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# THE MOLECULAR EPIDEMIOLOGY OF TUBERCULOSIS IN MONTREAL AND BRITISH COLUMBIA

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**To all those who contribute to the fight against TB**

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# ABSTRACT

Though the incidence of tuberculosis (TB) in Canada is low, TB remains a problem in specific population subgroups-most notably the foreign-born, who account for a steadily increasing proportion of all cases. The foreign-born represented 80% of all patients diagnosed with active TB on the island of Montreal in 1997 and 75% of all cases in British Columbia.

The extent to which foreign-born persons transmit TB to the Canadian-born is not known. Using DNA Restriction Fragment Length Polymorphism (RFLP), this study sought to estimate the proportion of active cases of TB among the Canadian-born potentially attributable to foreign-born sources.

*M. tuberculosis* isolates from patients diagnosed with active TB in British Columbia and Montreal from July 1,1995 through June 30, 1997 were characterized by RFLP analysis, using the standard IS6110 technique. Patients whose isolates had identical DNA fingerprints were considered to be clustered (confirmed by spoligotyping for isolates with $\leq 5$ IS6110 copies), indicating recent infection and potential person-to-person transmission.

Of the 706 isolates analyzed, 54 clusters were identified composed of 147 patients. Foreign birth was found to be negatively associated with clustering. HIV positive status, intravenous drug use, and alcohol use were found to be associated with an increased incidence of clustering.

Of the 54 clusters found, 9 (17%) had both foreign- and Canadian-born members and in only 4 (7%) was there potential transmission from a foreign-born source case to a total of 5 Canadian-born persons. The estimated proportion of active TB among the Canadian-born potentially attributable to recent transmission from foreign-born cases was 0.8% in British Columbia, and 12% in Montreal.

# RÉSUMÉ

Malgré la faible incidence de la tuberculose (TB) au Canada, cette maladie demeure un problème important pour certains sous-groupes de la population canadienne--notamment les personnes nées à l'étranger. Ces dernières représentent une proportion croissante des cas actifs, soit 80% à Montréal et 75% à Vancouver, en 1997.

La fréquence de la transmission de la TB par des patients nés à l'étranger envers les Canadiens de naissance est inconnue. Avec le typage de l'ADN (technique RFLP), cette étude avait comme but l'évaluation de la proportion des cas actifs parmi les Canadiens de naissance qui seraient possiblement reliés à des contacts avec des tuberculeux nés à l'étranger.

Les souches de *Mycobacterium tuberculosis* provenant des patients ayant reçu un diagnostic de tuberculose active en Colombie Brittanique et à Montréal, entre le 1er juillet 1995 et le 30 juin 1997, furent analysées par la technique de typage IS6110. Les résultats furent confirmés par la technique de spoligotypage, pour les souches ayant $\leq$ 5 copies de la séquence IS6110. On considéra que les patients dont les souches démontraient des empreintes d'ADN identiques firent partie de "grappes" de souches ("clustered"). Ces grappes indiquèrent une infection et donc une transmission récentes.

Parmi 706 cas de TB active, 54 grappes furent identifiées (équivalent à 147 patients). Les patients nés à l'étranger firent moins souvent partie des grappes que les Canadiens de naissance. Tel que prévu, on nota une association entre l'appartenance aux grappes et l'infection au VIH, ainsi qu'avec l'utilisation de la drogue intraveineuse et de l'alcool.

Parmi ces 54 grappes de souches, 9 (17%) impliquèrent des patients nés à l'étranger et des Canadiens de naissance ensemble. Dans seulement 4, on constata une transmission possible provenant de patients index nés à l'étranger, envers des Canadiens de naissance (5 personnes, au total). La proportion de cas de la TB active chez les Canadiens de naissance qui seraient possiblement dus à des patients index nés à l'étranger fut donc basse--0.8% en Colombie Brittanique, et 12% à Montréal.

# Chapter 1: INTRODUCTION

Tuberculosis (TB), an airborne disease, is a major global health burden. Over 95 % of the estimated 8 million new active cases of TB, diagnosed each year, occur in the developing world. In TB Canada is mainly a disease of the foreign-born, the elderly, and the Aboriginal. The Canadian-born non-Aboriginal have a very low incidence of TB. Canada admits over 200,000 immigrants every year, the majority of who are from TB endemic areas. These foreign-born continue to have high incidence rates of TB after arrival; this could result in transmission of TB to a largely susceptible population, which has never been exposed. Thus TB among the foreign-born is a potential public health concern for other segments of the population. There is only limited data regarding this transmission. Studies in the United States (US) show little evidence of transmission from foreign-born individuals to the American born population while a study in the Netherlands produced contrary results. This necessitates investigating the impact of TB among the foreign-born on the Canadian-born.

Traditional methods for studying TB transmission involve tuberculin skin testing of contacts of patients with active TB. This is more suited for close contacts (usually household) who are easily identified and rates of transmission are high. However tuberculin skin testing is less helpful to detect transmission in the broader community, because rates of transmission are low and it is difficult to link tuberculin reactors to specific source cases. Transmission of TB between foreign-born and Canadian-born persons would most likely occur in a community setting, where exposure is less obvious. Restriction fragment length polymorphism (RFLP) identifies unique DNA "fingerprints" of the *M. tuberculosis*. This technique offers a more reliable method of studying TB transmission as it can tell whether two patients have the same strain of bacilli (which has been taken to mean person to person between these individuals). This study used RFLP analysis to investigate whether there was a spill over of TB from the foreign-born (who have a high incidence) to the Canadian-born individuals in British Columbia and Montreal.

# Chapter 2: BACKGROUND

## 2.1 General considerations

The World Health Organization (WHO) estimates that one-third of the world's population or 1.7 billion persons are currently infected with *M. tuberculosis* (Kochi, 1991). There are an estimated 8 million new cases of active TB every year and *M. tuberculosis* kills more people than any single infectious agent. In 1998, TB was responsible for 2.9 million deaths (WHO 1999): compared to 2.3 million from HIV/AIDS. Between 1993 and 1996 the number of cases of active TB worldwide increased by 13 % (WHO 1998) largely because of an increase in endemic areas (like Africa) in association with HIV infection.

In industrialized countries, the rates of tuberculosis declined steadily until the mid 1980s. After this time, the incidence plateaued and in some countries like the United States of America, the rates rose (Centers for Disease Control (CDC), 1993). This was thought to be due to HIV co-infection, deteriorating social conditions, withdrawal or reduction of funding for public health activities, and program failures (Raviglione et al., 1995; Cantwell et al., 1994; Rieder et al., 1994). An increasing level of social deprivation among subpopulations of the developed world, particularly the homeless, is contributing to the increasing incidence of disease (Davies, 1995). In addition, in tandem with immigration patterns, an increasing proportion of TB in developed countries occurs among the foreign-born who come from countries with high incidence of disease. The elderly non-immigrants, who have a high prevalence of TB infection because they grew up in the era when TB was still common in developed countries, are also manifesting a high incidence of disease. An increasing level of social deprivation among subpopulations of the developed world, particularly the homeless, contributed to an increasing incidence of disease (Davies, 1995).

## 2.2 Bacteriology

TB is a chronic infectious disease caused by *Mycobacterium tuberculosis (M. tuberculosis)*. Man is the only natural host for *M. tuberculosis*. The organism is quite hardy and can survive for long periods under adverse circumstances. Although it is classified, by laboratory criteria, as an obligate aerobe, *M. tuberculosis* can persist without multiplying with a very limited oxygen supply. Its nutritional requirements are few, as evidenced by growth on simple salt solutions with ammonia as a source of nitrogen and glucose as a carbon source. Its hardy nature may be partly responsible for the potential for recurrence after apparently successful treatment. *M. tuberculosis* poses special therapeutic challenges. It is a slow growing bacterium, reproducing every 17-18 hours in ideal conditions. Probably because of this slow growth, antituberculous therapy is a prolonged process, lasting 6 months or more. This has significant implications for patient adherence to treatment and hence for transmission in the context of treatment non-compliance.

## 2.3 Transmission

TB is a contagious disease. Like the common cold, it spreads through the air. In nearly all instances, TB infection is acquired by inhalation of a tubercle bacillus contained in an airborne particle (droplet nucleus) small enough to reach the alveoli. Transmission of *M. tuberculosis* is influenced by features of the source case, the potential recipient of the bacilli (the contact), and the environment they share. An additional important factor may be the infectivity of the organism.

### 2.3.1 The Source case

A patient shedding the bacteria in the lungs or upper airways spreads the bacilli. This happens when the patient talks, sneezes, sings or coughs. Coughing is the most effective mechanism for producing droplet nuclei, so patients who cough more frequently have a greater potential for transmitting the organism than those who do not cough or cough

infrequently (Loudon RG et al., 1969). Not all patients with TB are infectious. Contagiousness depends on communication between the active lesion and a bronchus. Such communication usually results from the rupture of a tuberculous pulmonary cavity into a bronchus: this enables the bacilli to be expectorated in the sputum.

The most important factor contributing to infectiousness is the number of organisms contained in the lungs of a source case, hence expelled into the surrounding air. This can be inferred from the extent and morphology of the lung lesions as shown by a chest x-ray. Cavitary lesions have about twice the bacterial load of solid nodular lesions (Canneti, 1965). This is best estimated by microscopic examination of properly stained sputum. Patients who have organisms in their sputum, detected by direct smear microscopy, are more contagious than those with negative smears but positive cultures. It should be noted, however, that even culture-negative patients can be contagious.

Anti-TB therapy strongly affects the infectiousness of a source case. Once suitable treatment has begun, and the organism is sensitive, then transmission of infection is reduced, because of a rapid fall in the bacterial load in the lungs. Studies have shown that within two weeks of multiple drug therapy, the bacterial load decreases by over 99 % (Hobby GL et al., 1973; Jindani A et al., 1980). In addition, therapy reduces coughing. Loudon and Spohn showed that coughing was reduced by 40 % after one week of treatment, and by 65 % after two weeks. Such a reduction in the cough reduces the number of bacilli released into the environment, and consequently reduces infectiousness.

### 2.3.2 Circumstances of exposure (contact)

Crowding and intimacy of contact are important determinants of transmission. People who are close together and are exposed for prolonged periods are more likely infected (Kuemmerer et al., 1967). US statistics show that rates of both clinical disease and tuberculin reactivity are much greater among close (generally household) than casual (generally non household) contacts (Centers for Disease Control, 1985). However, it has

become increasingly clear that brief, often unrecognized contacts can be sufficient to permit transmission-particularly if the source case is highly infectious.

### 2.3.3 Environmental factors

Environmental factors influence transmission of the bacilli. It has been shown that under standard conditions of temperature and humidity indoors, about 65 % of aerosolized *M. tuberculosis* survived for three hours, about 52 % for six hours, and approximately 30 % for nine hours (Loudon RG et al., 1969). Removal of these droplets by ventilation or filtration, or killing them by ultraviolet light reduces transmission. An investigation of an outbreak of TB conducted in a submarine (Houk et al., 1968) suggested that a lack of sunlight, poor ventilation with re-circulated air, contributed to the development of the micro epidemic.

### 2.3.4 Host factors

Both nonimmunologic and immunologic factors defend the potential host from acquiring new TB infection. Of these, the most potent is the specific immunologic defense mounted following the first acquisition of infection with the tubercle bacilli (Bates, 1982). The cell-mediated immune response to the first tuberculous infection generally checks the proliferation of the original organisms. This protection is incomplete, especially among immune-compromised individuals. In addition, animal studies have shown that external challenges from subsequently inhaled mycobacteria are met with substantially increased resistance that generally prevents new exogenous infection from occurring (Lurie, 1933). These findings have been corroborated by observations that an already infected person who is in contact with an infectious case is much less likely to develop active disease than is an uninfected contact. Though the cell mediated immunity develops after infection, and is specific for the infecting organisms, sensitization with heterologous mycobacterial antigens such as the bacillus of Calmette and Guérin (BCG) or

nontuberculous bacteria may increase the responsiveness to *M. tuberculosis* and thus may offer some protection against subsequent infection (Koch-Weser et al., 1985).

## 2.4 Pathogenesis

There are three main stages in the pathogenesis of TB:

### 2.4.1 Primary infection

A susceptible individual who inhales aerosolized droplets may become infected with the bacilli. Once the bacilli reach the alveolar surfaces, they are ingested by pulmonary alveolar macrophages. If these are unable to kill the bacilli, they multiply within the macrophages, which later rupture. Subsequent to the rupture, T-cell mediated macrophages and monocytes are attracted to this focus, the ensuing lesion is called the Ghon focus. This together with the enlarged regional lymph nodes constitutes the primary complex (Collins et al., 1997). The primary infection may often result in subclinical, or a mild, self-limited illness.

Bacilli may spread from the primary complex to more distant sites via lymphatics and blood vessels. Tuberculous meningitis may develop- generally about three months after infection. Infection of the lymph nodes, with matted lymphadenopathy is also a possible sequel. Widespread dissemination can occur leading to miliary tuberculosis with small granulomas, resembling millet seeds, throughout the body. Occasionally, the primary focus or enlarged lymph node may erode into a bronchus causing a spreading endobronchial infection or a grossly enlarged node may compress a bronchus, causing lobar or segmental collapse.

Primary infection may progress in two ways depending on the immune competence of the host; 1) it may become latent TB infection or, 2) it may result in progressive disease.

### 2.4.2 Latent TB infection (Dormant State)

In over 95 % of infected non-immunocompromised individuals, the primary complex heals. The monocytes and macrophages form granulomas within which bacillary multiplication is counterbalanced by their death. This may be due to inhibitory factors and unfavorable conditions (Edward, 1999). Mycobacteria, in this steady state, may persist in these granulomas for several years or even decades. Most infected persons will die with their bacilli in this state.

### 2.4.3 Active disease

TB disease develops by one of two pathogenic sequences: 1) direct progression from recently acquired infection to disease, and 2) reactivation of previously acquired, latent infection.

#### 2.4.3.1 Progressive Primary Disease

Primary infection may directly progress to active disease if the immune system fails to contain the disease. This occurs in about 5% of cases within the first year of infection (Styblo, 1985), in the remaining 95% the host defenses are sufficient, at least initially, to prevent development of disease.

#### 2.4.3.2 Reactivation (Post-primary) Disease

In 5 -10 % of those initially infected, disease occur from reactivation of dormant bacilli occurring 6 months to many years after infection (Styblo, 1985). This results in reactivation pulmonary or extrapulmonary TB disease. The usual site for re-activation is the apical region of the lung. Unlike primary disease, reactivation pulmonary TB is usually characterized by tissue necrosis and the formation of cavities.

Extra pulmonary sites of disease may include the bones, skin, kidney, gastro-intestinal tract, and other organs.

Active pulmonary TB is the contagious form of the disease. This is because the patient can shed the bacteria in form of airborne droplets into the environment. Extra-pulmonary TB such as genitourinary TB, TB of the bones or of the skin is usually not contagious.

2.4.4 Risk factors for developing disease

Not all persons who get infected develop the disease. Several factors have been identified which increase the risk of active disease.

With the advent of the HIV epidemic, rates of TB have dramatically increased in some places. The specific targeting of CD4 helper cells by HIV renders the host uniquely susceptible to TB. Primary progression from new infection to disease occurs more frequently and more rapidly in HIV infected persons (Selwyn PA et al., 1989; Edlin BR et al., 1992; Daley CL et al., 1992; Fischl et al., 1992). In addition, infection with HIV greatly increases the likelihood of reactivation of latent TB infection, thereby leading to clinical TB. In the HIV negative individual with tuberculous infection, the lifetime risk of developing active TB is 5-10 % while in the HIV positive individual it is at least 50 % (World Health Organization, 1999). On a global level, HIV is the most important cause for the increased incidence of TB in the last 10 years.

Diseases such as lymphomas that interfere with cell-mediated immunity and immunosuppressive drugs also increase the risk of tuberculosis. Diabetes mellitus, severe undernutrition, low body weight, and silicosis can also increase the risk of developing active disease.

In spite of the number of risk factors for disease, the majority of cases diagnosed have none of these abnormalities or characteristics. There are likely to be other yet unidentified factors that promote the progression of infection to disease or enhance reactivation.

## 2.5 Epidemiology

Early this century, TB was a major cause of morbidity and mortality in Canada. The mortality rate was 84 per 100,000 in 1924, and reported incidence reached a high of 119 per 100,000 in 1946 (Health Canada 1997). With improvements in living conditions, public health programs and the advent of antibiotic therapy, the rates of disease and resulting death have decreased sharply since the 1940s. The reported incidence and mortality rates in 1995 were 6.5 and 0.4 per 100,000 respectively. However the decades-long trend of declining incidence has leveled off since 1987.

Certain groups in Canada are at increased risk of the disease, including the Aboriginal (Status Indians, non-Status Indians, Metis, Inuit and Innu), foreign-born residents from countries with high incidence of TB, disadvantaged inner-city residents, and HIV-infected individuals. In 1995, the reported TB incidence rate was 1.9 per 100,000 for non-Aboriginal Canadian born individuals, whereas those for Status Indians, all Aboriginal peoples and foreign-born residents were 44.5 per 100,000, 29.4 per 100,000 and 20.4 per 100,000 respectively (Health Canada 1997)

The impact of HIV infection on the epidemiology of TB in Canada has not been well defined, although so far it does not appear to be significant. By the end of 1996, a total of 606 individuals reported to have the acquired immune-deficiency syndrome (AIDS) also had a diagnosis of TB, representing 4.2% of the total number of AIDS cases reported. However this figure most likely underestimates the extent of HIV/TB coinfection because unlike AIDS, HIV infection per se is not reportable in all provinces. In HIV infected persons, active TB frequently develops long before the onset of other opportunistic infections so that HIV may not be suspected. Although recommended, HIV testing is not consistently done in TB patients. This can lead to an underestimate of coinfection. Groups such as injection drug users are particularly at risk for HIV/TB coinfection.

Antituberculous drug resistance is emerging as a major problem in many areas of the world. In Canada, there are limited national data regarding the extent of the problem. A national study conducted in 1993-94 showed that 8.7 % of the TB isolates studied were resistant to at least one of the commonly used anti-tuberculous drugs, and 0.6 % were multidrug-resistant TB defined as resistance to isoniazid, and rifampicin with or without any other drug resistance. Given this low background frequency of resistance, shared resistance patterns in clustered patients may be confirmation of transmission of infection from one to the other(s).

### 2.5.1 TB in immigrants

Enarson et al showed that immigrants to Canada in the 1960s manifested rates of TB parallel to those in their country of origin (Enarson et al., 1979). In addition, more recent reports from other developed countries indicate that the rates of active cases of TB are higher in the foreign-born individuals compared to the rates in the indigenous people. In the United Kingdom, 1988 TB rates were 135/100,000 in Indian-born persons, 101/100,000 in Pakistani- and Bangladeshi-born compared with 4.7/100,000 in the population born in the United Kingdom (Ormerod et al., 1994). In addition, the proportion of cases of active disease that occurs in the immigrant population is increasing. In the US, 34.7 % of all new cases occurred in the foreign born in 1995 up from 20 % in 1986 (McKenna et al., 1995) and TB incidence is 13 times higher among the immigrant population than among the native born (Iralu et al., 1991).

In Canada the proportion of reported cases among the foreign-born has increased, between 1980 when 35 % of all reported cases were foreign-born, and 1995 when the proportion had increased to 58 %. Most of these were from countries with a high prevalence of disease. In Montreal, people born outside Canada accounted for 78.2 % of all cases reported between 1992 and 1996 (Regíe régionale de Montreál-Center 1998), this despite the fact that they make up only 23.5 % of the total population. In British Columbia, between 1982-1985, immigrants from Japan, Korea, the Philippines, India,

China and Hong Kong made up 3.7 % of the total population but contributed a quarter of all the cases of active TB (Wang et al., 1989).

Through immigration, individuals infected with TB in their childhood bring with them an increased risk for reactivation. It is theorized that the stress of immigration, personal or social disruption, and poor nutrition may enhance reactivation, especially within the first five years of immigration (Powell et al., 1981; Barr et al., 1994). Some individuals may become infected in congregate settings immediately before or after immigration e.g., refugee camps and shelters. It is now believed that recent infection, acquired during a visit to the country of origin, plays a major role in the incidence of disease among the foreign-born (McCathy O R, 1984).

## 2.6 Traditional methods of studying TB transmission

### 2.6.1 Follow-up of contacts

The most basic way of tracing the spread of TB is follow-up of contacts to see if they develop active disease. Even if disease develops fairly soon after contact with a TB patient, it is not possible to be completely certain whether infection was acquired from the suspected source.

### 2.6.2 Contact tracing and investigation

Another method of studying TB transmission has been tuberculin skin testing of contacts. Tuberculin sensitivity provides an indication of an individual's exposure to mycobacterial antigens in the past and as such is an indicator of infection. In fact, the tuberculin skin test is the only way of detecting the majority of TB infections. Purified protein derivative for *M. tuberculosis* is injected on the volar aspect of the forearm (Mantoux test) and the area of induration (the result of cell-mediated immunity) is measured after 48 to 72 hours.

Though tuberculin skin testing can tell whether someone is infected with TB, it cannot tell whether this is a new or an old infection. This may create a problem in interpretation of results. However if a conversion from a negative test to a positive test can be documented then recent infection is likely and it is possible to be more confident about the source of infection.

A major concern about the Mantoux test is the specificity and sensitivity of the test. There can be false negative tests among those infected. This may be a result of concurrent infections like HIV, other infections (bacterial and viral), live virus vaccination, immunosuppressive drugs and diseases, burns, recent surgery, and may also be seen in the very young or the elderly. In addition, loss of antigen potency, incorrect administration, and incorrect reading may lead to a false negative test. Even when the test is applied and read correctly, results of tests in patients with active TB and no apparent immunosuppression, at the time of initial diagnosis show that only 80 to 85 % have reactions of 10mm or more (Holden et al., 1971). Thus, a negative test cannot be used in isolation to exclude TB. Testing for anergy used to be recommended but was later found to yield inconsistent results (Chin et al., 1996) and is now neither recommended nor routinely done.

False positive tests on the other hand may be a result of infection with nontuberculous mycobacteria. Administration of BCG vaccine can also cause a reaction greater than 9mm. In general, BCG-induced reactions, though not necessarily smaller, wane more quickly than reactions caused by natural infections. The size of the reaction varies greatly depending on the strain of BCG used, its potency when given, the age of the recipient, and the means of administration. Because of these variations, BCG vaccination renders the interpretation of the tuberculin skin test more difficult.

The method of contact tracing and testing depends on the concentric circles approach, which compares the infection rate among the contacts to that expected. Contacts of the patient are identified and categorized as to how close they are to the case. They then

undergo tuberculin skin testing. Those closest to the case are investigated first. If their prevalence of infection is higher than expected, then the next level of contacts is similarly investigated. This is continued until the actual prevalence of infection is equal to the expected.

Several drawbacks to the method of contact tracing exist. There may be many contacts of a patient with active TB, especially if that patient has been very mobile. In this situation it becomes impossible to trace all the contacts especially the brief social contacts. Thus, not all the possible candidates are identified and tested. Usually only the very close contacts can be identified and subsequently tested. In addition the definition of "excess" rate of infection is imprecise because exact estimates of the expected prevalence of infection are not available for most populations.

### 2.6.3 Tuberculin surveys

Tuberculin surveys have been done at community level to investigate for TB infection. These are conducted without knowledge of source cases or contacts. This involves tuberculin skin testing of all individuals in a community where it is suspected that there is an increased prevalence of infection. The draw back to this method is that it is labour intensive and it does not identify active disease that is transmissible (which is the real concern).

## 2.7 RFLP typing of *M. tuberculosis*

The limitations of traditional methods of tracing transmission of TB infection have led to efforts to find techniques, which provide better identification of chains of transmission of *M. tuberculosis*. RFLP fingerprinting is one such method.

### 2.7.1 RFLP technique

RFLP typing is a technique, which enables distinct DNA "fingerprints" to be made, which reflect the genetic constitution of the organism being typed. The technique of RFLP typing depends on the existence of multiple copies of identical sections of DNA (insertion sequences) within the bacterial chromosomes. These insertion elements are present in variable numbers and location (resulting in a high degree of polymorphism) in different strains. A comparison of the number and location of these fragments forms the basis of this method. To accomplish this an enzyme (a restriction endonuclease), which recognizes a sequence of base pairs, unique to the insertion sequence, and cuts the DNA at that point. To detect such sequences a labeled segment of DNA (a probe) is used. The probe is complementary to (and hybridize with) part of the insertion sequence.

A standardized method for TB RFLP typing is now well accepted (van Embden et al., 1993). In this method, the insertion element IS6110 is used. This is a member of the IS3 family which is found in virtually all members of the *M. tuberculosis* complex and is restricted to this group of organisms (Cave MD et al., 1994; Otal et al., 1991). There are several other insertion elements but IS6110, a 1355 base pair segment of DNA is the one most commonly used. It is recognized and cleaved by the restriction endonuclease (*Pvu*II). The probe then recognizes a section of this insertion sequence.

RFLP typing requires obtaining a sample from a patient with active disease, culturing it to grow the *M. tuberculosis*, extracting the mycobacterial chromosomal DNA, digesting the DNA with *Pvu*II, and separating the resultant fragments by electrophoresis. The position of the fragments of DNA on the gel used for electrophoresis depends on their molecular weight. Different molecular weight fragments arise because of varying distances between insertion sequences and the numbers of insertion sequences. These fragments are denatured into single-stranded DNA while still in situ on the electrophoresis gel. The fragments of single-stranded DNA are blotted onto a nylon membrane and a labeled single-stranded probe DNA added. This probe hybridizes to specific sections of IS6110 allowing the

positions of the fragments to be determined. Thus a banding pattern is obtained which reflects the number of copies of IS6110 and their position within the chromosome. This pattern is unique to a particular strain and so isolates sharing the same pattern are considered to belong to an RFLP cluster which is interpreted to mean that transmission was from one member of the cluster to another , but could also be from a common source, unidentified and not part of a known cluster (Small PM et al., 1994). Belonging to a cluster on RFLP analysis has been taken to occur when the isolates have 5 or more identical IS6110 bands after electrophoresis. Isolates with less than 5 bands yield indeterminate results necessitating the use of secondary techniques.

The consistency of electrophoresis gels may vary both within and between gels so that fragments of DNA of the same molecular weight may travel different distances. Internal markers are used which have fragments of known molecular weight against which the movement of the mycobacterial isolates are standardized. This allows comparison of large numbers of isolates.

Computer software is used to analyze banding patterns when the number of isolates is large because visual comparison of the isolates is difficult. Patterns are standardized with reference to markers and similarity co-efficients between isolates are produced (a similarity co-efficient of 1.0 theoretically indicates identical patterns). Dendrograms can be constructed which graphically represent the similarity co-efficients between isolates, allowing them to be clustered into groups of similar patterns (Godfrey-Faussett et al., 1992). Visual inspection can then be used to decide which isolates among these groups are identical or differ by only one or two bands (Plikaytis et al., 1992; Scmid et al., 1990).

### 2.7.2 Diversity and conservation of RFLP patterns

Patterns of IS6110 have been found to be stable and to be unchanged during passage through guinea pigs (Hermans PW et al., 1990) and humans (Cave MD et al., 1994), even when drug resistance patterns have changed (Rastogi et al., 1990). Cave et al demonstrated

by using restriction endonuclease analysis that the DNA sequence of IS6110 is highly conserved among the multiple copies present in a single strain, between different strains, and between species within the *M. tuberculosis* complex. The DNA "fingerprint" of a particular strain of TB is conserved for at least two years (Cave et al., 1991; Otal et al., 1991). This allows the method to be used with a high degree of confidence.

If RFLP patterns were fully stable there would be no diversity. However occasional mutations causing transposition or replication of insertion sequences result in strains with different patterns. This mutational capacity is responsible for the creation of different DNA "fingerprints" over time, as new strains evolve. The natural rate of these transpositions is low (Thierry et al., 1990). The great diversity of fingerprints is likely to be a reflection of gradual genetic drift over centuries. If the insertion sequences were more readily transposed over short periods of time, then the usefulness of this technique would be diminished because, during the time of transmission, fingerprint patterns might substantially change.

Strains from various geographical regions have been typed by this method. Results demonstrate that in areas where the incidence of TB is low, there is a greater degree of polymorphism among the isolated strains. This occurs in most European countries (van Soolingen D et al., 1991; Torrea et al., 1995), where the majority of cases of TB result from the reactivation of lesions due to previously acquired infections. Conversely, in countries where the incidence of disease is high, many cases of TB are due to new transmission. This results in a lower degree of polymorphism among the isolated strains (Hermans et al., 1995; van Soolingen et al., 1999).

### 2.7.3 Interpretation of RFLP patterns

A number of issues of interpretation of RFLP patterns are not yet resolved. If isolates are found to have identical fingerprints containing many copies of IS6110, this has usually been thought to indicate transmission among the individuals concerned (Frieden et al., 1996). Likewise, markedly different patterns are accepted to indicate unrelated strains. However,

when two isolates have similar but not identical patterns, there are two possible interpretations: it is possible that the two isolates diverged before the respective patients became infected and that the similarity is therefore a manifestation of shared history without direct transmission. Conversely it may be that genetic change of the organism occurred during the process of transmission, and thus isolates with subtle differences such as a single band difference, could still represent direct transmission. In support of this possibility, subtle changes have occasionally been detected over time in strains isolated from the same chronically diseased people (Cave MD et al., 1994).

Consequently, the epidemiological interpretation of similar fingerprints remains controversial. Some investigators define transmission on the basis of identical fingerprints (Small PM et al., 1994; Alland D et al., 1994), while others accept limited changes such as single band additions, deletions, and shifts (Barnes Peter F et al. 1996; Friedman et al., 1996). Several reasons for "false positive" results with respect to RFLP patterns exist, e.g. laboratory cross-contamination (Small P et al. 1993), limited strain heterogeneity in closed populations (Braden C R et al. 1997), and remote rather than recent transmission.

### 2.7.4 Limitations of RFLP typing

In general most isolates carry 10 to 15 copies of the IS6110 fragment, and the numbers and locations of the IS elements vary between strains, resulting in a high degree of polymorphism (Thierry et al., 1990). However, there are some *M. tuberculosis* isolates that have few (Fomukong et al., 1994; van Soolingen D et al., 1991) or no copies (Yuen et al., 1993) of IS6110 in their genome. Previously conducted molecular and epidemiologic studies have concluded that a clonal relation cannot be inferred to exist between strains with only one copy of IS6110 (Hermans et al., 1991; Yuen et al., 1993). This compromises the method and necessitates the use of other techniques to distinguish between such strains (van Soolingen et al., 1993). A minimum number of 5 IS6110 bands has thus been required to determine that two or more isolates are clustered based on RFLP analysis alone. This is because of evidence that the number of false positive clusters (i.e. identical RFLP

patterns without any transmission likelihood between the patients) increases when the number of bands is less than 5 (CDC 1993).

RFLP typing can help describe an outbreak of TB but cannot determine how many people have developed latent infection. It is only feasible in those individuals with active TB; these are a small percentage of those infected. It is generally accepted that around 5 % of infected immunocompetent individuals develop active disease in two years following infection and a further 5 % develop disease in later life. Tuberculin testing remains the best way of tracking latent infection.

Because RFLP typing depends on culturing *M. tuberculosis* to provide enough DNA, it is a slow process. Standard culture techniques may take six weeks or longer. This is not important in epidemiological studies but it limits its use as a clinical tool and in emergency investigations of outbreaks. In addition the expense involved makes it a realistic tool for research or public health in developed countries only.

If only a limited number of isolates in a given community are typed some of the clusters may be missed. Some of the patients may appear to have unique isolates simply because the other patients with whom they have identical RFLP patterns have not been typed. So, for RFLP analysis to be more informative, most of the isolates from the TB patients in that community need to be typed and compared.

### 2.7.5 Supplemental method of analyzing mycobacterial isolates, Spoligotyping

In view of the limitations of RFLP analysis, spoligotyping has been developed as one of the supplemental methods used to confirm clustering in isolates with less than five IS6110 bands.

The method of spoligotyping is based on DNA polymorphism present at one particular chromosome locus, the "Direct Repeat" (DR) region, which is uniquely present in M.

tuberculosis complex bacteria. This locus was first described in *Mycobacterium bovis* where the DR region consisted of 36 base pairs, interspersed by non-repetitive DNA spacers, each 35 to 41 base pairs in length.

In contrast to the DR s, the spacers are usually present only once in the DR region, but occasionally some are found twice, either separated by one or several DR's and other spacers. One DR and neighboring non-repetitive spacer is termed "Direct Variant Repeat" (DVR). When the DR regions of several strains were compared, it was observed that the order of the spacers is about the same in all strains, but deletions, and/or insertions of spacers and DR's occur. The mechanism, by which spacers and copies of the DR are generated, is unknown.

The method of spoligotyping detects the presence or absence in the DR region of 43 spaces of known sequence, by hybridization of PCR-amplified spacer DNA to a set of immobilized oligonucleotides, representing each of the unique spacer DNA sequences. The method derives its name from spacer oligotyping.

The level of differentiation by spoligotyping is less compared to IS6110 fingerprinting for strains which have five or more IS6110 copies (ISOGEN Bioscience BV, Spoligotyping manuals), but higher for strains with less than 5 copies. Therefore this method has been reserved for use in isolates with less than five IS6110 copies i.e. used as a secondary analysis.

### 2.7.6 The application of RFLP analysis to TB epidemiology

RFLP typing of *M. tuberculosis* strains is important for epidemiological study because it allows patients with TB to be linked and outbreaks to be traced to their sources with greater precision. The discriminatory power (the ability to distinguish between unrelated strains) of the technique has been found to be satisfactory. The number of strain types, which can be

defined and the relative frequencies of these different strains (Hunter et al., 1988) determine the discriminatory power of this method.

In population studies the number of isolates which are involved in clusters, and the overall degree of similarity between strains has been used to infer the extent of, and risk factors for TB transmission. The RFLP method has been used by epidemiologists to investigate the relative importance of new infection vs. reactivation, and to estimate the risk factors for recently acquired infection in large populations. This is important for public health purposes because TB control strategies can be focused on the groups at highest risk of transmitting or being infected by TB.

The epidemiological utility of RFLP typing increases with increase in size of the area under study. In such settings, RFLP typing is the most suitable method of detecting strain similarity hence of studying transmission. Unfortunately, RFLP is only useful for studying TB disease and not infection.

An epidemiological study conducted over a long interval would best be conducted using RFLP typing. This is because with a long interval, patients recall of their contacts would be limited. The high degree of specificity of RFLP technique makes it superior to contact tracing and tuberculin skin testing, which has a high degree of false positive results. However for this method to be more useful in the study of TB transmission, most of the isolates in a community must be typed and compared in order not to miss identical strains.

### 2.7.7 The contribution of RFLP typing to study TB transmission

The use of RFLP analysis to identify the pathways of TB transmission within a community is based on the premise that epidemiologically unrelated cases will have occurred as a result of the reactivation of latent infection and thus have unique RFLP patterns, whereas cases that are linked as a consequence of recent infection will have the same patterns (appear in a defined cluster).

The combination of RFLP analysis and conventional epidemiologic investigation has improved understanding of the transmission of TB. Comparison of *M. tuberculosis* fingerprints from TB strains isolated during circumscribed outbreaks has demonstrated matching patterns among persons who were clearly infected from a common source (Hermans PW et al., 1990; van Soolingen D et al., 1991; Daley CL et al., 1992; Rastogi et al., 1992; Beck-Sague C et al., 1992; Edlin BR et al., 1992; Godfrey-Faussett et al., 1992; Pearson ML et al., 1992; Coronado V et al., 1993; Dwyer et al., 1993). These studies suggest that patients with the same *M. tuberculosis* RFLP pattern can constitute an epidemiologically linked cluster. Furthermore, because TB developed during a relatively short period in the patients who are clustered, clustering has been taken to indicate recent infection and rapid progression to clinical illness (Chevrel-Dellagi et al., 1993). Tracing identical strain types with RFLP typing has been used to demonstrate transmission of TB in hospital outbreaks (Beck-Sague C et al., 1992; Jereb et al., 1993), HIV care institutions (Kent et al., 1994; Daley CL et al., 1992), shelters for the homeless (Centers for Disease Control (CDC), 1991; Dwyer et al., 1993), prisons (Centers for Disease Control (CDC). 1992), and in entire communities (Alland D et al., 1994; Small PM et al., 1994).

Molecular fingerprinting has suggested that TB can be transmitted during brief contact between persons who do not live or work together (Dwyer et al., 1993; Genewein A et al., 1993; Tabet et al., 1994). Studies in the US have shown that clustered patients are more likely to be HIV-infected, born in the US, younger, Hispanic, and poorer than those with unique RFLP patterns are. However RFLP typing is biased towards detection of transmission among subgroups at high risk of rapid progression to active disease following infection. Hence studies conducted in populations with high proportions of HIV infected individuals show frequent clustering.

Frieden et al conducted a study (Frieden et al., 1993) on TB patients reported in New York City in April 1991 to describe their characteristics. They carried out RFLP analysis of 344 isolates and found foreign-birth to be associated with a decreased risk for clustering. The

main drawback to this study was the one-month period of the survey which could lead to an under estimation of recent transmission (i.e. within two years) because patients may be epidemiologically linked but not have positive cultures in the same month and it did not specifically address foreign-born to US born transmission.

The first study to investigate the impact of TB in the foreign-born on the local population was conducted in the Netherlands. It is also the largest community- based RFLP study conducted to date. Borgdorff and his colleagues analyzed isolates from 2435 patients diagnosed in the Netherlands between January 1993 through July 1995 (Borgdorff et al, 1998). This study specifically investigated the foreign-born as a source of TB infection for the local population (Dutch-born). In a novel method, they estimated the probability that a patient could be the source of infection in a given cluster. This was done by taking into consideration the incidence rate of sources in the subpopulation to which the person belonged (defined by age, sex, and nationality) and multiplying it by the probability that the source would lead to secondary cases (this probability was assumed to depend on nationality alone). They then estimated a transmission index and effective reproductive rate (the effective reproductive rate was associated with rapid progression, and, by implication, with recent transmission).

Using this approach, they estimated that 32% of the 623 Dutch cases were a result of recent transmission, and half of these or 17% (95% confidence interval, 9-25%) of the total were, attributable to recent transmission from non-Dutch patients. One of the drawbacks of this study is that nationality was based on an individuals' current status and not ethnic background. This means that some foreign-born individuals who had immigrated to Netherlands were considered of Dutch nationality. This could have led to a bias in the estimate of foreign-born to Dutch transmission, since some of the "Dutch" were infact foreign-born.

In the first study to specifically address the issue of TB in the foreign-born in the US, Daniel Chin and colleagues (Chin et al., 1998) conducted a community based RFLP study in San Francisco to specifically determine the factors contributing to TB incidence in the US-born and foreign-born. RFLP analysis of TB isolates from 367 patients was done and epidemiologic investigation conducted by chart reviews and patient interviews to determine the likelihood of transmission between clustered patients. 21 % of all the patients were clustered, and 60 % of the clustered were US-born. Of all the clustered cases only 39 % had 'definite' or 'possible' transmission linkages with another clustered case, as determined by epidemiologic investigation. These cases were more likely to be US-born. The clustered cases were further subdivided into 'source' and 'secondary' cases based on the likely duration of infectiousness. In addition, some of the cases were classified as 'initial' if epidemiologic and genotyping investigations revealed them not to have resulted from recent infection in San Francisco. All the foreign-born cases were initial cases, suggesting that disease in this group was a result of reactivation of latent infection acquired prior to their arrival in San Francisco. Only 2 out of 19 new cases of TB in the US born were a result of transmission from the foreign-born. The US-born cases produced more secondary cases. This was taken to suggest that either a greater percentage of US-born cases are transmitting the disease or the US-born population is more likely to acquire TB infection and rapidly develop disease, or both.

The authors noted that their study might have had several shortcomings. The fact that they limited analysis to a 2-year period meant that those who were infected during that time but did not develop active disease were not included in the analysis. In addition all culture negative cases would have been ignored. This study used many exclusion criteria (which were justified), resulting in the exclusion of 67.4% of all the available isolates. A major shortcoming of this work was uncertainty surrounding relatedness of most cases- no obvious link is not equivalent to definitely unrelated. This significant loss of information must have resulted in an underestimate of the ongoing transmission, and might have

resulted in the conclusion that the foreign-born were not responsible for disease in the US-born.

The high proportion of patients with a history of AIDS among the clustered patients in all the US studies make them not truly representative of the Canadian situation where TB is not as strongly associated with HIV infection.

## 2.8 Summary of background

Overall, there is a very low incidence of TB in Canada, however TB remains a significant problem among specific groups of the population, such as the foreign-born. TB could very easily be transmitted from the foreign-born, who have a very high incidence of disease, to the Canadian-born who have very low prevalence of infection and so are susceptible if exposed. Evidence of such transmission is contradictory. Traditional methods of studying TB transmission-contact tracing and tuberculin skin testing have been found to be inadequate. RFLP typing offers a refined method of studying this transmission, although it has to be coupled with traditional epidemiologic investigation for transmission to be determined.

# Chapter 3: STUDY HYPOTHESIS AND OBJECTIVES

## 3.1 Study hypothesis

Among adults, transmission of pulmonary TB infection from the foreign-born to the Canadian-born, with progression to active disease, is responsible for 20% of the disease in Canadian-born individuals in British Columbia and Montreal.

## 3.2 Principal Study Question

To estimate the proportion of TB disease among Canadian-born individuals that could be attributed to transmission of infection from foreign-born individuals with active pulmonary disease.

## 3.3 Secondary Study Questions

1) What are the risk factors for belonging to a RFLP defined cluster?
2) Are the risk factors for clustering identical for Montreal and British Columbia?
3) Do the identical risk factors have the same magnitude of influence in the respective cities?
4) Do the risk factors for clustering vary when the population of patients is defined differently (18-49 years only vs. all ages, major cities vs. entire province)?
5) Is there evidence of TB transmission between Vancouver, Montreal and Toronto?

# Chapter 4: METHODS

## 4.1 Context

This thesis project arises within the context of a collaborative study conducted in the province of British Columbia, island of Montreal and the city of Toronto to determine the extent of TB transmission using RFLP typing. This was the first analysis of the data. The author was responsible for obtaining the data, cleaning it, and performing the statistical analysis.

## 4.2 Overall Study Design

This was a population-based cross- sectional study, with patient accrual over a two-year period from July 1st 1995 to June 30th 1997.

## 4.3 Study population

### 4.3.1 Principal study population

The source population included all patients with TB, diagnosed between July 1st, 1995 and June 30th 1997 who were reported to the public health departments in the three study areas. These were the patients resident in the province of British Columbia, the Island of Montreal, and in the downtown core of Toronto (the original city of Toronto). Patients were considered eligible for the study if they; a) had culture-proven active TB (at least 1 positive culture) within the study period, b) their isolate could be RFLP typed in the central laboratory at the University of Edmonton, in Alberta.

All adult TB patients (above 18 years) from the study areas were considered the principal study cohort for this thesis. Younger patients were excluded because their infection is thought to arise mainly from household contacts. Foreign- to Canadian-born transmission would most likely occur in a community setting, and infection would be mostly from non-household contacts.

All adult patients were included in order to utilize all the available information on RFLP patterns, and hence maximize the sensitivity of this method (as discussed in Section 2.7.4). The inclusion of as many patients as possible in the study areas was expected to increase the ability to detect clustering because individuals might have visited or lived outside city limits prior to diagnosis-they may have infected others or themselves have been infected outside the city. In addition it would be expected that clustered cases would encompass all ages. Inference about transmission from the foreign- to the Canadian-born was based on this cohort.

No RFLP analysis had been done for patients younger than 18 years or older than 49 years diagnosed in the core of downtown Toronto during the study period. In addition, the different public health units in the city were merging to form one public health department. As a result of this restructuring, only 49 isolates of TB were sent for RFLP analysis. These were considered too few, and consequently the data from the city of Toronto was not included in the inferences about foreign- to Canadian-born transmission of infection or in the investigation of risk factors for clustering. It was only used in the investigation of inter-provincial clustering.

4.3.2 Impact of modification of the study population.

During the first 6 months of the study (second half of 1995) all patients on the island of Montreal had their mycobacterial DNA fingerprinted. However priority was later given to patients between 18 to 49 years old partly because of logistical reasons but mainly because the investigators wanted to target the age group at highest risk for HIV infection and TB clustering. This had been revealed by the Peter Small and David Alland studies in the United States (US). Consequently only 18 to 49 year old TB patients had their mycobacterial isolates analyzed in 1996 and 1997.

For the province of British Columbia, this had not been the case. All patients during this study period had undergone RFLP analysis, and their results were available. As mentioned in Section 4.4.1 above, Toronto had results of 49 patients aged between 18 and 49 years, and was excluded from any further analysis.

In order to answer some of the secondary study questions of this research, the principal study population was modified according to age and geographical location.

**Table 1.** Notation for the different types of TB patient cohorts considered in this study as a result of the modification of the inclusion criteria

| | British Columbia | | Island of Montreal |
|---|---|---|---|
| | Vancouver City | Other areas | |
| All patients | $V_1$ | $O_1$ | $M_1$ |
| 18-49 year olds | $V_2$ | $O_2$ | $M_2$ |

4.3.2.1 Effect of age restriction

In order to investigate the effect of age restriction, the patient populations in British Columbia and Montreal were divided into two groups a) patients of all ages, and b) patients aged between 18 and 49 years only. This resulted in two patient cohorts for these study areas.

This was considered necessary because, as already mentioned, studies conducted in the US had revealed an increased incidence of clustering among younger patients who had an increased incidence of risk factors associated with TB transmission e.g. HIV infection. Based on these findings, this study decided to investigate the effect of restricting the study population to patients aged between 18 to 49 years only. This was in order to determine the effect on patient characteristics and on the risk factors for clustering.

From January 1st 1996 to June 31st 1997, logistical considerations had allowed only patients aged 18 to 49 to undergo RFLP analysis in Montreal, so information on RFLP analysis was not available for all the eligible patients. Consequently this restriction could only be investigated in British Columbia.

#### 4.3.2.2 Effect of place of residence at the time of diagnosis of TB

An attempt was made to study the effect of place of residence, at the time of diagnosis, on patient characteristics and on the risk factors for cluster formation. This was in order to investigate the differences between transmission of TB in rural and urban settings. It is believed that an increased incidence of HIV/TB coinfection in urban settings would result in an increased transmission of TB infection and rapid progression to active disease. This would be expected to be associated with more cluster formation.

Differences between major urban centers and other areas of the provinces could only be studied among TB patients from British Columbia, for whom there was RFLP information for those from Vancouver (the major urban setting in British Columbia) and on patients from other areas in British Columbia. The TB patients from British Columbia were thus divided into 2 groups; 1) Patients who were resident in Vancouver at the time of diagnosis of disease, and 2) Those resident in other areas of the province at the time of diagnosis

## 4.4 Patient recruitment and routine data collection

TB is a mandatory reportable disease in Canada. Each time a case of TB is diagnosed, it is reported to the public health unit of that province. Once a case is reported to the public health unit, a public health nurse opens a file and gathers information on demographics and medical history. In addition the nurse gathers information on level of contagiousness (smear status, cough, duration of cough, HIV serostatus, and TB risk factors) and identifies close contacts for screening purposes. The nurse attempts to ensure that the

patient is adequately treated (number of drugs, dosage, follow-up) and will only close the file when the entire treatment is finalized. All this information (except HIV status) is entered in a computer databank.

In each city, a member of the study team coordinated data collection with the public health departments. All the TB cases diagnosed within the study period and reported to the public health departments, were included in the study if they met the eligibility criteria. The public health staff was responsible for contact tracing and for keeping a register of all the cases. They collected routine demographic data e.g. age, sex, race or ethnicity, country of birth, and address at the time of diagnosis. Specific information about TB included the date of diagnosis, sites of disease, and results of microbiologic studies.

All necessary data, for this thesis, were obtained in a non-nominal form from the public health TB database, and so there was no contact with the patients. A study identification number generated solely for this project by the provincial laboratory, and necessary to link the laboratory and the epidemiological data, identified the specimens submitted for DNA fingerprinting. Since the public health staff retained all the unique patient identifiers, anonymity of the patient was guaranteed so informed consent was not necessary. A data extraction questionnaire was developed for the purpose of obtaining information from the public health database (Appendix 1).

Ethical approval for this study was obtained from the respective ethics committees in the 3 cities.

## 4.5 Laboratory data

### 4.5.1 Diagnosis of TB and preliminary laboratory work

The preliminary laboratory confirmation of active TB disease was the responsibility of the TB treatment centers and the respective public health laboratories. All preliminary positive cultures were confirmed at the provincial reference laboratory by Lowenstein-Jensen cultures (the gold standard). The provincial labs were also responsible for determining antibiotic susceptibility.

### 4.5.2 RFLP analysis

Dr. Dennis Kunimoto conducted DNA fingerprinting for all study patients at the University of Alberta, in Edmonton. All patients recruited into the study had one of their initial isolates sent for RFLP analysis. This was carried out using the standardized IS6110 technique (van Embden, et al., 1993). In order to produce the fingerprint, the M. tuberculosis DNA was cut using a restriction endonuclease, *Pvu*11 that consistently recognized and cut at a specific sequence of base pairs, the restriction site. The resulting fragments were then separated according to size by electrophoresis. The separated fragments were transferred onto a membrane (Southern blotting) for hybridization with a probe that recognized the DNA sequence of IS6110.

The DNA was extracted by vortexing in the presence of siliconized glass beads followed by successive phenol/chloroform extractions and ethanol. The DNA was then digested with 10 units of *Pvu*II and electrophoresed on a 0.8% agarose gel. Included in every lane was a 1 Kb DNA ladder (Gibco BRL, Gaithersburg, MD). A control strain was included in every lane of every gel to serve as a standard and ensure uniformity between the gels. The gel was transferred to nylon, cross-linked with UV light and probed with an IS6110 probe. Bands were visualized using a chemiluminescent method (Boehringer Mannheim) and autoradiography. The blot was stripped and re-probed with a labeled 1 Kb DNA ladder and similarly visualized. Images were digitized by a video camera system called

IMAGER (Appligene, Illkirch, France). The 2 images (IS6110 and 1 Kb ladder) were superimposed on each other using Gelcompare software (Applied Maths, Kortrijk, Belgium), to allow precise molecular weight determinations and inter-gel comparisons. Bands for each isolate were manually confirmed. Patterns of bands were compared by the Gelcompare software using the unweighted pair-group method of arithmetic averages (UPGMA). All isolates matched as identical or similar by computer were then visually confirmed.

4.5.3 Spoligotyping

All isolates with less than five IS6110 copies, found to be identical on RFLP analysis, underwent spoligotyping to confirm or disprove their similarity. By spoligotyping one can detect the presence or absence of spacers of known sequence.

The first step in the method is to amplify the DR region of a given strain by PCR. The primers used are based on the sequence of the DR, and allow the amplification of the spacer(s) between the DR targets. The obtained PCR products differ in length because of two reasons. First, the product contains several spacers and the DR's in between the primers anneal to the DR's not next to each other. Second, the product itself can act as a primer, and become elongated with one or more DVRs. Therefore, the PCR product providers no reliable information about the spacer order or total length of the DR region. A biotin-labeled reverse primer is used, so that all the reverse strands synthesized are biotin labeled. Oligonucleotides derived from the known spacers in the DR cluster are covalently linked to an activated membrane in parallel lines. PCR products are hybridized perpendicular to the oligo lines. After hybridization, the membrane is incubated in streptavidine peroxidase, which binds to the biotin label on the PCR products. Detection of hybridization signals is optimized by the enhanced chemiluminescence (ECL) detection system. The peroxidase present on the streptavidine catalyzes a reaction resulting in the emission of light, which can be detected by autoradiography of the membrane. This blot is referred to as reversed line blot. The presence of spacers was

visualized on film as black squares after incubation with streptavidine peroxidase and ECL detection. The pattern of spacers of the different strains on the film is compared for similarity.

## 4.6 Statistical analysis

Data was entered and analyzed with SAS (SAS Inc., Cary, North Carolina) and EpiInfo (version 5, Centers for Disease Control).

### 4.6.1 The outcome variable

The primary outcome variable for the logistic regression was membership in an RFLP-defined cluster and this was dichotomous i.e. clustered vs. non-clustered. A cluster was defined as two or more patients whose TB isolates had identical banding patterns on RFLP analysis, and more than five IS6110 bands (copy numbers), or with less than five IS6110 bands and identical spoligotype patterns. Isolates with unique fingerprints were deemed non-clustered.

Mixed clusters, which were the clusters used to infer transmission between the foreign- and the Canadian-born, were those that had both foreign- and Canadian-born members.

Inter-provincial clustering was considered as one of the secondary outcomes in this study. This was defined as two or more patients from cities of different provinces belonging to the same cluster.

### 4.6.2 Independent Variables

The primary independent variable was country of birth, dichotomized into Canadian- or foreign-born. Covariates considered included sex, age, HIV serologic status, Aboriginal status, history of contact with a TB patient, pulmonary site of disease, smear positive pulmonary disease, anti-tuberculous drug resistance, use of alcohol, intravenous drug use,

occupation as a health care worker, and underlying medical illnesses (diabetes mellitus, malignancy, renal disease, gastrointestinal surgery, renal transplant, silicosis, and malnutrition).

### 4.6.3 Univariate analysis

Univariate analyses were conducted to estimate the association of the respective independent variables with clustering. To test if the differences in means of continuous variable were statistically significant, Student's t-test was used. Chi-square tests were carried out for categorical variables. Fisher's exact tests were used when the cell values were less than five (Breslow NE, 1987). Variables significantly associated with clustering ($P<0.05$) were subsequently included in the logistic regression analysis.

### 4.6.4 Bivariate analysis

Bivariate analysis was carried out to determine the relationship between the independent variables in order to determine if any factors were significantly correlated. Pearson correlation coefficients were calculated for continuous variables like age and duration of stay in Canada (for the foreign-born) while Spearman's correlation coefficients were calculated for the categorical variables like gender, and site of disease. A correlation matrix was generated.

### 4.6.5 Stratified analysis

In order to investigate for confounding and effect modification, stratified analysis was carried out. This was done to find out if the other covariates were confounding the relationship between country of birth (foreign- vs. Canadian-born) and cluster membership. The risk for cluster membership, after stratifying for each of the independent variables in turn, was determined for the foreign-born as compared to the Canadian-born. The respective odds ratios for clustering, and their 95% confidence intervals were compared between the two strata of the independent variable under

consideration. This was supplemented by examining the 2 by 2 tables after stratifying for these variables to determine if there were any obvious differences.

The possibility of effect modification was evaluated after stratifying for all the independent variables in the study. Breslow-Day test of homogeneity was performed to check whether the odds ratios were constant across the different strata. Breslow-Day probabilities were then calculated to check for interaction (Breslow NE, Day, NE, 1987). A probability of less than or equal to 0.05 would indicate that the probabilities across the strata were not homogenous and that the particular variable was an effect modifier of the relationship between country of birth and clustering. This was confirmed by examining the respective 2 by 2 tables.

### 4.6.6 Multivariate Analysis

Multivariate analysis was performed to estimate the effect of each independent variable on the risk of clustering, after controlling for all the other variables, and to select a model that would best predict cluster membership. Since the outcome variable was categorical, logistic regression was used.

Country of birth was included in the multivariate analysis, as a dichotomous variable (foreign- vs. Canadian-born), and as and as a 3-level variable with Canadian-born non-Aboriginal as the referent group compared to Canadian-born Aboriginal, and foreign-born. The first strategy was to determine if foreign-birth, per se, was a predictor for clustering while the second strategy was to determine if Aboriginal status was a significant predictor for clustering. All other variables were considered as dichotomous except age, which was evaluated as a continuous variable.

### 4.6.7 Modeling Process

The goal of the modeling was to find a parsimonious model that would best explain the data. This is because the fewer the number of variables in the model, the more likely the resultant model is to be numerically stable, and more easily generalized.

Variables that were a priori expected to be associated with clustering were included in the logistic regression. Age and gender were also entered in the model, as is standard practice.

Stepwise logistic regression was used for model selection (SAS® User's Guide: Statistics. Version 5 Edition, 1989). This is a particularly useful and effective data analysis tool when the outcome being studied is relatively new, as is the case with TB clustering in Canada. The relative importance of the various covariates is not well known and the associations with the outcome not well understood.

## 4.7 Assessing transmission between the foreign- and Canadian-born

The primary study question was to estimate the extent of transmission of infection between foreign- and Canadian-born TB patients. This was estimated from mixed clusters. Clusters with only Canadian-born patients or only foreign-born patients were not used.

In order to estimate the extent of transmission of TB infection from foreign- to Canadian-born individuals, the mixed clusters were scrutinized for several criteria, which could suggest such transmission. Then based on these criteria, each mixed cluster was rated as having possible transmission between foreign- and Canadian-born, or unlikely to have any such transmission.

### 4.7.1 Evidence for/against transmission between foreign- and Canadian-born individuals

As already mentioned, RFLP defined clustering has been considered an indication of recent transmission of TB (Peter Small, et al. 1994). In order to identify transmission of infection between the foreign-born and the Canadian-born the following factors were considered:

(a) Site of disease.

Pulmonary disease leads to exhalation of aerosol droplets, which contain *M. tuberculosis* organisms. Extra-pulmonary TB is not considered contagious.

(b) Date of diagnosis.

For patients belonging to the same mixed cluster, it was assumed that the first case diagnosed with TB was the one most likely to have transmitted to the other(s).

(c) Duration of interval between times of diagnosis.

The time interval between the dates of diagnoses of disease in the Canadian- and the foreign-born was another consideration. If the interval was 6 or more months, there was a reasonable possibility that transmission occurred from the first case diagnosed (the source case), to the case diagnosed later (the secondary case). On the other hand, if the time interval between the diagnosis of 2 cases was less than 6 months the direction of transmission was more difficult to assess.

(d) Year of immigration to Canada

The time of arrival of the foreign-born TB patient would be used to determine if it were possible for the foreign-born to have transmitted the disease to the Canadian-born. If the foreign-born individual immigrated to Canada after the diagnosis of TB in the Canadian-born individual, then the foreign-born could not have possibly infected the Canadian born.

### 4.7.2 Degree of certainty about transmission

In order to make inferences on the likelihood of transmission between the foreign- and the Canadian-born TB patients, the mixed clusters were categorized into 2 groups based on the criteria described 4.8.1:

a) Probable transmission from foreign-born to Canadian-born

At least two of the following criteria had to be present

1. Foreign-born patient had TB diagnosed at least 6 months before the Canadian-born
2. Foreign-born individual entered Canada before the diagnosis of TB in Canadian-born individual
3. TB in the foreign-born was contagious (pulmonary TB)

b) Unlikely to have transmission from the foreign- to the Canadian-born

Could have any of the above criteria, but the absence of the others would not be supportive of possible transmission from the foreign-born to the Canadian-born.

After this categorization, the proportion of Canadian-born individuals belonging to the mixed clusters with probable evidence of transmission (from foreign- to Canadian-born) was used to make inference about the importance of foreign-born TB on the Canadian-born individuals. The attributable fraction would be calculated thus:

$$A_F = \frac{\text{Number of Canadian-born persons with TB in all mixed clusters rated as having probable risk of transmission from the foreign- to the Canadian-born}}{\text{Total number of Canadian-born persons with TB}}$$

This attributable fraction represented the fraction of TB in the Canadian-born attributable to transmission from foreign-born.

### 4.7.3 Investigation of inter-provincial clustering

Transmission of TB infection between the three different provinces, British Columbia, Quebec, and Ontario was investigated. Individuals found to be clustered with others from another province were evaluated to find out their general characteristics. This analysis was descriptive. Characteristics that were considered included;

1. Country of birth
2. Age
3. Sex
4. Drug resistance pattern
5. Date of diagnosis
6. Presence of risk factors e.g. HIV infection, intravenous drug use.

# Chapter 5: RESULTS

## 5.1 Study population

### 5.1.1 Principal study cohort

The study population consisted of all patients reported to the public health departments, 18 years of age or older, with culture confirmed active TB, living in the province of British Columbia, on the Island of Montreal and downtown Toronto; diagnosed between July 1st 1995 and June 30th 1997.

**Table 2.** Study population

| | Montreal | British Columbia | | Toronto |
|---|---|---|---|---|
| | | Vancouver | Other areas | |
| Total TB cases reported | 442 | 205 | 263 | 272 |
| Culture positive TB, all ages | 358 | 205 | 263 | - |
| All isolates analyzed, N (%) | 238 (67 ) | 205 (100 ) | 263 (100) | 49 * |
| Culture positive TB, 18-49 | 244 | 115 | 104 | 153 |
| Isolates analyzed (18-49), N (%) | 205 (84) | 115 (100) | 104 (100) | 49 (32) |

*This information was not available for the city of Toronto*
** Only 18 to 49 year old patients' isolates underwent RFLP analysis*

Most cultures in Toronto were not available for RFLP analysis because of the restructuring of the public health departments already mentioned in section 4.3.2.

#### 5.1.1.1 The characteristics of patients from British Columbia

The Canadian-born were the largest single group of the patients (N=121), making up 26% of all TB patients. Of these 42% were clustered. Chinese-born patients were the next most frequent (N=83) making up 18 % of all TB cases, with 11% of them clustered. The other frequent countries of origin were India (12%), Vietnam (8%) and Philippines (7%). Most of the other foreign countries had less than 1% representation.

5.1.1.2 The characteristics of patients from Montreal

In Montreal, RFLP analysis had been done for all patients diagnosed from July 1st to December 31st, 1995 (N=87, 36%) but was only done for patients aged 18 to 49 years in 1996 and 1997 (refer to Section 4.3.2). Of the 238 patients in Montreal, the most frequent country of birth was Haiti (N=59) making up 25% of all TB patients of whom 36% were clustered. The Canadian-born were the second largest group (N=39) constituting 16% of all TB cases, of these 35% were clustered. Other significant groups were those born in Vietnam (4 %) and those born in the Philippines (4 %).

A subgroup of the TB patients from Montreal (N=87), diagnosed between July 1st and December 31st 1995 lacked information regarding certain risk factors such as HIV infection, and intravenous drug use. Patients with and without this information were compared. In order for this comparison to be meaningful, only patients of the same age (18 to 49) were compared.

**Table 3.** Comparison of characteristics of patients, aged 18-49 years, from Montreal with information on risk factors with those without that information

| | Patients with No information On risk factors N=55° | Patients with information on risk factors N=151 | P-value* |
|---|---|---|---|
| Clustered, N (%) | 16 (29) | 33 (22) | 0.36 |
| Males, N (%) | 33 (60) | 79 (53) | 0.43 |
| Mean age, years (SD) | 32.4 (8.2) | 32 (8.1) | NS |
| Drug resistance, N (%) | 7 (13) | 23 (15) | 0.82 |
| Foreign-born, N (%) | 47 (86) | 137 (91) | 0.30 |
| Mean years in Canada, (SD) | 6.2 (6.3) | 6.1 (6.3) | NS |
| Pulmonary TB, N (%) | 40 (73) | 105 (70) | 0.73 |
| Smear positive, N (%) | 20 (61) | 44 (44) | 0.69 |
| Drug resistance, N (%) | 6 (15) | 14 (13) | 0.79 |

** Fisher's exact test ( categorical variables) and Students' t-test (continuous variables)- 2-sided*

*° Only patients aged 18-49 were included in this group (all 1996/97 patients were 18-49)*

5.1.1.3 A comparison of patients characteristics in the two main study areas

The characteristics of the primary cohorts in British Columbia and Montreal were compared. However it must be noted that most of the Montreal study population included only 18-49 year old patients (those diagnosed in 1996 and 1997)

**Table 4.** A comparison of patients' characteristics in the 2 study areas (all ages)

| | Montreal, all patients N=238 | British Columbia N=468 |
|---|---|---|
| Males, N (%) | 131 (55) | 260 (56) |
| Mean age, years (SD) | 36.2 (14.6) | 50.5 (20.7) |
| Drug resistance, N (%) | 34 (14) | 41 (9) |
| Canadian-born non-aboriginal, N (%) | 34 (14) | 83 (17) |
| Aboriginal, N (%) | - | 49 (11) |
| Foreign-born, N (%) | 204 (86) | 335 (74) |
| Mean years in Canada, (SD) | 7.2 (8.4) | 10.5 (12.2) |
| Pulmonary TB, N (%) | 162 (69) | 318 (68) |
| Smear positive, N (%) | 86 (58) | 158 (52) |
| HIV positive, N (%) | 8 (3)* | 26 (6) |
| Alcohol use, N (%) | 9 (4)* | 44 (9) |
| IVD¥ use, N (%) | 3 (1)* | 22 (5) |
| Underlying disease, N (%)* | 1 (0.4)* | 59 (13) |
| History of contact, N (%) | 24 (10)* | 74 (16) |
| Health care worker, N (%) | 2 (0.8)* | 17 (4) |
| Clustered, N (%) | 54 (23)* | 93 (20) |

¥ *Intravenous drug*

* *Diabetes Mellitus, Malignancy, Gastro-intestinal surgery, Renal disease*

- *No data available*

* *Available for a subset of the population diagnosed in 1996/97 (N=158).*

5.1.2 Modified inclusion criteria

5.1.2.1 Age restriction

When the age inclusion criterion was modified, two cohorts of patients were obtained in each study area; a) TB patients 18 to 49 years of age ($V_2$, and $M_2$ in Table 1), and b) TB patients of all ages ($V_1$, and $M_1$, in Table 1).

Analysis of the effect of this age restriction was only informative for the province of British Columbia where TB patients of all ages had undergone RFLP analysis (unlike in Montreal where most of the RFLP analysis was restricted to a younger age group).

**Table 5.** Comparison of the characteristics of patients-effect of age restriction (>50 years vs. 18-49 years old patients, British Columbia)

| | British Columbia (18-49) N=219 | British Columbia (>50 years) N=229 |
|---|---|---|
| Clustered, N (%) | 56 (26) | 36 (16) |
| Males, N (%) | 115 (53) | 135 (59) |
| Drug resistance, N (%) | 22 (10) | 18 (8) |
| Aboriginal, N (%) | 30 (14) | 16 (7) |
| Foreign-born, N (%) | 153 (72) | 171 (77) |
| Mean years in Canada,(SD) | 6.4 (7) | 16.2 (12) |
| Pulmonary TB, N (%) | 143 (65) | 162 (71) |
| Smear positive, N (%) | 61 (45) | 90 (57) |
| HIV positive, N (%) | 21 (10) | 3 (1) |
| Alcohol use, N (%) | 24 (11) | 21 (9) |
| IVD use, N (%) | 18 (8) | 3 (1) |
| Underlying disease, N (%) | 13 (6) | 45 (20) |
| History of contact, N (%) | 37 (17) | 35 (15) |
| Health care worker, N (%) | 8 (4) | 9 (4) |

Analysis revealed that TB patients above the age of 50 had lower proportions of clustered patients, Aboriginal individuals, HIV positive patients, and intravenous drug users, but had significantly more patients with underlying medical diseases (Table 5 above).

5.1.2.2 Modification of geographic inclusion criteria

This analysis was done to compare TB patients residing in major cities and those from other areas in British Columbia.

**Table 6.** A comparison of patient characteristics- all ages, city of Vancouver vs. other areas of British Columbia

| | From Vancouver N=205 | From outside Vancouver N=263 | P-value |
|---|---|---|---|
| Clustered, N (%) | 49 (24) | 46 (17) | 0.11 |
| Mean age, years (SD) | 35.1 (9) | 34.2 (9) | 1.00 |
| Males, N (%) | 111 (54) | 156 (58) | 0.51 |
| Drug resistance, N (%) | 19 (9) | 22 (9) | 0.87 |
| Aboriginal, N (%) | 20 (10) | 29 (12) | 0.65 |
| Foreign-born, N (%) | 148 (74) | 187 (74) | 1.00 |
| Mean years in Canada (SD) | 6.4 (7) | 7 (7) | 1.00 |
| Pulmonary TB, N (%) | 149 (73) | 170 (65) | 0.07 |
| Smear positive, N (%) | 69 (49) | 89 (54) | 0.43 |
| HIV positive, N (%) | 18 (9) | 8 (3) | **<0.001** |
| Alcohol use, N (%) | 20 (10) | 25 (10) | 1.00 |
| IVD use, N (%) | 18 (9) | 5 (2) | **<0.001** |
| Underlying disease, N (%) | 21 (10) | 38 (15) | 0.21 |
| History of contact, N (%) | 28 (14) | 46 (18) | 0.31 |
| Health care worker, N (%) | 7 (3) | 10 (4) | 1.00 |

*NB: P-values significant at the 0.05 level are in bold print*

Patients resident outside of the city of Vancouver at the time of TB diagnosis were less likely to be HIV positive, and to be using intravenous drugs than those resident in Vancouver. A similar analysis could not be done for Montreal because there was no RFLP analysis done for patients not resident on the island of Montreal at the time of TB diagnosis.

## 5.2 Cluster membership

### 5.2.1 Results of RFLP analysis

IS6110 RFLP analysis, alone, identified a total of 49 clusters in all the TB patients from the 3 study areas. These were clusters of patients having identical RFLP patterns with more than 5 IS6110 bands. Of these 14 were from Montreal, 32 from British Columbia, and 3 from Toronto.

### 5.2.2 Results of Spoligotyping

Isolates found to be identical on RFLP analysis but with five or less IS6110 bands were considered to have low copy numbers, and had to be analyzed with a more discriminative method, spoligotyping, to determine if they were identical or not.

Spoligotyping revealed 5 additional clusters in Montreal and none in British Columbia (Table 7) bringing the total number of clusters to 54. Overall, British Columbia had more isolates with low copy numbers, as was expected since most of its foreign born population was from South East Asia where TB isolates have been reported to have low copy numbers.

**Table 7.** Summary of the results of spoligotyping of isolates found to be identical but with 5 or less IS6110 bands

| Number of IS6110 bands | British Columbia | | Montreal | |
|---|---|---|---|---|
| | Number of identical isolates on RFLP analysis | Identical on spoligotyping, N (%) | Number of identical isolates identified on RFLP analysis | Identical on spoligotyping, N (%) |
| 5 | 0 | 0 (0) | 4 | 3 (75) |
| 4 | 0 | 0 (0) | 7 | 5 (71) |
| 3 | 4 | 0 (0) | 0 | 0 (0) |
| 2 | 2 | 0 (0) | 2 | 0 (0) |
| 1 | 21 | 0 (0) | 19 | 6 (31) |
| TOTAL | 27 | 0 (0) | 32 | 14 (44%) |

There were 4 isolates with 5 bands identical on RFLP analysis in Montreal. Spoligotyping revealed 3 to be identical. This confirmed that they were truly clustered. Similarly there were 7 isolates with 4 IS6110 sequences found to be identical on RFLP typing with 5 of them confirmed identical by spoligotyping. Of the 19 isolates found to be identical on RFLP analysis (but having only 1 IS6110 band), 6 were identical on spoligotyping.

The decreasing proportion of isolates confirmed to be identical on spoligotyping after RFLP analysis (Table 7, last column) illustrates that the chance of similarity of RFLP patterns when the IS6110 sequences are few is higher .

## 5.3 Risk factors for Clustering

Statistical analysis was carried out to determine the factors that were significant predictors of cluster membership.

### 5.3.1 Univariate analysis for the British Columbia patients

Table 9 shows that in British Columbia, Canadian-birth, aboriginal status, HIV positive status, intravenous drug abuse, and alcohol use were associated with an increased risk for clustering.

**Table 8.** Univariate analysis: Clustered vs. Non-clustered (British Columbia, all ages)

| | Clustered N=93 (20%) | Non-clustered N=375 (80%) | P-value/OR | 95% CI |
|---|---|---|---|---|
| Males, N (%) | 55 (60) | 206 (55) | 1.21 | 0.76-1.92 |
| Mean age, years (SD) | 48 (18.1) | 51 (21.3) | P=0.16 | - |
| Aboriginal, N (%) | 24 (30) | 25 (7) | **5.01** | **2.69-9.31** |
| Drug resistance, N (%) | 8 (9) | 33 (9) | 0.96 | 0.43-2.16 |
| Foreign-born, N (%) | 38 (43) | 297 (81) | **0.17** | **0.11-0.28** |
| Mean years in Canada,(SD) | 8.4 (7.9) | 10.8 (12.6) | P=0.27 | - |
| Pulmonary TB, N (%) | 62 (67) | 257 (69) | 0.92 | 0.57-1.49 |
| Smear positive, N (%) | 32 (54) | 126 (51) | 1.15 | 0.65-2.03 |
| HIV positive, N (%) | 14 (15) | 12 (3) | **5.36** | **2.39-12.03** |
| Alcohol use, N (%) | 19 (20) | 26 (7) | **3.47** | **1.81-6.55** |
| IVD use, N (%) | 16 (17) | 7 (2) | **10.9** | **4.35-27.45** |
| Underlying disease, N (%) | 10 (11) | 49 (13) | 0.80 | 0.39-1.65 |
| History of contact, N (%) | 17 (18) | 57 (15) | 1.25 | 0.69-2.67 |
| Health care worker, N (%) | 4 (4) | 13 (4) | 1.25 | 0.40-3.93 |

*NB: Odds ratios with 95% confidence intervals excluding 1 are in bold print.*
*OR = Odds Ratio*

This analysis was repeated for TB patients from the city of Vancouver in order to determine the predictive factors in a city setting. It revealed that the same factors were still significant predictors for cluster membership (Table 9).

**Table 9.** Clustered vs. Non-Clustered (City of Vancouver, all ages)

| | Clustered N=49 (24%) | Non-clustered N=156 (76%) | P-value/OR | 95% CI |
|---|---|---|---|---|
| Males, N (%) | 28 (58) | 83 (53) | 1.23 | 0.64-2.37 |
| Mean age, years (SD) | 47.5 (15) | 47.9 (21.2) | P=0.89 | - |
| Aboriginal, N (%) | 11 (23) | 9 (6) | **4.92** | **1.90-12.77** |
| Drug resistance, N (%) | 7 (14) | 12 (8) | 2.0 | 0.74-5.40 |
| Foreign-born, N (%) | 21 (45) | 127 (83) | **0.17** | **0.08-0.35** |
| Mean years in Canada,(SD) | 9.2 (8.3) | 11.3 (11.3) | P=0.33 | - |
| Pulmonary TB, N (%) | 38 (78) | 111 (71) | 1.40 | 0.66-2.98 |
| Smear positive, N (%) | 22 (63) | 47 (44) | 2.12 | 0.97-4.67 |
| HIV positive, N (%) | 12 (25) | 6 (4) | **8.12** | **2.86-23.03** |
| Alcohol use, N (%) | 10 (20) | 10 (6) | **3.74** | **1.46-9.63** |
| IVD use, N (%) | 14 (29) | 4 (3) | **15.2** | **4.72-48.99** |
| Underlying disease, N (%) | 3 (6) | 18 (12) | 0.50 | 0.14-1.78 |
| History of contact, N (%) | 6 (12) | 22 (14) | 0.85 | 0.32-2.23 |
| Health care worker, N (%) | 2 (4) | 5 (3) | 1.29 | 0.24-6.84 |

The same analysis was done for 18 to 49 year old patients from Vancouver (Table 10). This was in order to enable comparison with the Montreal cohort (which was mainly made up of 18 to 49 year old patients). Once again, the same factors, plus age, were found to be significant predictors of clustering.

**Table 10.** Univariate analysis: Clustered vs. Non-clustered (Vancouver 18-49 years)

| | Clustered N=32 (28%) | Non-clustered N=83 (72) | P-value/OR | 95% CI |
|---|---|---|---|---|
| Males, N (%) | 19 (59) | 39 (47) | 1.65 | 0.72-3.77 |
| Mean age, years (SD) | 39.9 (6.7) | 33.2 (9) | **P<0.001** | - |
| Aboriginal, N (%) | 10 (33) | 7 (9) | **5.29** | **1.79-15.64** |
| Drug resistance, N (%) | 4 (13) | 7 (8) | 1.55 | 0.42-5.71 |
| Foreign-born, N (%) | 11 (38) | 65 (80) | **0.14** | **0.06-0.36** |
| Mean years in Canada,(SD) | 8.9 (9.6) | 6.0 (6) | P=0.35 | - |
| Pulmonary TB, N (%) | 23 (72) | 54 (65) | 1.37 | 0.56-3.35 |
| Smear positive, N (%) | 12 (57) | 18 (35) | 2.44 | 0.87-6.90 |
| HIV positive, N (%) | 11 (34) | 6 (7) | **6.72** | **2.23-20.31** |
| Alcohol use, N (%) | 9 (28) | 6 (7) | **5.02** | **1.62-15.59** |
| IVD use, N (%) | 11 (34) | 4 (5) | **10.35** | **2.99-35.80** |
| Underlying disease, N (%) | 0 | 7 (8) | - | - |
| History of contact, N (%) | 4 (13) | 14 (17) | 0.70 | 0.21-2.33 |
| Health care worker, N (%) | 1 (3) | 3 (4) | 0.86 | 0.09-8.59 |

In order to investigate whether there were any differences in the risk factors in a major urban setting and other areas, the cohort of patients outside the city of Vancouver was evaluated for the risk factors for clustering (Table 11).

**Table 11.** Clustered vs. Non-clustered (Other areas in British Columbia, all ages)

| | Clustered N=44 (17%) | Non-clustered N=219 (84%) | P-value/OR | 95% CI |
|---|---|---|---|---|
| Males, N (%) | 27 (61) | 123 (57) | 1.21 | 0.63-2.36 |
| Mean age, years (SD) | 48.7 (21.2) | 53.5 (21.0) | P=0.17 | - |
| Aboriginal, N (%) | 13 (31) | 16 (7.6) | **5.44** | **2.37-12.46** |
| Drug resistance, N (%) | 1 (2) | 21 (10) | 0.22 | 0.02-1.64 |
| Foreign-born, N (%) | 17 (41) | 170 (80) | **0.17** | **0.08-0.34** |
| Mean years in Canada,(SD) | 7.3 (7.2) | 10.4 (13.6) | P=0.15 | - |
| Pulmonary TB, N (%) | 24 (55) | 146 (67) | 0.60 | 0.31-1.16 |
| Smear positive, N (%) | 10 (42) | 79 (56) | 0.57 | 0.24-1.37 |
| Drug resistance, N (%) | 0 | 18 (8) | - | - |
| HIV positive, N (%) | 2 (5) | 6 (3) | 1.69 | 0.33-8.66 |
| Alcohol use, N (%) | 9 (21) | 16 (7) | **3.26** | **1.34-7.96** |
| IVD use, N (%) | 2 (5) | 3 (1) | 3.43 | 0.56-21.15 |
| Underlying disease, N (%) | 7 (16) | 31 (14) | 1.15 | 0.47-2.80 |
| History of contact, N (%) | 11 (25) | 35 (16) | 1.75 | 0.81-3.79 |
| Health care worker, N (%) | 2 (5) | 8 (4) | 1.26 | 0.26-6.13 |

This analysis revealed that only Canadian-birth, aboriginal status, and alcohol use were associated with a significant risk of clustering. Notably, HIV infection and intravenous drug use were no longer significant predictors of clustering, as few patients had these characteristics.

**Table 12.** Summary table of the odds ratios for clustering in the different populations of British Columbia

| Odds Ratios | British Columbia (All ages) N=468 | British Columbia (18-49) N=219 | Vancouver (All ages) N=204 | Vancouver (18-49) N=115 | Outside Vancouver (All ages) N=263 | Outside Vancouver (18-49) N=104 |
|---|---|---|---|---|---|---|
| Males | 1.13 | 1.34 | 1.23 | 1.65 | 1.21 | 0.97 |
| Aboriginal | **4.83** | **5.69** | **4.92** | **5.29** | **5.44** | **5.70** |
| Foreign-born | **0.18** | **0.17** | **0.17** | **0.14** | **0.17** | **0.26** |
| Drug resistance | 1.10 | 0.86 | 2.00 | 1.55 | 0.22 | 0.30 |
| Pulmonary TB | 0.90 | 0.82 | 1.40 | 1.37 | 0.60 | 0.39 |
| Smear positive | 1.09 | 1.29 | 2.12 | 2.44 | 0.57 | 1.83 |
| Drug resistance | 0.96 | 0.77 | 2.00 | 1.86 | - | - |
| HIV positive | **5.20** | **6.04** | **8.12** | **6.72** | 1.69 | 3.55 |
| Alcohol use | **3.34** | **4.31** | **3.74** | **5.02** | **3.26** | 3.00 |
| IVD use | **10.6** | **9.84** | **15.2** | **10.35** | 3.43 | 7.18 |
| Underlying disease | 0.78 | - | 0.50 | - | 1.15 | - |
| History of contact | 1.21 | 1.33 | 0.85 | 0.70 | 1.75 | 2.52 |
| Health care worker | 1.22 | 0.42 | 1.29 | 0.86 | 1.26 | - |

***NB**: Odds ratios with 95% confidence intervals that excluded 1 are in bold.*

Table 12 illustrates that the Canadian birth, and Aboriginal status were found to be significant predictors of clustering in all groups considered. Notable differences were that HIV infection and intravenous drug use were not significant predictors for clustering in the population of patients resident outside Vancouver.

### 5.3.2 Univariate analysis for Montreal patients

Univariate analysis was conducted for the TB patients from the Island of Montreal (Table 13). As had been the case in British Columbia, Canadian birth was associated with increased risk of clustering.

Other risk factors for clustering e.g. HIV positive status, intravenous drug use, and aboriginal status could not be evaluated for the entire cohort because not all patients had information about the pertinent risk factors. Patients diagnosed in 1995 (37% of total cohort) lacked this information.

**Table 13.** Univariate analysis: Clustered vs. Non-clustered (Montreal all patients)

| | Clustered N=46 (19%) | Non-clustered N=192 (81%) | P-value/OR | 95% CI |
|---|---|---|---|---|
| Males, N (%) | 27 (59) | 104 (55) | 1.20 | 0.63-2.31 |
| Mean age, years (SD) | 32.7 (12.9) | 37.3 (14.9) | P=0.03 | – |
| Drug resistance, N (%) | 3 (7) | 31 (16) | 0.36 | 0.11-1.24 |
| Foreign-born, N (%) | 33 (72) | 171 (89) | **0.32** | **0.14-0.68** |
| Mean years in Canada,(SD) | 6.5 (6) | 7.4 (8.9) | P=0.44 | – |
| Pulmonary TB, N (%) | 34 (74) | 128 (68) | 1.32 | 0.64-2.75 |
| Smear positive, N (%) | 21 (68) | 65 (55) | 1.72 | 0.74-3.95 |
| Drug resistance, N (%) | 2 (6) | 20 (16) | 0.34 | 0.08-1.52 |

When analysis was restricted to 18-49 year olds, foreign birth was once again associated with a decreased risk for cluster membership. (Table 14).

**Table 14.** Univariate analysis: Clustered vs. Non-clustered (Montreal 18-49 years)

| | Clustered N=36 (18%) | Non-clustered N=163 (82%) | P-value/OR | 95% CI |
|---|---|---|---|---|
| Males, N (%) | 21 (58) | 88 (54) | 1.19 | 0.57-2.48 |
| Mean age, years (SD) | 31.7 (9) | 32.7 (8) | P=0.50 | – |
| Drug resistance, N (%) | 3 (8) | 26 (16) | 0.49 | 0.14-1.65 |
| Foreign-born, N (%) | 28 (78) | 150 (92) | **0.30** | **0.12-0.80** |
| Mean years in Canada,(SD) | 6.7 (6) | 6.1 (6) | P=0.58 | – |
| Pulmonary TB, N (%) | 24 (67) | 114 (72) | 0.80 | 0.36-1.71 |
| Smear positive, N (%) | 16 (70) | 58 (54) | 1.93 | 0.74-5.04 |
| Drug resistance, N (%) | 2 (8) | 16 (14) | 0.58 | 0.12-2.60 |

Univariate analysis was also conducted for the cohort of patients with more detailed epidemiologic data (N=151). This was an attempt to determine the influence of risk factors like HIV infection on the risk of cluster formation. There were only 2 health care workers in this group, 1 Aboriginal patient, and 1 patient with underlying medical illness. These variables were therefore excluded from the univariate analysis.

HIV seropositivity and Canadian birth were found to be associated with an increased risk for clustering, as had been the case in the TB patients from the city of Vancouver (Table 15).

**Table 15.** Clustered vs. Non-clustered, Montreal 18-49 years (with more detailed information)

| | Clustered N=23(15%) | Non-clustered N=128 (85%) | P-value/OR | 95% CI |
|---|---|---|---|---|
| Males, N (%) | 12 (53) | 64 (50) | 1.09 | 0.36–1.64 |
| Mean age, years (SD) | 32.2 (8) | 32.0 (8) | P=0.87 | – |
| Drug resistance, N (%) | 2 (9) | 20 (16) | 0.52 | 0.12–1.58 |
| Foreign-born, N (%) | 17 (74) | 115 (90) | **0.24** | **0.08–0.76** |
| Mean years in Canada,(SD) | 7.5 (7) | 5.8 (6) | P=0.25 | – |
| Pulmonary TB, N (%) | 16 (70) | 85 (66) | 1.37 | 0.45–2.37 |
| Smear positive, N (%) | 10 (63) | 45 (53) | 2.70 | 0.95–7.37 |
| Drug resistance, N (%) | 1 (6) | 12 (15) | 0.41 | 0.10–2.30 |
| HIV positive, N (%)[‡] | 7 (30) | 9 (7) | **15.0** | **2.65–85.01** |
| Alcohol use, N (%) | 3 (13) | 6 (5) | 3.10 | 0.75–11.7 |
| IVD use, N (%) | 3 (13) | - | – | – |
| History of contact, N (%) | 3 (13) | 20 (16) | 0.81 | 0.27–2.26 |

*‡ Only 46 patients (30 %) had been tested for HIV*

**Table 16.** Summary table for the odds ratios for clustering, obtained from the univariate analysis of Montreal TB patients

| Odds Ratios | Montreal All patients N=238 | Montreal 18-49 N=205 |
|---|---|---|
| Males | 1.20 | 1.19 |
| Drug resistance | 0.36 | 0.49 |
| Foreign-born | **0.32** | **0.30** |
| Pulmonary TB | 1.32 | 0.80 |
| Smear positive | 1.72 | 1.93 |
| Drug resistance | 0.34 | 0.38 |

***NB**: Odds ratios that had been found to be associated with 95% confidence intervals not including 1 are in bold.*

Considering the odds ratios in Table 16, it can be noted that the odds ratios were similar in all patients and in the restricted group. Canadian birth was consistently a significant predictor for clustering. As expected, there was no statistically significant difference in the mean ages of the clustered patients compared to the nonclustered in the restricted age group (P=0.50) but there was a statistically significant difference in the cohort of all ages (P=0.03). The mean duration of stay in Canada for the foreign-born patients was not different for the clustered and the non-clustered patients in both population groups.

### 5.3.3 Stratified Analysis

#### 5.3.3.1 British Columbia (all ages)

A stratified analysis was done for the principal cohort in order to determine whether some covariates were modifying or confounding the association between clustering and the main predictor variable-foreign-birth.

The study population was stratified for each of the independent variables and odds ratios were calculated for each of the strata. The strata specific odds ratios were compared to the adjusted odds ratio (Mantel-Haenszel) obtained from stratified analysis. The Mantel-Haenszel odds ratios were found to always fall within the range of the strata specific values (except for HIV which was borderline possibly because of a zero cell value). This revealed that there was no significant confounder for the association between country of birth and cluster formation. The lack of a confounding effect was confirmed by looking at the cell values of the respective 2 by 2 tables and comparing the distribution of the risk factors among the clustered and the non-clustered.

**Table 17.** Stratified analysis to check for confounding and effect modification in the British Columbia cohort of TB patients (all ages)

| Variable | | CB* Clustered Yes | CB* Clustered No | FB* Clustered Yes | FB* Clustered No | OR | 95% CI | $OR_{MH}$ | 95% CI | Breslow-Day Prob. |
|---|---|---|---|---|---|---|---|---|---|---|
| IVD | Yes | 15 | 6 | 1 | 1 | 0.40 | 0.02-7.48 | 0.26 | 0.14-0.34 | 0.72 |
| | No | 36 | 63 | 39 | 294 | 0.23 | 0.13-0.39 | | | |
| Alcohol | Yes | 17 | 21 | 2 | 4 | 0.62 | 0.10-3.80 | 0.21 | 0.13-0.35 | 0.20 |
| | No | 34 | 48 | 38 | 291 | 0.18 | 0.11-0.32 | | | |
| HIV+ | Yes | 9 | 13 | 0* | 3 | 0.41 | 0.25-0.68 | 0.21 | 0.12-0.33 | 0.34 |
| | No | 38 | 60 | 40 | 292 | 0.22 | 0.13-0.37 | | | |

*CB = Canadian-born*

*FB = Foreign-born*

*$OR_{MH}$ = Mantel Haenszel Odds Ratio*

** SAS Logit estimators use a correction of 0.5 in every cell of these tables that contained a zero*

*NB: 1) Some patients had no information on risk factors*

Breslow–Day tests for homogeneity were performed, after stratifying, to check for effect modification. For a given covariate, a Breslow-Day probability of less than or equal to 0.05 would have indicated that the odds ratio across the strata were not homogenous and that the covariate in question was an effect modifier of the association between country of birth and clustering. All the tests performed revealed values greater than 0.05. This suggested that there was no effect modification, which was confirmed by looking at the cell values of the respective 2 by 2 tables.

5.3.3.2 Montreal cohort (18 to 49 years)

Stratified analysis was similarly done for Montreal patients. However in Montreal only 18 to 49 year old patients were investigated. This was because of 2 reasons:

1. Patients of all ages in Montreal underwent RFLP analysis in 1995 only. This study only included the second half of 1995. This group of patients made up only one third (37%) of the total Montreal cohort.

2. The subgroup of patients diagnosed in the second half of 1995 lacked information on some of the pertinent risk factors e.g. HIV status, and intravenous drug use.

In view of the above, stratified analysis was done for only those members of the study sample diagnosed in 1996 and the first half of 1997, who were all 18 to 49 years of age. Results of the analysis conducted are shown in Table 19.

Once again there was no evidence of any covariate confounding the association between place of birth (foreign-born vs. Canadian-born) and cluster formation. However some of the cell values were zero compromising the estimates provided by the logit estimator.

**Table 18.** Stratified analysis to check for confounding and effect modification, Montreal TB patients, 18-49 years old

| Variable | | CB* Clustered Yes | CB* Clustered No | FB* Clustered Yes | FB* Clustered No | OR | 95% CI | $OR_{MH}$ | 95% CI | Breslow-Day Prob. |
|---|---|---|---|---|---|---|---|---|---|---|
| **HIV+** | Yes | 4 | 0 | 4 | 8 | 3.00 | 1.35-6.77 | 0.45' | 0.001-0.82 | 0.15 |
| | No | 0 | 1 | 2 | 27 | 1.10 | 0.97-1.19 | | | |
| **Alcohol** | Yes | 3 | 0 | 0 | 4 | -* | -* | 0.21' | 0.05-0.82 | 0.50 |
| | No | 2 | 6 | 11 | 75 | 0.44 | 0.08-2.46 | | | |

*CB = Canadian-born*

*FB = Foreign-born*

$OR_{MH}$ *= Mantel Haenszel Odds Ratio*

**SAS Logit estimators not computed because more than 50% of the expected cell values contained a zero*

' *Values not accurate estimates because of the zero cell values*

5.3.4 Multivariate analysis

Multivariate analysis, using logistic regression, was used to estimate the influence of each risk factor on clustering after adjusting for all the other factors.

5.3.4.1 Checking for collinearity (British Columbia)

The principal risk factor in this thesis was country of birth. After all the other variables were checked for confounding, they were then checked for their collinearity with each other (Table 20 below).

**Table 19.** R values for Spearman's and Pearson's correlation tests of the independent variables of the TB patients from British Columbia

| | HIV + | Sex | Age | IVD use | Pulmonary disease | Drug resistance | Alcohol |
|---|---|---|---|---|---|---|---|
| **Canadian-birth** | 0.55 | 0.34 | 0.23 | 0.53 | 0.25 | 0.20 | 0.50 |
| **Aboriginal** | 0.24 | 0.17 | 0.20 | 0.35 | 0.19 | 0.14 | 0.64 |
| **HIV +** | | 0.22 | 0.05 | 0.79 | 0.14 | -0.14 | 0.06 |
| **Gender** | | | 0.06 | 0.13 | 0.23 | -0.01 | 0.18 |
| **Age** | | | | 0.08 | -0.01 | 0.05 | 0.24 |
| **IVD use** | | | | | 0.16 | -0.04 | 0.23 |
| **Pulmonary disease** | | | | | | -0.22 | 0.22 |
| **Drug resistance** | | | | | | | -0.04 |

There was no significant collinearity detected between the variables except for HIV positive status and intravenous drug use. These were the only potential confounding factors but stratified analysis had revealed that they were not.

5.3.4.2 Checking for collinearity (Montreal)

A similar analysis for the Montreal cohort (all ages) revealed no significant collinearity. The numbers of patients with HIV results was small, and there was no reporting of intravenous drug use. The collinearity of these two factors (which can be assumed a priori) was not verified in Montreal because of lack of information.

**Table 20.** R values for Spearman's and Pearson's correlation tests for the independent variables of all the TB patients from Montreal

| | Gender | Drug resistance | Pulmonary disease | Smear positive TB |
|---|---|---|---|---|
| **Canadian-birth** | 0.24 | 0.66 | 0.11 | 0.49 |
| **Gender** | | 0.53 | 0.10 | 0.52 |
| **Drug Resistance** | | | 0.73 | 0.48 |
| **Pulmonary TB** | | | | 0.04 |

5.3.5 Results of the logistic regression modeling

Logistic regression modeling was conducted separately for British Columbia and Montreal because it was expected that the patients in these 2 areas had different patterns of exposure and transmission. Backwards, stepwise logistic regression was used.

5.3.5.1 Modeling results for British Columbia

Logistic regression analysis for the population of patients from British Columbia revealed that age (considered as a continuous variable) and intravenous drug use were significant predictors of clustering.

**Table 21.** Final model for British Columbia

| Variable | Adjusted OR (95% CI) |
|---|---|
| Canadian-born non-aboriginal | Reference category |
| Foreign-born | 0.29 (0.07, 1.18) |
| Canadian-born aboriginal | 1.12 (0.25, 5.01) |
| Age | 1.28 (1.04, 1.42)[♦] |
| Intravenous drug use (Yes vs. No) | 5.83 (1.26, 27.0) |

[♦]*Age considered as a continuous variable. Each unit change in the beta parameter estimate reflected the effect of a change of 1 year.*

Canadian-birth was modeled as a dummy variable with, aboriginal and foreign-birth being compared to the reference category, Canadian-born non-aboriginal. None of these 2 comparison groups had a statistically significant odds ratio when compared to the non-Aboriginal Canadian-born.

Other factors included in the modeling but not selected by the modeling process included, gender, site of disease, drug resistance, history of contact, and HIV positive status. The fact that HIV positive status was excluded from the final model could be a result of its high collinearity with intravenous drug use.

5.3.5.2 Modeling results Montreal

Subsets logistic regression was done for the Montreal cohort that had information on the risk factors of interest. This group of patients included those diagnosed after 1995 and all of them were between the age of 18 and 49 years.

Logistic regression revealed that after adjusting for all variables, the statistically significant variable in the Montreal cohort was smear-positive disease. Canadian birth was found to be a statistically significant predictor even after adjusting for all other factors. However unlike in the British Columbia logistic regression modeling, country of birth was included as a 2-level variable (foreign-born vs. Canadian-born). Other factors included in the modeling but not significant in the final model were age, gender, and site of disease.

**Table 22.** Final model for Montreal

| Variable | Adjusted OR (95% CI) |
|---|---|
| Foreign-born, (Yes vs. No) | 0.34 (0.15, 0.77) |
| Smear positive disease (Yes vs. No) | 2.93 (1.00, 4.39) |
| Drug resistance (Yes vs. No) | 0.37 (0.08, 1.66)* |

** Subsets logistic regression with SLentry and SLstay of 0.3 included resistance in the model despite the fact that it had not been a significant predictor on univariate analysis.*

## 5.4 Investigation of possible transmission between foreign- and Canadian-born TB patients

### 5.4.1 Inferring transmission from foreign-born to Canadian-born

In order to make an inference about the direction of transmission of TB infection between the foreign-born and the Canadian-born, only those clusters that had both foreign-born and Canadian-born members were investigated in detail. There were only 9 such clusters, also called mixed clusters.

**Table 23.** Composition of clusters

| | Number of Clusters (Number of members) | |
|---|---|---|
| | Montreal | Vancouver |
| Canadian members only | 1 (2) | 13 (35) |
| Foreign-born members only | 12 (32) | 11 (23) |
| Canadian- and foreign-born members | 5 (11) | 4 (9) |

*NB: Some individuals in both study areas lacked information about country of birth*

### 5.4.2 Characteristics of "mixed clusters"

The characteristics of the patients constituting the clusters, which had both foreign-born and Canadian-born cases (mixed clusters), were explored in detail to find out if an inference could be made about the transmission of TB from the foreign-born to the Canadian-born, using the criteria already described in the methods. (Refer to the following Tables).

**Table 25.** 1st British Columbia Cluster with both Canadian and foreign-born members

| | First case | Second case | Comments/Observations |
|---|---|---|---|
| **Country of birth** | Canada | Chile | 1. This cluster had only these two members. The foreign-born had smear pulmonary disease, the form that is contagious. This makes him the most likely source of infection in this cluster.<br>2. The Canadian-born patient was immunocompromised and so was susceptible to infection. This is supported by her young age, which suggests that her disease is unlikely due to reactivation.<br>3. The Chilean patient arrived in Canada before diagnosis of disease in the Canadian-born individual, so he could be the source of infection.<br>4. The interval between diagnosis of TB in these two patients is only 3 months so it is difficult to determine the direction of transmission based on this interval.<br>5. The presence of drug resistance in the Canadian-born cannot be explained and does not support acquisition of infection from the foreign-born. It however doesn't rule it out. |
| **Site of disease** | Extra-pulmonary | Pulmonary | |
| **Date of diagnosis** | Apr/1997 | Jun/1997 | |
| **Date of onset of symptoms** | Negative | Positive | |
| **Smear results** | NA | 1977 | |
| **Year of immigration** | 29 | 45 | |
| **Age, years** | Female | Male | |
| **Gender** | Positive | Negative | |
| **Chest x-ray** | Yes | Yes | |
| **Drug resistance** | None | Yes | |
| **CONCLUSION** | The foreign-born patient was most likely the source of infection in this cluster. | | |

**Table 26.** 2nd British Columbia cluster with both foreign- and Canadian-born TB patients

| | First case | Second case | Comments/Observations |
|---|---|---|---|
| **Country of birth** | Canada | Philippines | 1. This cluster had 8 members, with only 1 foreign-born patient.<br>1. The foreign-born patient was diagnosed with TB 4 months after the first patient diagnosed in this cluster, and had extra-pulmonary disease. This makes it unlikely that she could have been the source of infection.<br>2. The first patient diagnosed in this cluster (Canadian-born) had smear positive pulmonary disease and was most likely the source of infection.<br>3. All the Canadian patients were from Vancouver.<br>4. There were only 2 Aboriginal people in this cluster<br>6. No risk factors predisposing to TB transmission were observed |
| **Site of disease** | Pulmonary | Extra-pulmonary | |
| **Date of diagnosis** | Sept 1995 | Dec 1995 | |
| **Date of onset of symptoms** | Positive | Positive | |
| **Smear results** | N/A | 1993 | |
| **Year of immigration** | 36 | 45 | |
| **Age, years** | Male | Female | |
| **Gender** | Negative | Negative | |
| **Chest x-ray** | No | No | |
| **Drug resistance** | No | No | |
| **CONCLUSION** | The Canadian-born patient was most likely the source of infection in this cluster and not the foreign-born | | |

**Table 27.** 3rd British cluster with both foreign-born and Canadian-born members

| | First case | Second case | Comments/Observations |
|---|---|---|---|
| **Country of birth** | Africa | Canada | 1. This cluster had 6 members, with only I foreign-born patient whose exact country of origin was not known.<br>2. This foreign-born patient was diagnosed with TB 8 months after the first patient diagnosed in this cluster, but had extra-pulmonary disease. This makes it unlikely that he could have been the source of infection.<br>3. The first Canadian-born patient diagnosed had pulmonary disease and might have been the source of infection.<br>4. 4 of the 5 Canadian-born patients were from Surrey and lived close to each other (2 had the same 6-digit postal code, and 3 had the same 3-digit postal code.<br>5. There were no distinctive risk factors identified linking the members in this cluster. |
| **Site of disease** | Extra-pulmonary | Pulmonary | |
| **Date of diagnosis** | June 1995 | February 1996 | |
| **Smear results** | Positive | Negative | |
| **Year of immigration** | 1972 | N/A | |
| **Age, years** | 48 | 50 | |
| **Gender** | Male | Male | |
| **HIV status** | Negative | Negative | |
| **IVD use** | No | Yes | |
| **Drug resistance** | No | No | |
| **CONCLUSION** | The foreign-born patient was unlikely to be the source of infection in this cluster. | | |

**Table 28.** 4th British Columbia cluster with both foreign-born and Canadian-born members

| | First case | Second case | Comments/Observations |
|---|---|---|---|
| **Country of birth** | Canada | China | 1. This cluster had 4 members from 2 countries; China, and Canada.<br>2. The Chinese-born patient was diagnosed with TB 4 months after, the first patient diagnosed in this cluster (Canadian-born), and had extra-pulmonary disease. This makes it very unlikely that she could have been the source of infection.<br>3. The first Canadian-born patient diagnosed had smear positive pulmonary disease and might have been the source of infection.<br>4. The Canadian-born patient first diagnosed was elderly and his disease was probably the result of reactivation .<br>5. There were no distinctive risk factors identified linking the members in this cluster. |
| **Site of disease** | Pulmonary | Extra-pulmonary | |
| **Date of diagnosis** | January 1996 | April 1996 | |
| **Smear results** | Positive | Positive | |
| **Year of immigration** | N/A | 1972 | |
| **Age, years** | 69 | 51 | |
| **Gender** | Male | Female | |
| **HIV status** | Negative | Negative | |
| **IVD use** | No | No | |
| **Drug resistance** | No | No | |
| **CONCLUSION** | The foreign-born patient was unlikely to be the source of infection in this cluster. | | |

**Table 29.** 1st Montreal cluster with both foreign- and Canadian-born TB patients

| | First case | Second case | Comments/Observations |
|---|---|---|---|
| **Country of birth** | Canada | Vietnam | 1. This cluster was made up of 2 patients only<br>2. The Vietnamese-born patient was diagnosed 1 year after the Canadian-born so is very unlikely to be the source of infection in this cluster.<br>3. The Canadian-born had smear positive pulmonary disease with cavitary lesions on the chest x-rays. These lesions are associated with a high degree of contagiousness. Hence this patient may have been the source of infection in this cluster. |
| **Site of disease** | Pulmonary | Pulmonary | |
| **Date of diagnosis** | October 1995 | November 1996 | |
| **Date of onset of symptoms** | October 1995 | October 1996 | |
| **Smear results** | Positive | Positive | |
| **Year of immigration** | N/A | 1979 | |
| **Age, years** | 36 | 39 | |
| **Gender** | Male | Male | |
| **Chest x-ray** | Abnormal (Cavitary) | Abnormal | |
| **Drug resistance** | No | No | |
| **CONCLUSION** | The foreign-born patient was not the source of infection in this cluster. | | |

**Table 30.** 2nd Montreal cluster with both foreign- and Canadian-born TB patients

| | First case | Second case | Comments/Observations |
|---|---|---|---|
| **Country of birth** | Canada | Vietnam | 1. This cluster was made up of 4 patients with only 1 foreign-born member.<br>2. All the Canadian-born patients were diagnosed with disease before the Vietnamese born patient.<br>3. The first diagnosed Canadian-born patient had pulmonary disease with abnormal chest x-rays. This patient was most likely the source of infection in this cluster.<br>4. The Vietnamese patient had extrapulmonary disease and was diagnosed last, so he was not the source of infection. |
| **Site of disease** | Pulmonary | Extra-pulmonary | |
| **Date of diagnosis** | October 1995 | February 1997 | |
| **Date of onset of symptoms** | October 1995 | Unknown | |
| **Smear results** | Negative | Negative | |
| **Year of immigration** | N/A | 1975 | |
| **Age, years** | 58 | 23 | |
| **Gender** | Female | Male | |
| **Chest x-ray** | Abnormal | No record | |
| **Drug resistance** | None | None | |
| **CONCLUSION** | The foreign-born patient was not the source of infection in this cluster. | | |

**Table 31.** 3rd Montreal cluster with both foreign- and Canadian-born TB patients

| | First case | Second case | Comments/Observations |
|---|---|---|---|
| **Country of birth** | North Korea | Canada | 1. This cluster was made up of 3 patients with only 1 foreign-born member.<br>2. TB was first diagnosed in the North Korean-born individual (4 months earlier than the first Canadian-born diagnosed)<br>3. The North Korean-born patient had smear positive pulmonary disease with evidence of cavities on chest x-rays. This is a very contagious form of the disease.<br>4. The North Korean-born patient arrived in Canada long before disease developed in the Canadian-born individuals. Thus this patient could certainly have been the source of infection in this cluster. |
| **Site of disease** | Pulmonary | Pulmonary | |
| **Date of diagnosis** | June 1995 | October 1995 | |
| **Date of onset of symptoms** | May 1995 | October 1995 | |
| **Smear results** | Positive | Not reported | |
| **Year of immigration** | 1975 | N/A | |
| **Age, years** | 21 | 19 | |
| **Gender** | Male | Female | |
| **Chest x-ray** | Abnormal (Cavitary) | Abnormal | |
| **Drug resistance** | | | |
| **CONCLUSION** | The foreign-born patient was most likely the source of infection in this cluster. | | |

**Table 32.** 4th Montreal cluster with both foreign- and Canadian-born TB patients

| | First case | Second case | Comments/Observations |
|---|---|---|---|
| **Country of birth** | Haiti | Canada | 1. This cluster was made up of 3 patients with 2 patients from Haiti.<br>2. TB was first diagnosed in a Haitian-born individual (16 months before the Canadian-born)<br>3. The Haitian-born patient had smear positive pulmonary disease with evidence of cavities on chest x-rays. This is a very contagious form of the disease.<br>4. Symptoms of disease in the Canadian-born developed after diagnosis of disease in the Haitian-born individual. |
| **Site of disease** | Pulmonary | Pulmonary | |
| **Date of diagnosis** | October 1995 | February 1997 | |
| **Date of onset of symptoms** | June 1995 | January 1997 | |
| **Smear results** | Positive | Positive | |
| **Year of immigration** | 1981 | N/A | |
| **Age, years** | 21 | 18 | |
| **Gender** | Male | Male | |
| **Chest x-ray** | Abnormal (Cavitary) | Abnormal (Cavitary) | |
| **Drug resistance** | None | None | |
| **CONCLUSION** | The foreign-born patient was most likely the source of infection in this cluster. | | |

**Table 33.** 5th Montreal cluster with both foreign- and Canadian-born TB patients

| | First case | Second case | Comments/Observations |
|---|---|---|---|
| **Country of birth** | Romanian | Canada | 1. This cluster was made up of only these 2 patients.<br>2. TB was first diagnosed in the Romanian-born individual (4 months before the Canadian-born)<br>3. The foreign-born patient had smear positive pulmonary disease with evidence of cavities on chest x-rays. This is a very contagious form of the disease.<br>4. Symptoms of disease, and diagnosis of TB in the Canadian-born developed after diagnosis of disease in the Romanian-born individual. |
| **Site of disease** | Pulmonary | Pulmonary | |
| **Date of diagnosis** | August 1995 | November 1995 | |
| **Date of onset of symptoms** | June 1995 | October 1995 | |
| **Smear results** | Positive | Negative | |
| **Year of immigration** | 1992 | N/A | |
| **Age, years** | 28 | 72 | |
| **Gender** | Male | Male | |
| **Chest x-ray** | Abnormal (Cavitary) | Abnormal | |
| **Drug resistance** | None | None | |
| **CONCLUSION** | The foreign-born patient was most likely the source of infection in this cluster. | | |

**Table 33.** Summary table of the categorization of mixed clusters based on the likelihood of transmission of TB from the foreign-born to the Canadian-born

| | British Columbia | Montreal |
|---|---|---|
| Number of mixed clusters | 4 | 5 |
| Clusters with possible transmission from the foreign- to the Canadian-born | 1 | 3 |
| Clusters unlikely to have transmission from the foreign- to the Canadian-born | 3 | 2 |
| Total number of Canadian-born patients in mixed clusters with possible foreign- to Canadian-born transmission, (x) | 1 | 4 |
| Total number of Canadian-born patients in the principal study cohort, all ages (y) | 132 | 34 |
| ATTRIBUTABLE FRACTION, (x/y) | 0.008 | 0.12 |

From Table 33 above, it can be noted that the foreign-born were potentially associated with more transmission to Canadian-born individuals in Montreral (12% in Montreal vs. 0.8% in British Columbia).

There were no mixed clusters with possible transmission of TB infection from a foreign-born patient to a Canadian-born individual in Vancouver city. Therefor the attributable fraction for the city of Vancouver , based on the RFLP results available for this study, was zero.

## 5.5 Investigation of possible transmission between patients from different provinces (inter-provincial clustering)

There were 16 patients who were found to be clustered with patient(s) from other provinces. These inter-provincial clusters also included all the other patients (in each respective city) who were clustered with the patient(s) from the same city. For example, if patient (A) from Vancouver was clustered with patients (B) and (C) from the same city, and was also clustered with patient (D) from Montreal, then the inter-provincial cluster would be comprised of all these 4 patients.

An analysis of the 5 inter-provincial clusters revealed that there were no obvious epidemiologic links between the clustered patients. A detailed post hoc epidemiologic investigation would be necessary to determine if these clusters were evidence of inter-provincial TB transmission or were a result of a patient infected in one city but diagnosed in another (as after relocation) or were purely a coincidence.

**Table 34.** Summary of the inter-provincial Clusters

| Cluster | No. of members | Place of residence | Dates of diagnosis | Place of birth | Year of arrival in Canada | Site of disease | Age | Sex |
|---|---|---|---|---|---|---|---|---|
| 1 | 3 | Vancouver | Oct 1995 | China | 1993 | Pulmonary | 73 | M |
| | | Toronto* | Jan 1995 | Canada | - | Pulmonary | 42 | M |
| | | Toronto* | Oct 1996 | Canada | - | Pulmonary | 46 | M |
| 2 | 2 | Vancouver | Nov 1996 | Vietnam | 1986 | Pulmonary | 82 | F |
| | | Toronto | Nov 1996 | Canada | - | Pulmonary | 32 | M |
| 3 | 5 | Montreal* | Oct 1995 | Canada | - | Pulmonary | 58 | F |
| | | " | Nov 1995 | " | - | " | 71 | M |
| | | " | Sept 1996 | " | - | " | 45 | M |
| | | " | Feb 1997 | North Korea | 1965 | Extrapulm. | 23 | M |
| | | Vancouver | Feb 1996 | Britain | 1968 | Pulmonary | 33 | M |
| 4 | 4 | Montreal | May 1995 | Phillipines | 1990 | Bones | 44 | M |
| | | Vancouver | Dec 1995 | " | 1982 | Extrapulm. | 40 | M |
| | | Delta (BC) | Apr 1997 | " | 1986 | Pulmonary | 38 | M |
| | | Burnaby (BC) | May 1997 | China | 1992 | Pulmonary | 81 | F |
| 5 | 2 | Montreal | Aug 1995 | Pakistan | 1995 | GUT | 47 | M |
| | | Vancouver | Jul 1997 | Afghanistan | 1988 | Pulmonary | 61 | M |

*M=Male*
*F=Female*
*BC=British Columbia*
*GUT= Genito urinary Tract*

* *Clustered together in the same province/city*

# CHAPTER 6 DISCUSSION

## 6.1 Summary of results

Typing of 754 *M. tuberculosis* isolates from adult (≥18 years) TB patients from British Columbia, Montreal, and Toronto revealed that 148 (20%) patients were clustered. Of the 467 patients from British Columbia 20% were clustered while 23% of the 238 patients from Montreal were clustered, and 18% of the 49 patients from Toronto were clustered. Because of the few patients in Toronto, they were only considered in the investigation of inter-provincial clusters.

There were 54 clusters identified ranging in size from 2 to 5 patients. Excluding the 9 clustered patients from Toronto, 71 of the 139 clustered patients (51%) were foreign-born. There was a significant negative association between foreign birth and cluster membership in Montreal, and British Columbia. Other variables associated with cluster membership, which did not appear to confound the primary association, were HIV positive status, and intravenous drug use. Restricting the analysis to 18 to 49 year old patients, as expected, resulted in a higher prevalence of HIV positive patients, intravenous drug use, and history of previous contact with a TB patient but fewer individuals with chronic illnesses. However, the factors found to be associated with clustering did not change with this age restriction.

There were 9 clusters, which included a total of 34 foreign- and Canadian-born patients together. In only 4 of these clusters was there epidemiologic evidence of probable transmission of infection from the foreign-born to the Canadian-born; the latter represented only 5% of all Canadian patients. The estimated proportion of active TB among the Canadian-born potentially attributable to recent transmission from foreign-born cases was 0.8% in British Columbia, and 11% in Montreal. Overall, these results suggest that TB in the foreign-born has a small impact on the Canadian-born population.

## 6.2 Patient selection

This study targeted adult patients (≥ 18 years) because its goal was to investigate transmission of infection from the foreign- to the Canadian-born, which is most likely to occur in the community setting. Childhood infection would usually result from household contacts.

TB patients of all ages had their isolates analyzed by RFLP in British Columbia. In Montreal all isolates were analyzed between July and December 1995 (87 patients -37% of the total Montreal population studied) but only isolates from 18 to 49 year olds were analyzed in 1996 and 1997. This was because the younger adults were likely at higher risk for new acquisition and transmission of TB. Earlier US studies had revealed that clustered patients were more likely to be young than the non-clustered (Small 1994, Alland et al. 1994, Kent A et al 1995, Braden CR et al 1997, Barnes PF et al 1997). For example in the New York population mean age was 36 years for the clustered vs. 46 for the non-clustered, P=0.001(Alland et al. 1994) and in the San Francisco study it was 40.8 vs. 48.4, P <0.001 (Small 1994).

The failure to conduct RFLP analysis for older patients during most of the study period in Montreal resulted in loss of about 30% of potential cases in 1996 and 1997. The exclusion of elderly patients, who are more likely to have reactivation of infection with respect to the population as a whole, might have resulted in an overestimation of recent transmission and consequently resulted in an over estimation of foreign-born to Canadian-born transmission.

### 6.2.1 Effect of age restriction on determinants of clustering

The effect of age restriction was studied in British Columbia where results for RFLP analysis were available for all patients. To determine if the patients aged 18 to 49 were systematically different from patients older than 50 years of age; the two age strata were compared.

Statistically significant differences were observed, with a decreased prevalence in the above 50 age group of clustered patients (26% vs. 15%, P=0.01), intravenous drug use (8% vs. 1%, P<0.001), HIV positive status (10% vs. 1%, P<0.001), and Aboriginal status (14% vs. 7%. P<0.001). However this same group (above 50 years) had more underlying diseases (20% vs. 6%, P=0.02) than the group of patients below 50 years of age.

Most of the differences in these two populations were expected based on earlier findings in the US e.g. intravenous drug use, HIV positive status, and underlying diseases. However, these differences were not believed to have affected our principal study question, transmission from foreign- to Canadian-born individuals, because patients of all ages were considered in the inference about foreign- to Canadian-born transmission. The lower incidence of clustering in the older age group is likely due to reactivation of remotely acquired infection as the major cause of TB.

HIV positive status has been associated with a faster progression to disease after infection. The New York and San Francisco RFLP studies revealed that HIV positive status or AIDS disease were associated with an increased risk for clustering. The actual influence of HIV infection or AIDS on clustering was not established by this study. This is because there were few people tested for HIV infection. In Montreal there were 46 (30%) patients who had been tested for HIV. Thus there must have been an under estimation of the effect of HIV infection, however this would not be differential for the foreign-born or the Canadian-born, and would not affect our primary study question.

### 6.2.2 Effect of geographical location on determinants of TB transmission

The effect of place of residence at time of diagnosis was investigated. It was thought necessary to investigate the effect of residence in a major city (as compared to less urbanized area) on the risk factors for clustering because a) the foreign-born congregate in large cities, b) reports have shown that TB incidence rates are highest in major urban centers. The 1996/97 Annual Report of the Division of Tuberculosis Control of British

Columbia revealed that while the city of Vancouver had a rate of 24.4 and 26.8 per 100,000 people in 1996 and 1997 respectively, the rest of British Columbia had an average rate of less than 5 per 100,000 people, (BC Center for Disease Control 1997), and c) Risk factors for TB infection, and rapid development of active disease e.g. over crowding, homelessness, high levels of drug abuse, and HIV infection are more prevalent.

Living in Vancouver was significantly associated with an increased incidence of HIV positive status (9% vs. 3%, P<0.001), and intravenous drug use (9% vs. 2%, P<0.001). Notably, there were no differences in the proportion of foreign-born patients or in the degree of clustering. These findings could not be compared with any research findings from the US because all the studies concentrated either in the major cities (Small 1994, Alland et al 1994, Kent AS et al 1995, Frieden TR et al 1996, Barnes PF et al 1997, Chin DP et al 1998) or in rural communities (Braden CR et al 1997). It is expected that the high prevalence of other risk factors for TB infection, and progression to disease, would result in more Canadian-born being infected in settings not involving the foreign-born.

### 6.2.3 Study period

The accrual of patients over a two-year period is a limitation of this study. TB is a disease that has a latent phase of variable length after infection i.e. after acquisition of TB infection, disease may develop many years later. As such some of the transmission patterns may be missed during this two-year period. For example:

(a) A foreign-born patient may immigrate into Canada, transmit TB to a Canadian-born individual, who later develops the disease outside this time frame. At the time of RFLP analysis, the Canadian-born will be free of disease and so this transmission will be missed.

(b) Transmission may occur, but the newly infected contact never develops active disease. Again, transmission will be missed.

There are many possible situations that could result in missed transmission because of the short study period. On the whole, they would all lead to an underestimate of disease transmission. More specifically, this time period will result in a bias toward detection of transmission associated with factors, which promote faster development of disease after infection. The best example of this is HIV infection. Hence the shorter the time under study the stronger the association of HIV and clustering is likely to be. Not surprisingly HIV infection was strongly associated with clustering in both British Columbia (OR 5.36, 95% CI 2.39,12.03), and Montreal (OR 15, 95% CI 2.65, 85.01).

### 6.2.4 Conclusions about patient selection

From the foregoing discussion, it appears likely that the inclusion of all adult patients in the investigation of TB transmission in British Columbia resulted in an unbiased estimate of recent transmission. However in Montreal the cohort was largely restricted to younger individuals. So the overall extent of recent transmission in the population was likely to be an over estimate-since elderly patients are more likely to have reactivation disease.

## 6.3 Patient characteristics

Both cohorts had a large proportion of foreign-born patients, as was expected. Montreal had more foreign-born TB patients (86%) as compared to British Columbia (74%) and this might result in an a higher attributable fraction in this group. The more foreign-born TB patients in a population, the greater the probability that some Canadian-born will be infected by the foreign-born.

The Aboriginal people are associated with a high incidence of TB (27.4/100,000 in 1996, 34.5/100,000 in 1997; BC Center for Disease Control). Their presence in British Columbia (11% of the cohort) versus their absence in Montreal may mean that the Canadian-born (especially the aboriginal) are responsible for a large proportion of TB transmission in British Columbia (Canadian-born Canadian-born transmission) than in

Montreal. This would result in a lower proportion of disease among the Canadian-born originating from foreign-born persons.

It was noted from British Columbia that restriction to patients aged 18 to 49 years resulted in a higher prevalence of HIV infection, and intravenous drug use. Since HIV infection, and intravenous drug use have also been associated with an increased risk for acquisition of TB infection, the fact that the Montreal cohort was predominantly 18 to 49 suggests that there would be an increased detection of transmission of infection. This was indeed the finding. In Montreal the attributable fraction of TB in the Canadian-born as a result of possible transmission from the foreign-born was about 10 times higher than in British Columbia.

## 6.4 RFLP method of fingerprinting

### 6.4.1 The technique

The technique of RFLP finger printing has been found to be accurate in differentiating between different strains of *M. tuberculosis*. Copies of IS6110 appear to insert preferentially in a region of the chromosome which is rich in small segments of DNA which are multiply repeated with only a small amount of DNA between copies, so called direct repeats (Hermans et al. 1991). This results in a high degree of polymorphism on RFLP typing. However its degree of polymorphism significantly reduces when there are only a few copies of IS6110 in the DNA. This has meant that RFLP typing using IS6110 alone has been unable to distinguish between some strains, which have only one or two copies of IS6110 (van Soolingen et al 1991).

Difficulties have been encountered with IS6110 typing of Asian isolates of *M. tuberculosis*: this is because many strains from Vietnam (Yuen et. al. 1993) and the Indian sub-continent have only one or few copies of the IS6110. A few isolates of *M. tuberculosis* with no copies of IS6110 have been identified (van Soolingen et al, 1991).

The issue of low copy numbers was anticipated in British Columbia, where a large proportion of the foreign-born population emigrated from Asia. The Chinese-born constituted 18%, Indian-born 12%, Vietnamese-born 8%, and Philippines-born 15% of all TB patients. (Similarly in Montreal the number of patients from the Asia was high, with the Vietnamese-born and the Philippines-born being among the largest groups). A supplemental method of analysis, spoligotyping, was thus carried out. If this had not been done, 5 clusters would have been missed (based on the definition of clustering-which requires more than 5 identical IS6110 copies). This would have potentially resulted in missed transmission links.

### 6.4.2 Patient eligibility for RFLP analysis

The RFLP technique requires mycobacterial DNA, limiting the study of transmission to culture-positive active cases. Since not all infected persons develop active disease, not all transmission is evaluated. In some sense, RFLP must be viewed as complementary to tuberculin surveys, which detect latent infection but have major limitations because of poor specificity. Even when tuberculin testing is used optimally, its results can only indicate who is infected, not who is responsible for transmission. Hence tuberculin skin testing could never address the question of foreign-born to Canadian-born transmission.

### 6.4.3 Interpretation of results of RFLP typing

This study, like others previously conducted, considered clustered patients to have recently transmitted disease, and patients with unique RFLP patterns to be primarily due to reactivation of infection. The substantial diversity of RFLP patterns among members of the study population suggests that the probability would be low of chance occurrence of identical RFLP fingerprints among 2 unrelated cases, and would be even lower for clusters of 3 or more members. In relatively isolated and stable populations, there may be limited strain heterogeneity, which in the extreme could lead to "false positive" clusters (Braden CR et al. 1997). This could occur among the Aboriginal but is very unlikely in

clusters including both foreign- and Canadian-born individuals-the primary concern in this study.

## 6.5 Risk factors for clustering

### 6.5.1 Why analyze the risks for clustering separately for the 2 study areas?

The risk factors for clustering were investigated separately for British Columbia and Montreal in order to detect differences in the relative importance of the risk factors.

### 6.5.2 The risk factors for clustering

Univariate analysis revealed that Canadian birth, aboriginal status (for Canadian-born patients), HIV positive status, intravenous drug use, and alcohol use were significant risk factors for clustering in British Columbia.

Foreign-birth was associated with a decreased risk for cluster formation in both Montreal and British Columbia. Stratified analysis indicated that there was no factor that confounded the association between foreign birth and clustering. Most notably, HIV infection, which had not been found to be a predictor of clustering in San Francisco (Small 1994), did not mask the protective effect of foreign-birth. It supports the belief that most foreign-born reactivate infection acquired in their countries of birth, hence the unique patterns. In addition there is a decreased incidence of other factors related to acquisition and rapid progression to active disease (HIV infection, intravenous drug use) in this population.

The Aboriginal play a major role in the epidemiology of TB in British Columbia. This group has the highest incidence of TB in Canada. Hence, transmission among Canadian-persons (Aboriginal↔non-Aboriginal) might be more frequent. However, it is unlikely that there is a significant difference in the foreign-born→Canadian-born transmission

patterns as a result of an increased proportion of Aboriginal in Vancouver. The significance of Aboriginal status may be because it represents; a) endemic place of birth; b) crowding on reserves, and c) crowding and congregation in the urban environment.

After adjusting for all other factors using logistic regression, foreign birth was still associated with an decreased risk of clustering in Montreal but not in British Columbia, which might have been a result of categorizing Canadian-birth into Aboriginal, and non-Aboriginal thus reducing the power (due to the reduced numbers because of the sub-categorization).

## 6.6 Transmission of infection

### 6.6.1 Detection of transmission of TB infection

As already mentioned, this study, considered clustered patients to have recently transmitted or contracted disease, and patients with unique RFLP patterns to be primarily due to reactivation of infection. The study did not attempt to investigate for epidemiologic links between clustered patients. This was a weakness, because post hoc epidemiological information might have revealed that some of the transmission between the foreign-born and the Canadian-born was actually not present. As such, this study might have over estimated transmission patterns.

### 6.6.2 Transmission between the foreign- and Canadian-born patients

The principal study question was to determine the extent of TB transmission from the foreign-born to the Canadian-born. This study revealed that there were few clusters that had both foreign- and Canadian born individuals; 4 out of 32 in British Columbia, and 5 out of 19 in Montreal. There were even fewer clusters where the foreign-born might have been the source of infection (1 in British Columbia, 3 in Montreal). The estimated attributable fraction suggests that the foreign-born in Montreal are about 10 times more likely to account for new infection in the Canadian-born than in British Columbia. This

may be a reflection of patient selection (Section 6.2). However because RFLP analysis can only be used to analyze microbiologically confirmed cases, patients who become infected but whose infection remains latent during the course of the study were not identified. Reactivation of infection in these latently infected individuals will continue to produce overt disease for decades. Thus the true number of incidences of TB transmission that occurred between 1995 and 1997 is under estimated. There is however no reason to believe that this will differentially affect foreign-born Canadian-born transmission. So the attributable fraction calculated is a valid measure of the ongoing transmission.

The attributable fraction of TB in the Canadian-born as a result of transmission from the foreign-born was low in this study confirming that foreign-born TB does not have a significant impact on TB among the Canadian-born individuals, despite the preponderance of TB among foreign-born persons in Canada.

### 6.6.3 Conclusions about transmission from foreign- to Canadian-born

This study suggests that the foreign-born are responsible 12% of TB transmission to the Canadian-born in Montreal and 0.8% in British Columbia. This is in keeping with findings in Netherlands (Borgdorff et al 1998) which showed that 17% of the Dutch cases were attributable to the recent transmission from foreign-born individuals. However it contrasts with findings from 2 studies in San Francisco, which revealed epidemiologic separation between the US- and the foreign-born populations (Peter Small, 1994, Daniel Chin et al 1998). However one study did not attempt to calculate an attributable fraction (Peter Small, 1994), and the other found 4 clusters with definite transmission (foreign-born US born). Using their results, an attributable fraction of 2% can be calculated (Daniel Chin et al 1998).

In the Canadian context, the low attributable fractions may be a result of several factors;

a) The index of suspicion in the diagnosis of TB is much higher in the foreign-born. A foreign-born patient with symptoms of cough, fever, and weakness is more likely to be worked up for TB, diagnosed promptly and put on treatment. This individual would then quickly become non-infectious. When a Canadian-born patient presents with similar symptoms (especially if he/she smokes), it is possible that they would be attributed to other chest diseases, and a delay in diagnosis would occur which could promote the transmission of disease.

b) The foreign-born were less likely to belong to high-risk groups for TB transmission. In British Columbia there were more Canadian-born HIV positive individuals, and more Canadian-born individuals using intravenous drugs. All these are independent risk factors for TB transmission with development of active disease. Since RFLP typing is done for active cases, the foreign-0born will be under represented because of their low prevalence of these risk factors. Thus, infection in Canadian-born individuals may be from other Canadian-born individuals, often in the context of well known risks e.g. drug use etc.

c) The preponderance of TB among the Aboriginal.

### 6.6.4 Inter-provincial clustering

Not much could be deduced from the interprovincial clusters. A detailed epidemiological investigation would be necessary to determine how they came about. These clusters may have arisen as a result of relocation of one of the members of the cluster to another province after contact and transmission of infection. It could also be a result of casual contact between members of different provinces.

### 6.6.5 Public health implications

As in earlier reports, this study suggests that TB among the foreign-born does not have a significant impact on the indigenous population, it is well documented that the incidence of TB in developed countries is largely due to disease in the foreign-born. TB among the Canadian-born is most often due to Canadian-born Canadian-born transmission in the setting of conditions such as HIV infection and intravenous drug use, as was the case in San Francisco.

To address the problem of TB in Canada, special attention should be given to groups like HIV positive individuals, the Aboriginal, and intravenous drug users. Special programs should be set up for these groups; such as screening for infection with PPD, prompt diagnosis of active disease and successful completion of adequate therapy. Social programs should also to be strengthened to curb the spread of HIV infection, reduce the prevalence of intravenous drug use (a major problem in Vancouver), control the rise in homelessness (another challenge in the major cities especially Toronto). These programs need to be implemented in a targeted manner. In this way the acquisition and transmission of TB may be secondarily reduced.

The problem of TB in the foreign-born can also be addressed by some of the measures above, but in addition screening of potential immigrants for TB should continue as it reduces the risk of potential sources of infection in Canada. At this point the main reason to screen is to prevent disease among the immigrants and refugees themselves, as there is little evidence of transmission to the Canadian-born. However care must be taken not to concentrate financial/logistical resources on these screening programs at the expense of more productive strategies like screening of other high-risk groups, eg. HIV infected, injection drug users, etc., prophylaxis of those infected, prompt treatment of those with active disease, and surveillance of multidrug resistant strains.

The main challenge to TB control in the developed countries remains the unchecked spread of the disease in the developing world. It was well articulated by Anne Fanning in an editorial, in the Canadian Journal for Infectious Diseases (Anne Fanning, 1995) entitled "The impact of Tuberculosis in Canada: We are our brothers' keepers", that as long as TB remains a problem in some parts of the world, the developed countries are not safe. Canada, which admits over 200,000 people every year, is at risk. Though it is necessary that though public health facilities be strengthened at home, the developed countries should support the efforts of the World Health Organization and other international agencies in their fight against TB. This would be effectively done by facilitating the already existing National TB Control Programs.

## 6.7 Conclusion

The foreign-born do not seem to be causing a large proportion of the new cases among the Canadian-born. However, TB in the foreign-born constitutes a large proportion of all new cases reported. The public health system needs to develop measures that will adequately detect infection/disease in the foreign-born and ensure adequate treatment before they become a major source of infection for Canadian-born individuals. At the same time strategies should be focused on special risk groups among the Canadian-born e.g. the Aboriginal, AIDS patients, and intravenous drug users.

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# APPENDIX

CANADIAN RFLP STUDY
DATA ABSTRACTION FORM--HEALTH UNIT
PART 1

Study {ID Num}ber _____ Dead: <Y> DOD: <dd/mm/yy>

{Last n}ame at birth <A > {First n}ame <A >

{D}ate {o}f {b}irth: <dd/mm/yy> {Gender}: 1. Male (Code): #
2. Female

{Add}ress at time of Dx: {No}. _____ Street _____ Apt
City _____ Province <A > {Postal} Code _____

{Country} of {B}irth (First 4 Letters): <A >

{Country} of {L}ast Permanent Address (First 4 Letters): <A >
(if born outside Canada)

{Year} of {imm}igration to Canada: 19 ##

{Im}migration {Status}:
1. Citizen (Code) #
2. Landed Immigrant
3. Refugee
4. Visitor
5. Student Visa
6. Other

If CANADIAN-BORN, Is Patient {Aborig}inal? <Y> Status: <Y> Off Reserve: Y/N

{Occup}ation (Current or last full-time): _____

{H}ealth {C}are {W}orker:
1. Presently (Code) #
2. In the past
3. Never

Work in {Prison}s:
1. Presently (Code) #
2. In the past
3. Never

Present Status:
1. {Employed} (Code) #
2. Unemployed
3. Student

Language(s) Spoken:
{English} <Y>
{French} <Y>
{Other l}anguage (specify): _____

{Housing}:
1. Permanent House or Apartment (Code) #
2. Nursing Home
3. Temporary Residence
4. Homeless

How Many {Rooms} in the House or Apartment: ##

How Many {Persons} in Total Live in the Same Home: ###

Use of Homeless {Shelter} in Past Year: <Y>
If YES, {Name} of {Shel}ter: _____

Use of {Rooming H}ouse/Hotel in Past Year: <Y>
If YES, {Name} of {R}ooming {H}ouse: _____

MEDICAL HISTORY

1. TUBERCULOSIS

{Month} of Diagnosis (1-12): ## {Year} of Diagnosis: 19 ##

{Place} of {Dia}gnosis : _____
(Name of hospital/clinic)

Name of {MD} {Diag}nosing: _____

{Admit}ted to Hospital? <Y>

If YES: {Hosp}ital {Name}: _____
Duration (Number of {Days}): ###

{Site} of TB: 1. Pulmonary (Code): #
2. Extra-Pulmonary
3. Both

If EXTRA-PULMONARY: (Indicate all that apply)
Lymph {Nodes} <Y>
{Pleural} <Y>
{Bone}/Joints <Y>
{Abdo}minal <Y>
{Other} {sit}e (Specify): _____

{Method} of Diagnosis: 1. Microbiology (Code): #
2. Histology
3. Clinical/X-Ray

MICROBIOLOGY

Smear 1 {S1} {Type}: S1 1. Sputum (Code 1,2, or 3): #
S1 2. BAL
{S1} 3. {Other} (Specify): _____

{S1} 1. {Pos}itive (Code 1 or 2): #
2. Negative

{S1} {Date}: <dd/mm/yy>

Smear 2 {S2} {Type}: S2 1. Sputum (Code 1,2, or 3): #
S2 2. BAL
{S2} 3. {Other} (Specify): _____

{S2} 1. {Pos}itive (Code 1 or 2): #
2. Negative

{S2} {Date}: <dd/mm/yy>

Smear 3 {S3} {Type}: S3 1. Sputum (Code 1,2, or 3): #
S3 2. BAL
{S3} 3. {Other} (Specify): _____

{S3} 1. {Pos}itive (Code 1 or 2): #
2. Negative

{S3} {Date}: <dd/mm/yy>

Culture 1 {C1} {Type}: C1 1. Sputum (Code 1,2, or 3): #
C1 2. BAL
{C1} 3. {Other} (Specify): _____

{C1} 1. {Pos}itive (Code 1 or 2): #
2. Negative

{C1} {Date}: <dd/mm/yy>

Culture 2 {C2} {Type}: C2 1. Sputum (Code 1,2, or 3): #
C2 2. BAL
{C2} 3. {Other} (Specify): _____

{C2} 1. {Pos}itive (Code 1 or 2): #
2. Negative

{C2} {Date}: <dd/mm/yy>

Culture 3 {C3} {Type}: C3 1. Sputum (Code 1,2, or 3): #
C3 2. BAL
{C3} 3. {Other} (Specify): _____

{C3} 1. {Pos}itive (Code 1 or 2): #
2. Negative

{C3} {Date}: <dd/mm/yy>

{Sens}itive to {All} Drugs: <Y>

If NO, Resistant To (R-): {R-INH} <Y>
{R-RIF} <Y>
{R-PZA} <Y>
{R-ETHAM} <Y>
{R-STREP} <Y>
{R-OTHER} (Specify): {1}. _____
{R-OTHER} {2}. _____
{R-OTHER} {3}. _____
{R-OTHER} {4}. _____

{Tr}eatment: {Date} Begun: <dd/mm/yy>

Drugs Used (Tx-): {Tx-INH} <Y>
{Tx-RIF} <Y>
{Tx-PZA} <Y>
{Tx-ETHAM} <Y>
{Tx-STREP} <Y>
{Tx-OTHER} (Specify): {1}. _____
{Tx-OTHER} {2}. _____
{Tx-OTHER} {3}. _____
{Tx-OTHER} {4}. _____
{Tx-OTHER} {5}. _____

Self: <Y> Self%: ### Directed: <Y> Dir%: ###
{Treat}ing {MD}: _____
{MD} {Tel}. No.: <long distance>

Place of {Follow}-up: _____
Hospital/Clinic

{Clin}ic {Tel}. No. <long distance>

Self-Adm <Y>

2. DISEASES, DRUGS AND DISORDERS PREDISPOSING TO TB

Any {Predisp}osing Disorder? <Y>

If YES, Mark All That Apply:

{IV} {D}rug {A}buse: <Y>
{HIV} Sero{pos}itive: <Y>
{HIV} {Risk} Patient: <Y>
{AIDS}: <Y>

{Alcohol}ism: <Y>
{Diabetes} (on insulin): <Y>
{Malig}nancy (current): <Y>
{Rad}iation Therapy: <Y>
{Imm}uno{supp}ressive Therapy: <Y>

{Renal} {Dis}ease: <Y>
Renal {Dialysis}: <Y>
Renal {Transpl}ant: <Y>

{Silic}osis: <Y>
{Steroids}: <Y>
{Gastr}ectomy: <Y>
{GI} {Bypass} Surgery: <Y>
{Malnut}rition (Underweight): <Y>
{W}eigh{t} {Loss} (exceeding 10%): <Y>

{Other} Medical {P}roblems (Specify): {1}. _____
{Other P}rob {2}. _____
{Other P}rob {3}. _____
{Other P}rob {4}. _____
{Other P}rob {5}. _____

3. ANY {MED}ICATION{S}? <Y>

If YES, Specify {Med}ication {Name}s: {1}. _____
{Med Name 2}. _____
{Med Name 3}. _____
{Med Name 4}. _____
{Med Name 5}. _____

Taking {O}ral {C}ontraceptives at Time of Diagnosis? <Y>

{Pregnant} During Treatment? <Y>

4. PAST HISTORY REGARDING TB

{Prior} {TB D}iagnosis? <Y>

If YES, In What {Year} Was It {Prev}iously Diagnosed: 19 ##

In Which {Country} {D}iagnosed: _____

{Prior TB T}reatment? <Y>

If YES, Which Drugs (P-): {P-INH} <Y>
{P-RIF} <Y>
{P-PZA} <Y>
{P-ETHAM} <Y>
{P-STREP} <Y>
{P-OTHER} (Specify): {1}. _____
{P-OTHER 2}. _____
{P-OTHER 3}. _____
{P-OTHER 4}. _____
{P-OTHER 5}. _____

{Prior Con}tact/Known Exposure: <Y>

{Prior} Tuberculin {Tes}t: <Y>

If YES, Was the {Test POS}ITIVE? <Y>
{Millim}eters (if known): ##

{BCG Vacc}ination: <Y>

If YES, How Many {Times Vac}cinated: #
{Age} at Last {Vacc}ination (Years): ##