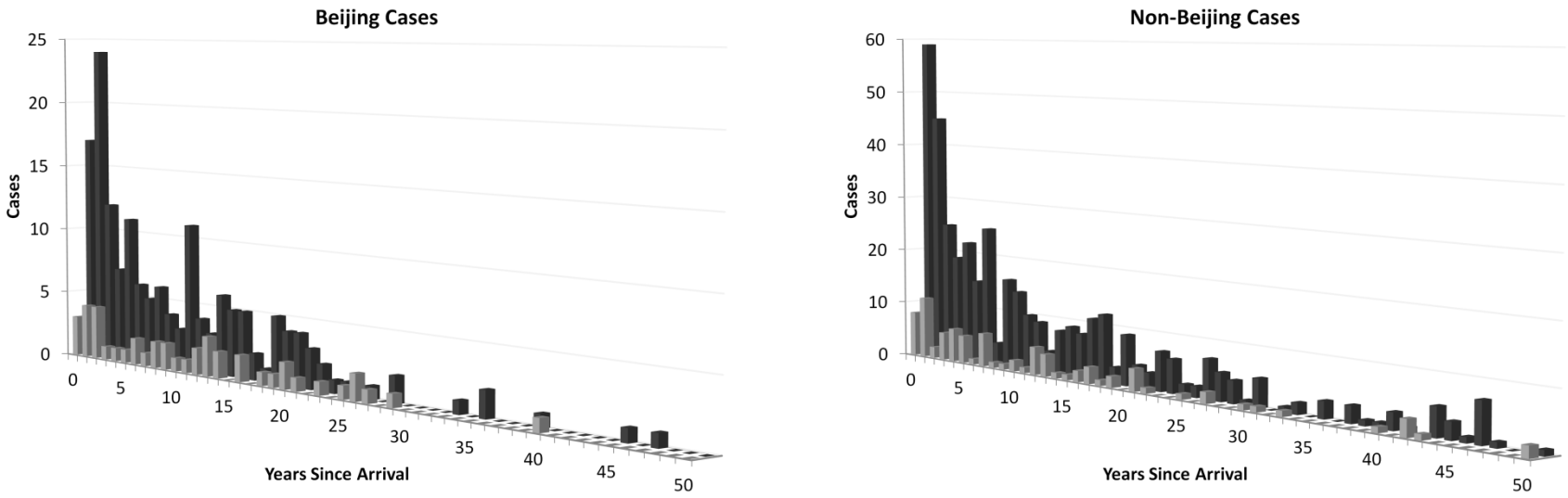
† Index cases in 1994 through June 30, 2004 and their associated secondary cases were eligible for inclusion.

Figure 5-1: Kaplan-Meier Estimates of the Probability of an Identical DNA Fingerprint Pattern Match in Respiratory TB Cases in Alberta, 1991-2007



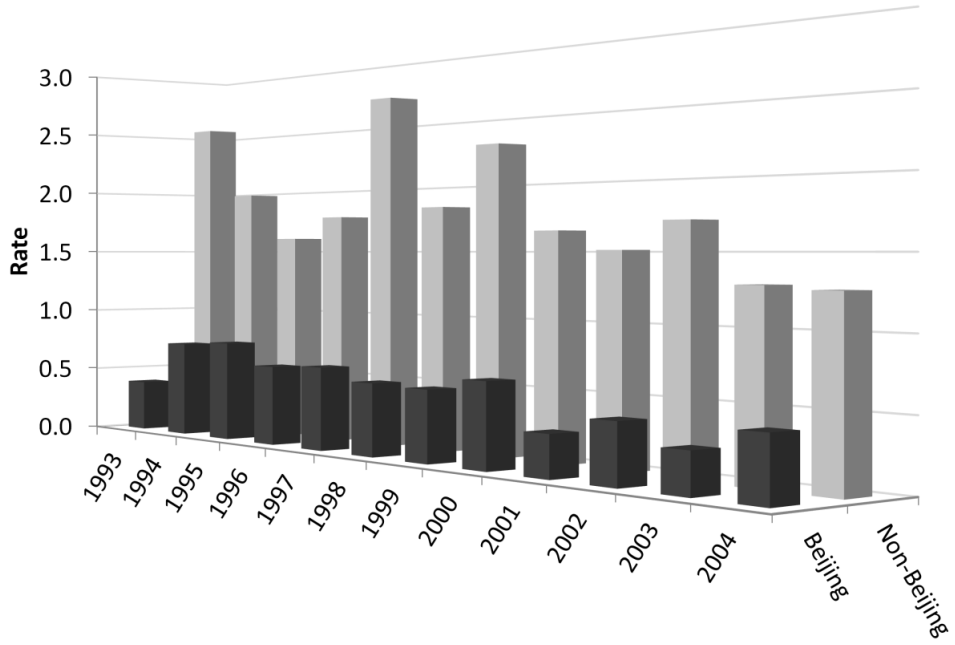
The estimated probabilities (solid line) and 95% confidence intervals (dashed lines) were derived through a Kaplan-Meier analysis of the time between the dates of diagnosis of cases with identical DNA fingerprint patterns as per IS*6110* RFLP analysis (as well as spoligotyping for isolates with ≤5 copies of IS*6110*). Cases with unique DNA fingerprint patterns (i.e. no other case had an identical DNA fingerprint pattern during the 1991-2007 period) were censored at the end of the study period (June 30, 2007).

Figure 5-2: Number of Foreign-Born Non-clustered and Clustered Cases According to *M. tuberculosis* Genotype and Time Since Arrival, 1991-2007



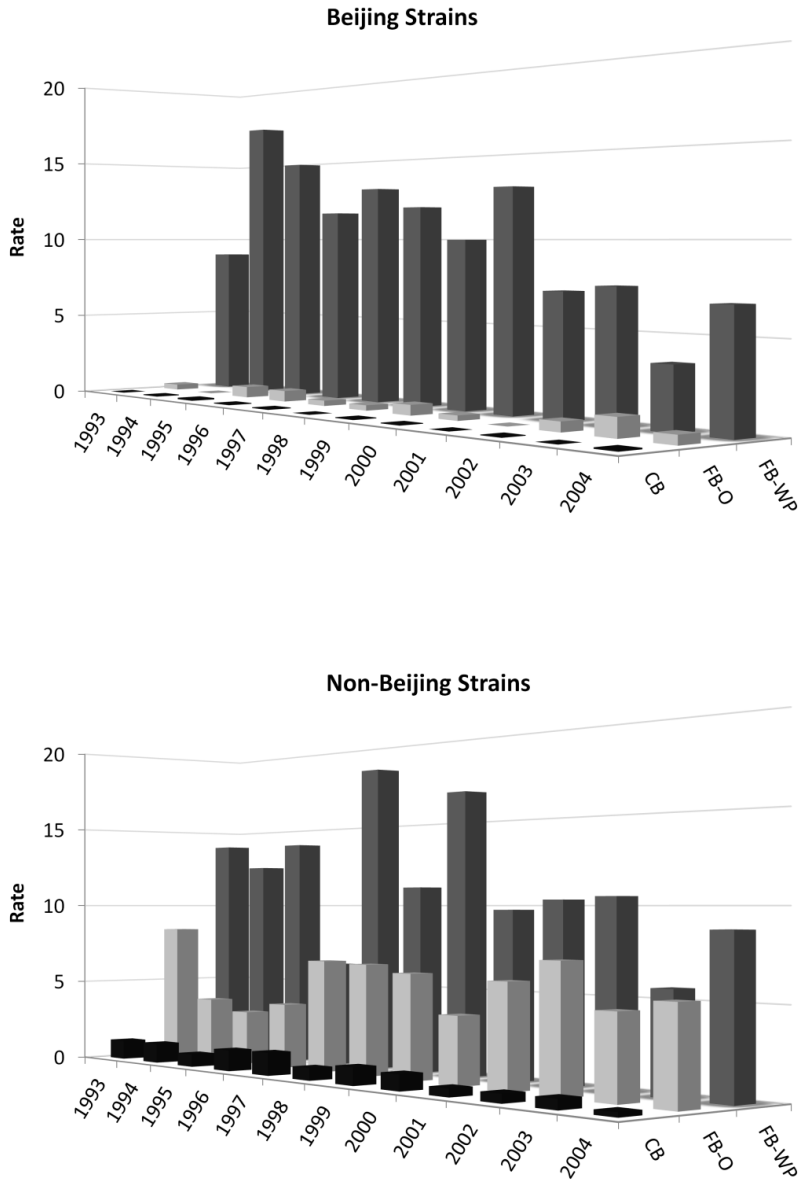
Clustered cases, gray; non-clustered cases, black.

Figure 5-3: Annual Incidence Rates of Beijing and Non-Beijing Index Cases in Alberta, 1993-2007



Incidence rates are per 100,000 person-years

Figure 5-4: Annual Incidence Rates of Beijing and Non-Beijing Index Cases by Population Group, 1993-2007



Rates are per 100,000 person-years.

Abbreviations: CB, Canadian-born; FB-WP, foreign-born individuals born in the Western Pacific region; and FB-O, foreign-born individuals born outside of the Western Pacific region

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CHAPTER 6: GENERAL DISCUSSION AND CONCLUSIONS

6.1 Summary of Research

6.1.1 Overview of Thesis Research Studies

The first of this thesis' two foci was aimed at furthering the knowledge base that is required to successfully prevent tuberculosis (TB) in the foreign-born, a necessary criterion for the overall advancement of TB elimination in immigrant-receiving countries. In this area of concentration, the profile of TB in the foreign-born in relation to general trends in TB incidence over a prolonged period in Canada was comprehensively examined. To do so, foreign-born TB incidence in Canada was methodically dissected so that the relative contributions and trends in TB incidence among immigrant groups were identified. Once the factors related to the relative constancy in foreign-born TB incidence were better understood, an immigrant group that comprises a high-yield target for the screening and treatment of latent TB infection (LTBI) was identified.

The project's second focus (within the second and third studies) originated from the repeated speculation that the Beijing family of strains is inherently more virulent and more transmissible than other *Mycobacterium tuberculosis* strain families. If these speculations are correct, and given the high probability of the importation of Beijing strains based on immigration patterns in Canada over the past five decades, TB resulting from Beijing strain infections could raise significant public health policy implications. The epidemiology and transmissibility of this family of strains was therefore studied in depth in the major immigrant-receiving province of Alberta, Canada.

6.1.2 Summary of Results

Tuberculosis disease (TB) among foreign-born individuals in Canada between 1986 and 2002 was comprehensively assessed in order to identify the relative contributions and trends in TB incidence among immigrant groups. The retrospective analysis of 18,275 foreign-born TB cases found an inverse association between TB incidence (case counts and rates) with increased time

since arrival regardless of immigrants' sex, age at arrival or country of birth group. For example, TB rates within the first 2 years since arrival were 2 to 3 times higher than those 3-5 years since arrival. This relationship resulted in immigrants who were within 5 years of their arrival contributing an almost equal number of TB cases annually as the much larger pool of immigrants who were more than 10 years since arrival despite a 3.5-fold difference in in-country person-years of observation. This latter finding was further explained by the remarkably stable and relatively low TB incidence that was observed among immigrants who were more than 10 years since arrival. In comparison, TB incidence within the first five years of arrival was highly dynamic and sensitive to changes in immigration level and, to a lesser extent, shifts in the source countries of new immigrants. Tuberculosis incidence was also associated with age at arrival and the incidence of TB in the immigrants' country of birth. Based on these findings, as well as recognition of the higher rates of serious adverse effects of standard treatment for latent TB infection (LTBI; 9 months of daily isoniazid) among those aged >64 years,[1-3] immigrants ≤2 years post-arrival who were aged 15-35 years at arrival and born within countries with TB incidence rates >50/100,000 population were recommended as ideal high-yield targets for the screening for LTBI.

To understand whether the importation and subsequent emergence of Beijing strains in high-income immigrant-receiving countries poses an increased public health threat, the second study compared the incidence and disease presentation of TB cases resulting from infections with Beijing strains to that of non-Beijing TB cases. Of the 1826 *M. tuberculosis* isolates in Alberta between 1991 and mid-2007 for which the lineage could be determined (99% of all culture-positive cases), 350 (19%) were Beijing strains and 298 (85%) of these were among individuals born in the Western Pacific region. Beijing strains had a particularly small presence in the Canadian-born population, accounting for 15 (4.4%) cases among Canadian-born non-Aboriginal individuals and 5 (1.7%) cases among Aboriginal peoples. An increased likelihood of respiratory TB among Beijing cases was of borderline significance. As well, Beijing strains were significantly more likely to be associated with polyresistant-TB cases and MDR-

TB compared to non-Beijing strains. Additional subgroup analysis found the associations between Beijing strains and polyresistant-TB to be significant among individuals born in the Western Pacific (compared to all others) and among those aged <35 years at diagnosis while an association with MDR-TB only occurred amongst those born in the Western Pacific. Apart from these findings, the disease presentations of Beijing and non-Beijing cases were similar in relation to infectiousness (sputum smear positivity, bacillary load, and lung cavitation) and monoresistance to first-line antituberculosis drugs.

The third and final study in this thesis used molecular epidemiological data to compare the transmissibility of Beijing and non-Beijing strains in Alberta between 1993 and mid-2007. Beijing strains were attributed with 197 of the 954 (21%) culture-positive respiratory TB cases included in this study of recent transmission. Although the overall likelihood for clustering was significantly lower for Beijing strains, a similar proportion of clustered cases were attributed to Beijing and non-Beijing strains within each population group (Canadian-born, foreign-born Western Pacific and foreign-born 'other'). The incidence rate of Beijing index cases was 70% less than that of non-Beijing index cases, both groups having a trend of declining annual rates of index cases over the study period. On average, an index case resulted in 0.13 secondary cases within 2 years. In adjusted analysis, the number of secondary cases was decreased if the index case was aged >64 years (versus ≤34 years) or born outside of Canada. The number of secondary cases was also not associated with Beijing strains, HIV co-infection or, among foreign-born cases, time since arrival.

6.2 Significance of Research

6.2.1 *Foreign-born Tuberculosis in an Immigrant-Receiving Country*

Routine screening and treatment for LTBI in foreign-born populations has previously been disregarded due to poor cost-effectiveness.[4] However, the identification of high-yield target groups would presumably be a way to improve the cost-effectiveness within any routine screening strategy for LTBI that is developed. This study identified the ideal high-yield targets for the routine

screening for LTBI among permanent residents ('immigrants') to be immigrants ≤2 years post-arrival who were aged 15-34 years at arrival and born within countries with World Health Organization (WHO) estimated rates of smear positive TB of >50 per 100,000 population. Although it has been recommended that immigrants within 5 years of arrival be screened,[5] the current study results suggest that screening efforts would be better utilized in the interval between the year of arrival and the second year following the year of arrival. In particular, TB rates in this interval were 2 to 3-fold higher than those in the 3-5 years since arrival interval regardless of immigrants' sex, age at arrival or country of birth group. The importance of earlier screening was further supported by the sensitivity analysis which explored the potential influence of prevalent active but unrecognized disease upon arrival on TB incidence rates in year 0-2.

Although immigrants aged >64 years at arrival maintained significantly higher rates of TB than the recommended target age group of 15-34 years, they are not appropriate targets for routine screening due to higher rates of serious adverse effects during standard treatment (daily isoniazid for 9 months).[1-3] Nevertheless, the high rate of TB among those aged >64 years at arrival and the subgroup analysis that found that TB rates progressively increased with each consecutive 5-year increase in age suggests an increased role for primary care providers in the screening for LTBI among older immigrants from high incidence countries. This would be especially relevant for older immigrants from high incidence countries who have other high risk factors for the development of active TB.[2]

This study also demonstrated that foreign-born TB incidence is influenced by shifts in immigration levels and, to a lesser extent, shifts in the source countries of new immigrants. With sustained sourcing from higher-incidence countries and projected increases in immigration for the foreseeable future,[6, 7] these findings suggest that foreign-born TB incidence will increase without a change in TB control programs to include targeted screening for LTBI. It also emphasizes the intensified need for the funding of efforts to enhance TB control programs in high incidence source countries.[8]

Additionally, the awareness that immigrants ≤5 years since arrival contributed almost the same number of TB cases annually as those who were >10 years since arrival will have important implications for the assessment of the program effectiveness of routine targeted screening. In particular, targeted screening can be expected to produce minimal reductions in national foreign-born incidence for several years.

6.2.2 The Epidemiological and Clinical Significance of Beijing Strains

This study revealed a marked presence of Beijing family of *M. tuberculosis* strains among culture-confirmed TB cases in the low incidence immigrant-receiving province of Alberta. By in large, TB cases resulting from infections with Beijing strains were limited to foreign-born individuals and, in particular, those born in the Western Pacific region. In addition to supporting the correlation between *M. tuberculosis* lineage and the host's country of origin/birth,[9-11] the minimal penetration of Beijing strains into the native-born population supports previous assertions that the foreign-born are not a significant source of TB in the native-born.[12-14]

The disease presentation of TB cases resulting from infections with Beijing strains was generally very similar to that of non-Beijing strains, especially in relation to sputum smear positivity, bacillary load, lung cavitation and immediately life-threatening forms of TB. That Beijing strains were associated with an increased likelihood of polyresistant-TB and MDR-TB emphasizes the importance of routine drug susceptibility testing and appropriate treatment management. In conjunction with the success of TB control in Alberta,[15] these findings imply that there is no reason to suspect that current strategies would be any less adept at controlling Beijing than non-Beijing strains.

The subgroup analysis conducted in this study found Beijing strains to be associated with an increased likelihood of MDR-TB among individuals born in the Western Pacific (compared to all others. Additionally, an association between Beijing strains and polyresistant-TB was of borderline significance among individuals born in the Western Pacific and of significance among those aged less

than 35 years at diagnosis. These findings suggest that the recommended targets for routine screening for LTBI pursuant to the study presented in Chapter 3 would also assist in mitigating the incidence of polyresistant and MDR-TB within the foreign-born population.

6.2.3 The Recent Transmission of Beijing and Non-Beijing Strains

This study confirmed that the foreign-born are not a significant source of *M. tuberculosis* transmission (including Beijing strains) despite having considerably higher index case rates than the native-born population. Rather, the vast majority of foreign-born TB cases were non-clustered and presumably resulted from the reactivation of infections acquired prior to immigration. This finding, combined with the inverse association between the number of non-clustered Beijing and non-Beijing cases rates and increased time since arrival, emphasizes the importance of the early screening for LTBI among foreign-born individuals.

Also of significance is that Beijing strains did not result in any more clustering than non-Beijing strains in a low incidence setting with effective TB control practices. As the transmission of *M. tuberculosis* occurs most frequently among cases with sputum smear positive disease and lung cavitation,[16, 17] the similarity in clustering is not unexpected given that Beijing cases were generally no more infectious than non-Beijing cases (Chapter 4 and Chapter 5).

In agreement with previous studies,[14, 17-21] the recent transmission of *M. tuberculosis* was unrelated to drug resistance and was lower for index cases that were foreign-born or older than 64 years. The current study then went on to expand on these previous findings by also determining that these common risk factors were independent of *M. tuberculosis* genotype (defined as Beijing or non-Beijing strains), HIV status and, among foreign-born individuals, time since arrival.

6.3 Strengths of the Research Studies

The methodological strength of the study investigating foreign-born TB incidence in Canada (Chapter 3) resulted from the analysis of data from national TB and immigration databases. Precise denominator data from the federal agency responsible for immigration negated the need to derive population estimates from census data or other population surveys. The analysis of national-level data from the Canadian Tuberculosis Reporting System also eliminated the impact of post-immigration (secondary) migration within Canada and other jurisdictional limitations encountered with province-specific studies.[19, 22-24] The acquisition of precise national-level numerator and denominator data over a prolonged study period enabled this investigation to be the most comprehensive report of foreign-born TB in Canada to date.

The two studies related to Beijing strains (Chapter 4 and Chapter 5) are the foremost studies of their kind in Canada as well as other low TB incidence immigrant-receiving countries. The analysis of a comprehensive provincial TB dataset derived through the amalgamation of TB Registry and mycobacteriology data was critical for the Beijing studies. In particular, the use of this dataset minimized selection and information bias. The assessment of genomic deletions (RD105) as a means of discriminating Beijing from non-Beijing strains significantly enhanced the accuracy of strain identification compared to the more common use of spoligotyping, mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) typing, or the comparison of IS*6110* restriction fragment length polymorphism (RFLP) patterns to international references strains. The accuracy of strain classification was further assured through secondary genotyping on a substantial convenience sample of isolates, several of which were genotyped at laboratories external to the Provincial Laboratory for Public Health. Another distinguishing feature of these studies was the inclusion of a measure of pathogen load (semi-quantitative scores for acid-fast bacilli (AFB) load on baseline sputum smears) as this presumably serves as a proxy for the infectiousness of the source.

As Alberta is one of four primary immigrant-receiving provinces in Canada, three of which have very similar immigration patterns (Alberta, Ontario and British Columbia), the results of the Beijing studies are anticipated to have national relevance. The generalizability of the study results to other low TB incidence immigrant-receiving countries will be influenced by the degree of similarity in immigration patterns.

6.4 Limitations and Implications for Future Research

Although novel contributions have been added by these studies to the field of foreign-born TB research, there are several limitations that could be addressed through future studies. First, the analysis of foreign-born tuberculosis in Canada was limited by the exclusion of foreign-born temporary residents. As TB incidence is highest within a relatively short period of time post-arrival among permanent residents, it would be beneficial to determine if similar rate patterns occur among temporary residents. It is anticipated that future studies in this area will identify additional high-yield targets for screening for LTBI. Similarly, another area for future study is the potential impact of migrant type (economic, family reunification, refugee, skilled worker, etc) on the distribution of TB in foreign-born populations as migrants may not equally reflect the TB incidence rates of their place of origin.

The availability of year of arrival only, without consistent identification of day and month, did not permit a more accurate evaluation of TB incidence rates within the first two years of arrival in any of the studies. A more precise assessment of TB incidence patterns within the first 2 year of arrival would be highly advantageous for TB control programs as it may enable the identification of a shorter period for targeted screening, a finding that could translate into logistical and cost benefit advantages.

In the studies related to the Beijing family of strains, the relatively small number of Beijing cases resulted in estimates of limited precision (for example, MDR-TB, HIV-TB co-infection, risk factors for transmission). Further

investigation of the associations between Beijing strains and these factors within larger datasets would be informative.

The transmission index used in the second Beijing study was more suitable for the quantification of recent transmission within an expansive study period than previously published indices.[12, 13, 25] Nonetheless, it shares many of the same limitations as other TB transmission indices as that it inadequately captures the complexities of transmission such as the mutation rates of the genetic markers, growth within the infectious populations, sampling proportions, and generation time.[26] There is a continued need to develop and validate a transmission index that can more accurately draw quantitative inferences from genotyped samples.[26]

Several additional issues are considered to be of importance for future research related to foreign-born tuberculosis. Population-specific variations in the association between Beijing strains and drug resistance (Chapter 4), as well as inconsistencies in the disease presentation of Beijing strains in the larger body of literature, may result from differences in the pathogenic characteristics of Beijing sublineages. It would therefore be beneficial for future population-based studies to examine the epidemiological and disease characteristics of cases in relation to Beijing sublineage. Another issue relates to the dynamic immigration policies of immigrant-receiving countries. In particular, it is important to determine if the recommended high-yield targets for screening for LTBI remain relevant within the context of continually shifting in immigration patterns and levels. Further, studies are needed to identify the potential reduction in TB incidence that could result from routine evidenced-based screening of these targets, including the indirect prevention of secondary cases. This information could then be used to inform cost-effectiveness analyses. Finally, a highly relevant and important area for future study is the identification of preventive therapy regimens that would support the routine screening of the highest-yield target group, namely immigrants aged >64 years at arrival who are within 2 years of arrival from a high incidence country of birth (>50 per 100,000 population).

6.5 Conclusions

Immigrant-receiving countries will continue to be challenged by TB in their foreign-born populations for the foreseeable future given the continued high global prevalence of TB and unprecedented levels of human migration. The identification of a high-yield target group suitable for the routine screening for LTBI provides a tangible starting point for the renewed consideration of evidence-based in-country screening strategies for the prevention of TB in the foreign-born. At the same time, it is reassuring that the importation and subsequent reactivation of Beijing strains generally pose similar challenges for TB control programs as other *M. tuberculosis* strain families. The one exception -- the increased occurrence of MDR-TB among individuals born in the Western Pacific and infected with Beijing strains -- serves as a stark reminder of the threats being posed by the global emergence of drug-resistant strains. Now is the opportune time to lessen this threat in immigrant-receiving countries through the provision of effective screening strategies in the foreign-born population.

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APPENDIX A: LOW TB INCIDENCE COUNTRIES WITH VERY HIGH HUMAN DEVELOPMENT

Low TB Incidence Countries (WHO estimated smear positive TB per 100,000 pop)*	
1) Monaco (1.3)	35) Belgium (7.1)
2) Iceland (1.9)	36) Czech Republic (7.2)
3) Barbados (2.2)	37) Luxembourg (7.2)
4) Bermuda (2.2)	38) Austria (7.3)
5) Cayman Islands (2.2)	39) Ireland (7.4)
6) Grenada (2.2)	40) British Virgin Islands (7.5)
7) Sweden (2.6)	41) Cuba (7.7)
8) Cyprus (2.8)	42) Dominica (7.8)
9) United States of America (2.9)	43) Costa Rica (7.8)
10) Canada (2.9)	44) France (8.1)
11) Australia (3.0)	45) Saint Lucia (8.2)
12) Jordan (3.1)	46) Cook Islands (8.3)
13) Antigua & Barbuda (3.3)	47) West Bank & Gaza Strip (8.3)
14) Norway (3.3)	48) United Arab Emirates (9.3)
15) Jamaica (3.4)	49) Turks & Caicos Islands (9.8)
16) Malta (3.6)	50) Lebanon (9.9)
17) Puerto Rico (3.6)	51) Greece (10.5)
18) San Marino (3.7)	52) Libyan Arab Jamahiriya (10.7)
19) Italy (4.3)	53) Albania (10.8)
20) New Zealand (4.3)	54) Mauritius (11.2)
21) American Samoa (4.4)	55) Samoa (11.2)
22) Israel (4.4)	56) Andorra (11.2)
23) Netherlands (4.4)	57) Slovenia (11.9)
24) Netherlands Antilles (4.4)	58) Syrian Arab Republic (12.2)
25) Montserrat (4.6)	59) Egypt (12.4)
26) Denmark (4.8)	60) Anguilla (12.5)
27) Finland (4.9)	61) Chile (12.5)
28) Switzerland (4.9)	62) Tunisia (12.5)
29) Trinidad & Tobago (5.0)	63) Tonga (12.8)
30) Saint Kitts & Nevis (5.3)	64) Uruguay (13.5)
31) United Kingdom (5.5)	65) Slovakia (13.9)
32) Germany (5.7)	66) Kuwait (14.0)
33) United States Virgin Islands (6.1)	67) Saint Vincent & the Grenadines (14.4)
34) Oman (6.6)	68) Iran (14.4)

APPENDIX A: LOW TB INCIDENCE COUNTRIES WITH VERY HIGH HUMAN DEVELOPMENT (Con't)

Very High Human Development (Index Score)[†]	
1) Luxembourg (0.958)	14) Andorra (0.934)
2) Norway (0.955)	15) Finland (0.934)
3) Liechtenstein (0.951)	16) Austria (0.933)
4) Canada (0.950)	17) Denmark (0.931)
5) Australia (0.946)	18) Spain (0.929)
6) Netherlands (0.946)	19) United Kingdom (0.929)
7) United States (0.945)	20) Ireland (0.929)
8) Sweden (0.944)	21) Germany (0.926)
9) Switzerland (0.943)	22) New Zealand (0.924)
10) Japan (0.942)	23) Italy (0.924)
11) Iceland (0.942)	24) Israel (0.905)
12) France (0.939)	25) Greece (0.903)
13) Belgium (0.937)	26) Brunei Darussalam (0.901)

Low TB Incidence Countries with Very High Human Development	
1) Andorra	12) Ireland
2) Australia	13) Israel
3) Austria	14) Italy
4) Belgium	15) Luxembourg
5) Canada	16) Netherlands
6) Denmark	17) New Zealand
7) Finland	18) Norway
8) France	19) Sweden
9) Germany	20) Switzerland
10) Greece	21) United Kingdom
11) Iceland	22) United States

***** Global TB database. Geneva: World Health Organization; 2010. (http://www.who.int/tb/country/global_tb_database/en/) (Accessed August 25, 2010).

[†] Human development reports. Getting and using data: Get data by country, indicator or table from the 2009 report. HDI trends and indicators (1980-2007). New York, NY: United Nations Development Programme; 2009. (http://hdr.undp.org/en/statistics/data/). (Accessed August 25, 2010).

APPENDIX B: ETHICS APPROVAL FOR THE STUDY OF BEIJING STRAINS IN ALBERTA

Re-Approval Form

Date:	January 12, 2011
Principal Investigator:	Richard Long
Renewal ID:	Pro00001161_REN3
Study Title:	Mycobacterium tuberculosis
Approval Expiry Date:	January 11, 2012
Sponsor/Funding Agency:	CIHR - Canadian Institutes for Health Research

The Health Research Ethics Board - Biomedical Panel has reviewed the renewal request and file for this project and found it to be acceptable within the limitations of human experimentation.

The re-approval for the study as presented is valid for one year. It may be extended following completion of the annual renewal request. Beginning 45 days prior to expiration, you will receive notices that the study is about to expire. Once the study has expired you will have to resubmit. Any proposed changes to the study must be submitted to the HREB for approval prior to implementation.

All study-related documents should be retained, so as to be available to the HREB on request. They should be kept for the duration of the project and for at least five years following study completion.

Sincerely,

S.K.M. Kimber, MD, FRCPC
Chair, Health Research Ethics Board - Biomedical Panel

Note: This correspondence includes an electronic signature (validation and approval via an online system).

APPENDIX C: PERMISSION TO REPRODUCE 'MAP MAKER' MAP

Re: Permission request

Sent: Thu 18/08/2011 1:35 AM

From: Map Maker [info@mapmaker.com]

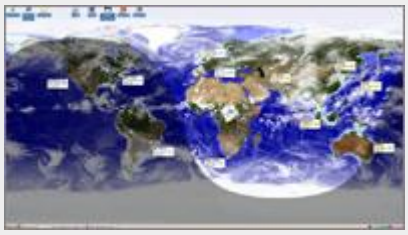
To: Deanne Langlois-Klassen

Deanne Langlois-Klassen,

You are very welcome to reproduce the map. If you do include a credited we'd prefer it if it read "the figure was created with Map Maker *Gratis* (www.mapmaker.com)."

Good luck with your thesis.

Eric Dudley (PhD!).



Map Maker Ltd, The Pier Carradale, Kintyre, Argyll, PA28 6SQ, United Kingdom
Telephone: [44] (0)1583 431 358, Fax: +44 (0)870 622 1428
web site: www.mapmaker.com

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