An Evaluation of
The Role of Tuberculosis DNA Fingerprinting
in Delineation of a Manitoba Outbreak

By

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AN EVALUATION OF THE ROLE OF TUBERCULOSIS DNA FINGERPRINTING IN DELINEATION OF A MANITOBA OUTBREAK

BY

LAWRENCE J. ELLIOTT

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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ABSTRACT

Statement of the Problem: DNA fingerprinting of *M. tuberculosis* (MTB) isolates became available as an epidemiologic tool in 1990, and its value and limitations in tuberculosis (TB) epidemiology are still being assessed. This molecular epidemiologic tool has not previously been applied to tuberculosis epidemiology in Manitoba.

Methods: The study design consisted of the sequential and independent application of both conventional epidemiologic methods and DNA fingerprinting methods in the investigation of a TB outbreak associated with a Manitoba shelter. Transmission patterns involved in the outbreak were first hypothesized based on conventional contact-tracing information. All available shelter-associated MTB isolates were then submitted for restriction fragment length polymorphism (RFLP) typing, and clusters of isolates with identical RFLP patterns were compared to the previously hypothesized transmission patterns, to either support or refute transmission.

Results: Conventional methods revealed that the risk of developing tuberculosis increased markedly with frequency of overnight stays at the shelter. DNA fingerprinting supported the conventionally-hypothesized transmission patterns in the majority (68%) of outbreak-associated cases, and further delineated the outbreak by refuting transmission in some cases and by detecting some previously unsuspected outbreak-related cases.

Conclusions: DNA fingerprinting was found to provide useful insights which complemented conventional outbreak investigation methods in delineating transmission patterns in this outbreak. In addition, the use of RFLP data in conjuntion with conventional data contributed further to understanding the evolution of the outbreak.

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My fellow Graduate Students in Community Health Sciences

1. Introduction

1.1 Background

Although the use of DNA fingerprinting of *Mycobacterium tuberculosis* isolates has been previously applied in outbreak investigations, it is a new technique with much yet to learn about its contributions and limitations to tuberculosis epidemiology. DNA fingerprinting of *M. tuberculosis* has not previously been utilized for epidemiologic purposes in Manitoba. In 1993, Manitoba Tuberculosis Program staff noticed an excess of cases of active tuberculosis (TB) in the users of a Manitoba inner-city shelter for homeless or alcohol-dependent men and women. In this study, both conventional outbreak investigation methods and DNA fingerprinting methods were independently applied in the investigation of the tuberculosis outbreak associated with the shelter, providing an opportunity for evaluation of the new molecular method and comparisons with the results of conventional methods.

1.2 Objectives

The objectives of this study are as follows:

1.21 To define the outbreak in terms of person, place and time, using conventional epidemiologic methods;

1.22 To determine if exposure to the shelter environment during the study period was associated with an increased risk of tuberculosis;

1.23 To form hypotheses regarding outbreak transmission patterns based on conventional epidemiologic methods; and

1.24 To determine if the evidence from DNA fingerprinting subsequently performed on the *M. tuberculosis* isolates associated with the shelter supports or refutes the transmission hypotheses postulated previously by conventional epidemiologic methods, and if this additional evidence is useful in delineation of the outbreak.

2. Review of the Literature

2.1 Global Burden of Tuberculosis

Worldwide, tuberculosis remains the most common single infectious cause of death, responsible for approximately 2.9 million deaths in 1990 (Kochi, 1991). The World Health Organization (WHO) estimates that tuberculosis accounts for 25% of disease-specific preventable mortality and is the leading cause of preventable mortality on the planet (Joseph, 1993). One third of the world's population - approximately 1.9 billion people - are estimated to have been infected with *M. tuberculosis*, with approximately eight million new active cases of tuberculosis annually (Joseph, 1993).

The global burden of tuberculosis weighs most heavily on developing countries. While tuberculosis incidence, prevalence and mortality declined rapidly in most industrialized nations in the last three decades, recent trends have led to great concern among public health officials in several developed countries (Reichmann, 1991 and 1993). In many industrialized countries, the rate of decline in TB incidence has slowed since the mid-1980s, and in the U.S., Japan, and 10 western European countries, tuberculosis incidence has actually increased (Kochi, 1991). It is estimated that between 1985-1991 in the U.S., 28,000 cases in excess of expectation based on previous tuberculosis trends were reported (Joseph, 1993; Reichmann, 1993). This reversal of the previous declining trends in these developed nations has been attributed to a combination of several factors, including the emergence of the acquired immune deficiency syndrome

(AIDS) epidemic and drug-resistant *M. tuberculosis* organisms, reduction in funding for casefinding and treatment, increase in urban poverty and homelessness, increased immigration from high prevalence countries, and decreased physician awareness and knowledge of tuberculosis (Reichmann, 1991 and 1993). The combined impact of these worrisome trends in developed countries, as well as the continuing high rates in most developing countries with the prospect of even higher rates due to the AIDS pandemic, led to the WHO's declaration of tuberculosis as a global emergency in 1993 (World Health Organization, 1993).

2.2 Burden of Tuberculosis in Canada

While Canada continues to report one of the world's lowest tuberculosis rates, this rate has stopped declining and has levelled off at approximately 7.0 to 7.5 cases of active tuberculosis per 100,000 persons per year since 1988 (Statistics Canada, 1993). Tuberculosis in Canada is concentrated in certain geographic regions, with those census divisions with very high rates (over 20 cases per 100,000 per year) being concentrated in the northern regions of the country, and those census divisions with moderately high rates (10-19 per 100,000) being located mainly in major metropolitan areas (Gaudette and Ellis, 1993). In the last 10 years, incidence rates were generally below the Canadian average in the Maritime Provinces (except Newfoundland), close to the Canadian average in Newfoundland, Quebec, Ontario and Alberta, and above the average in Manitoba, Saskatchewan, B.C. and the two northern Territories (Health and Welfare Canada, 1992).

The highest regional rates are observed in the North West Territories, followed by the Yukon Territory (Health and Welfare Canada, 1992).

Tuberculosis cases have been concentrated in four major risk groups in Canada: Aboriginal Canadians, immigrants from high-prevalence countries, residents of areas with low socio-economic status in major Canadian cities, and the elderly (Standards Committee of the Canadian National Tuberculosis Conference, 1987). The proportion of all Canadian tuberculosis cases originating in the immigrant or Aboriginal risk groups has been increasing since at least 1977 (Gaudette, 1989), reaching levels of 53% for immigrant groups and 19% for aboriginal groups by 1993 (Statistics Canada, 1993). Although the number of Canadians co-infected with the human immunodeficiency virus (HIV) and *M. tuberculosis* has not yet been precisely estimated due to the problem of under-reporting of tuberculosis in HIV-infected persons, Canadian TB authorities believe the rate of co-infection is very low relative to that in the U.S. (Health and Welfare Canada, 1992). As well, a very small proportion of all *M. tuberculosis* isolates in Canada has been found to be resistant to the commonly-used anti-tuberculous drugs, with these infections occuring almost exclusively in immigrants (Long et al, 1993). However, due to Canada's continuing pattern of immigration from high prevalence countries, the presence of current population groups (such as the Aboriginal population) with documented high prevalence of TB infection, and the prospect of increasing HIV coinfection, TB authorities are predicting that tuberculosis will continue to be a major problem in Canada (Fitzgerald, 1994).

2.3 Burden of Tuberculosis in Manitoba

Manitoba's overall tuberculosis incidence has roughly paralleled that of Canada as a whole, generally declining over the last three decades, and apparently levelling off at approximately 90 to 110 cases per year since 1988 (Statistics Canada, 1993; Sanitarium Board of Manitoba, 1994). This represents an incidence rate of approximately 9.0 per 100,000 population, slightly higher than the national rate. The major group at risk for tuberculosis in Manitoba is the Aboriginal population, with the proportion of total Manitoba cases occuring among Treaty Status persons increasing gradually from approximately 25% in 1980 to approximately 35% in 1993, and with Treaty and Nontreaty Aboriginals comprising 50% of all Manitoba cases in 1993 (Sanitarium Board of Manitoba, 1994). In contrast to the national situation, cases among recent immigrants comprised only 19% of the total Manitoba cases in 1993. Coinfection of HIV and TB had only been observed in one case in Manitoba by 1993 (personal communication, E.S. Hershfield, Director of Tuberculosis Control, Manitoba, 1995), and multi-drug resistance has not been a significant problem in Manitoba (Long et al, 1993).

2.4 Tuberculosis in the Homeless and Temporary Shelter Users

Tuberculosis has been documented to disproportionately affect homeless persons, clients of temporary shelters, and residents of single-room occupancy hotels since early in this century (Centers for Disease Control and Prevention - CDC- 1992; Lerner, 1993).

For example, the tuberculosis death rate among homeless persons and transients in New York City was documented at approximately 350 per 100,000 per year in 1930 (Lerner, 1993). A definition of "homeless" adopted for epidemiologic studies is: persons who do not have customary and regular access to a conventional dwelling or residence (CDC, 1992). The incidence of active tuberculosis in homeless persons has been estimated as being 10 to 50 times greater than that of the general population (Sherman et al, 1980; Patel et al, 1985; Slutkin et al, 1986; Nolan et al, 1991). Screening programs at selected shelters and clinics have found active tuberculosis prevalences of 1.6% to 6.8% in this population, and latent infection prevalences of 18% to 51% (Sherman et al, 1980; Slutkin et al, 1986; CDC, 1985; Barry et al, 1986).

The major demographic group comprising the homeless has historically been middle-aged men in the inner-city areas of large metropolitan centres, and tuberculosis in this group was until recently believed to consist almost exclusively of reactivation of latent infection (Nolan et al, 1991). However, frequent transmission of infection has been shown to occur within this population, including among individuals with a history of previous latent or active infection (CDC, 1985; Barry et al, 1986; Nardell et al, 1986). Several outbreaks of active tuberculosis have been associated with shelters, with identified contributing factors including crowding of clients and suboptimal ventilation, especially in winter (Nardell et al, 1986; Scheffelbein and Snider, 1988; Riley and Nardell, 1989; Nolan et al, 1991; Paul et al, 1993). Delayed diagnosis, a high incidence of cavitary, smear-positive tuberculosis, and non-compliance with treatment are additional features of tuberculosis associated with this population, further facilitating transmission

(Riley and Nardell, 1989; CDC, 1992).

2.5 DNA Fingerprinting of M. tuberculosis

Due to the re-emergence of tuberculosis as a major public health problem in recent years, the search for new technological tools to aid in the fight against the disease has intensified. One such tool that has been developed and increasingly applied since 1990 is the molecular method of DNA fingerprinting of isolates of *M. tuberculosis* to identify individual strains of the organism (Otal et al, 1991; Van Soolingen et al, 1991; Gicquel, 1993). Before 1990, phage typing was the only subtyping method available for *M. tuberculosis,* and this method did not gain widespread use due to its technical difficulty and lack of discriminatory power (Gicquel, 1993). The discovery of an insertion sequence now called *IS6110,* which is present in variable numbers and positions in the genome of different strains of MTB, led to the development of a new DNA fingerprinting method for strain identification (Van Soolingen et al, 1991). This method, which has gained wide acceptance in producing a discriminating "DNA fingerprint" from *M. tuberculosis* genetic material, is called **restriction fragment length polymorphism (RFLP) typing** (Van Soolingen et al, 1991; Van Embden et al, 1993). This term derives from the polymorphisms in the length of DNA fragments generated when the genetic material extracted from the isolates is digested by restriction enzymes and then separated by gel electrophoresis. When a labelled probe which attaches to the insertion sequence is added to the gel, a characteristic RFLP pattern, or DNA fingerprint, is generated.

It has been shown that distinct *M. tuberculosis* strains reliably generate unique RFLP patterns, presumably since the *IS6110* insertion sequence has transposed frequently enough over many years that it occurs in different numbers and positions in distinct strains (Van Soolingen et al, 1991). The RFLP pattern produced from a particular strain has also been found to remain essentially the same over a period of a few years, suggesting that the insertion sequence does not move frequently enough to make the pattern unstable. Thus, the RFLP method using the IS6110 probe is felt to be sufficiently stable and yet discriminating to provide useful results for epidemiologic analysis, and it was this standardized method which was recommended for general use at a recent Consensus Conference convened by the National Institutes of Health in the U.S.(Van Embden et al, 1993).

2.6 Epidemiologic Applications of DNA Fingerprinting of M. tuberculosis

The theoretical and empirical basis for applying the new molecular epidemiologic technique of RFLP analysis to tuberculosis is that clusters of isolates with identical RFLP patterns are believed to indicate recent transmission in most instances, as opposed to reactivation of tuberculosis (Genewein et al, 1993; Hamburg and Frieden, 1994). Evidence to support this concept has accumulated from the application of RFLP typing to outbreak investigations. The method was first used to complement conventional epidemiologic methods in investigating a small community outbreak of tuberculosis in Holland in 1991: all isolates from epidemiologically-linked cases were found to have an

identical RFLP pattern, whereas unrelated cases had different RFLP patterns (Van Soolingen et al, 1991). Using the same approach, Pearson et al (1992) used RFLP typing to confirm transmission of a multi-drug resistant strain of *M. tuberculosis* within a hospital. Daley et al (1992) used the method to confirm rapid transmission of the same strain among eleven HIV positive people in a residential facility in a period of 106 days.

Increasingly, the RFLP method is being used in population studies of large numbers of *M. tuberculosis* isolates from defined geographic areas to determine transmission patterns. In general, studies of isolates from developing countries have revealed clustering of identical isolates, suggesting recent transmission, while isolates from industrialized countries have been characterized by heterogeneity of RFLP patterns, suggesting infrequent transmission and frequent reactivation (Genewein et al, 1993). It has been shown that in Holland, where the incidence of tuberculosis is declining, all epidemiologically unrelated *M. tuberculosis* isolates have unique RFLP patterns (Hermans et al, 1990). These studies seemed to confirm the long-held suspicion that about 90 percent of active tuberculosis cases in industrialized countries result from reactivation of latent infection (Hamburg and Frieden, 1994).

However, two recent RFLP studies of large numbers of isolates from tuberculosis cases in San Francisco (Small et all, 1994) and the Bronx borough of New York City (Alland et al, 1994) revealed that fully one-third of the isolates were in clusters of identical patterns. The authors of both studies argue that this degree of clustering indicates recent transmission in approximately one-third of the tuberculosis cases in their populations. Both studies first defined their clusters based on identical RFLP patterns,

and then analyzed the demographic and other characteristics of the clustered cases, compared to the non-clustered cases. Both studies found that HIV infection or AIDS was the one factor most strongly associated with the clustered cases, indicating that the majority of tuberculosis cases in these HIV-infected patients resulted from recent transmission rather than reactivation. Other factors associated with recent transmission in these studies included living in poverty, belonging to certain ethnic or racial groups, being of younger age, and being infected with a drug-resistant strain of TB. The fact that the ethnic/racial groups associated with recent transmission were different in the two studies (Hispanic in San Francisco, Black in New York) was believed by Hamburg and Frieden (1994) to indicate that transmission was related to socioeconomic factors rather than to race itself.

2.7 Application of DNA Fingerprinting to Shelter-Associated Tuberculosis Outbreaks

Although outbreaks of tuberculosis in association with shelters for the homeless have previously been described, the use of molecular epidemiologic methods in investigation of tuberculosis associated with shelter users has been reported in only one study. Dwyer et al (1993) used RFLP analysis to confirm that isolates were identical from 18 of 19 cases occurring over a seven year period in men using shelters for the homeless in Melbourne, Australia. No comparison of conventional epidemiologic methods with the molecular method was made in the study.

3. Design and Methods

3.1 Study Design

The basic design of the study was a sequential application of conventional epidemiologic investigational methods followed by the new molecular epidemiologic method of DNA fingerprinting in the investigation of the outbreak, allowing comparison and evaluation of the epidemiologic contributions of the new method. The conventional and molecular methods were applied independently of each other. In other words, the conclusions and hypothesized transmission patterns arising from the conventional methods were stated before the DNA fingerprinting was performed, and the information gleaned from the conventional investigation was not made available to the laboratory performing the DNA fingerprinting.

The basic steps in the conventional outbreak investigation methods, to be described in more detail below, were: defining the study period, defining the study population, establishing case definitions, ascertaining cases, confirming that an outbreak occurred, describing cases in terms of person, place and time, and forming hypotheses regarding important exposure factors and transmission patterns. For purposes of this study, an outbreak is defined as a noticeable increase in the number of new cases of tuberculosis in the population under study, in comparison to the usual incidence in the same population.

Following the completion of the conventional outbreak investigation methods,

isolates of *M. tuberculosis* were forwarded for DNA fingerprinting to the laboratory of Dr. Dennis Kunimoto, in the Department of Medical Microbiology and Infectious Diseases, at the University of Alberta, Edmonton, Alberta. Two groups of isolates were sent for analysis: all available isolates from tuberculosis cases ascertained in the conventional investigation, as well as a sample of Manitoba *M. tuberculosis* isolates to serve as a "background" comparison group.

3.2 Study Hypotheses

The two major study hypotheses are as follows:

3.21 Exposure to the shelter environment was associated with an increased risk of tuberculosis; and

3.22 The pattern of tuberculosis transmission as determined by conventional epidemiologic methods will be supported by evidence from DNA fingerprinting of *M. tuberculosis* isolates.

3.3 Study Period

The period of time chosen for case ascertainment was January 1, 1990 to December 31, 1993. This period was chosen so as to include a pre-outbreak period as well as the outbreak duration.

3.4 Study Population

The study population from which cases were ascertained consisted of clients/users of the shelter during the study period, shelter staff, and potential tuberculosis contacts of these persons. For the purpose of the investigation, the definition of tuberculosis contacts used was that used by the Manitoba Tuberculosis program: all persons with whom a diagnosed tuberculosis patient has been in contact, at home, at work, or socially for a close and prolonged period of time since the diagnosed patient first developed the symptoms of tuberculosis (MacMorran, 1990).

The shelter is located in the inner-city Main Street area of the City of Winnipeg (pop. 600,000). This area has a high concentration of older hotels and beverage rooms. The shelter is the main facility used by the city's homeless, as well as by intoxicated persons brought in by police for short-term detoxification. Alcohol-dependent and homeless persons comprise the majority of shelter clients, who at the time of the study slept in two large rooms on mattresses spaced approximately 12 inches apart. There was also a common lounge area where clients could go to smoke and drink coffee. Meals were not served at the shelter. The staff offices and meeting rooms were in a separate area; however, this area shared a common ventilation system. Ventilation was provided by two rooftop forced-air units using 75-85% recirculated air. The 1992-93 annual report for the shelter reported that the shelter served an average of 84 clients per day, including an average of 46 in the common sleeping areas. The nine single room occupancy hotels in the inner-city Main Street area each housed an average of about 40 registrants per day, as reported by their managers.

3.5 Case Definitions

The following case definitions were used in case ascertainment (with the definition of a "contact" as defined in Section 3.4, above) :

SHELTER USER CASES: cases of active tuberculosis diagnosed between January 1, 1990 and December 31, 1993, among people who stayed at the shelter for one or more days during this period.

CONTACT CASES: cases of active tuberculosis diagnosed during the study period among the identified (non-shelter using) contacts of the Shelter User Cases.

SHELTER STAFF CASES: cases of active tuberculosis diagnosed during the study period among the shelter staff.

New infections with MTB (i.e. tuberculin converters who did not develop active tuberculosis) were not assessed in this study, as comprehensive tuberculin screening and followup was not logistically possible in the shelter-using population due to a high level of transiency, as found in previous studies (CDC, 1992).

3.6 Sources of Data

3.61 Manitoba Tuberculosis Registry Data

The Manitoba TB Registry maintains a database containing the demographic, diagnostic, treatment and contact information on all cases of TB diagnosed in Manitoba. For each case of tuberculosis diagnosed in Manitoba, TB Registry staff arranges for a list of contacts to be compiled (MacMorran, 1990). "Contacts" are defined as all persons with whom the diagnosed patient has been in contact, at home, at work or socially for a close and prolonged period since the diagnosed patient first developed the symptoms of tuberculosis. This contact list is prepared in consultation with the case-patient, staff at the Respiratory Hospital in Winnipeg where new patients are admitted, and local public health officials. Contact followup consists of surveillance for onset of symptoms of tuberculosis as well as tuberculin skin tests and/or chest x-ray at the time of identification and again approximately three months later.

3.62 Shelter Data

The shelter maintains a computerized database of all clients who have used the shelter's services. A change in the shelter's computer software system and data storage policy at the beginning of 1992 resulted in a limitation in the data available for this study: a list of all persons who used the shelter was available for the entire 1990-93 study period, whereas demographic data and shelter use frequency data for shelter users were available only for 1992 and 1993. A list of all shelter staff during the 1990-93 period

was also available.

3.63 *M. tuberculosis* Isolate Data

The Health Sciences Centre Mycobacteriology Laboratory in Winnipeg maintains a bank of *M. tuberculosis* isolates for all positive specimens sent in for culture to their lab; this includes isolates from all culture-confirmed cases of tuberculosis diagnosed in Manitoba. These isolates are coded by stock number, and the date of the positive culture is recorded.

3.64 RFLP Data

These data consisted of a list of matching and unique isolates, coded by the stock number described in the previous section, as determined in the laboratory of Dr. Dennis Kunimoto. Further details on these data are given in the DNA Fingerprinting Methods section, below.

3.7 Case Ascertainment

Ascertainment of **shelter user cases** was conducted by cross-referencing Manitoba TB Registry lists of active TB cases diagnosed during the study period with the shelter's registration list for the same period. **Contact cases** were ascertained by reviewing TB Registry contact lists for each of the above-identified shelter cases, to identify all such contacts who developed active TB during the study period. **Shelter staff**

cases were ascertained by cross-referencing TB Registry case lists with shelter staff lists for the study period. The **total shelter-associated case pool** was comprised of all shelter user cases, contact cases and shelter staff cases.

3.8 Data Extraction and Analysis of Conventional Epidemiologic Data

3.81 Descriptive Epidemiology of Cases

Demographic, clinical and bacteriologic data on all cases were extracted from TB Registry records, and entered into an Epi Info (CDC, Epi Info Software Package, Version 6.02) database for further analysis. Once the above database for all cases was assembled, the outbreak was analyzed in terms of time (generating an epidemic curve), place (generating a schematic map of cases by place of residence) and person (generating a table of demographic, clinical and bacteriologic characteristics of cases).

3.82 Analysis of Exposure to Shelter

Shelter use frequency by shelter user tuberculosis cases was compared to shelter users who did not develop TB, to determine if shelter exposure was associated with an increased risk of developing active TB. Shelter use episodes were restricted to those involving overnight stay at the shelter. Frequency of shelter use over the 1992-93 period was categorized into four categories: 1 day; 2-24 days; 25-50 days, and; more than 50 days. The rate of tuberculosis observed for each of these four categories was expressed as the number of cases per 1000 users, and the rate ratio using the "1 day" category as the

referent, was calculated for each category.

3.83 Transmission Patterns Hypothesized from Conventional Data

The available information from a review of TB Registry contact lists for all cases was used to generate hypotheses about transmission patterns involved in the outbreak. These hypotheses were then depicted schematically on a map of shelter-associated cases, using double-headed arrows to represent confirmed epidemiologic links (contacts). Clusters of epidemiologically linked cases (such as a socially-interacting group among the homeless cases) were depicted with labels (e.g. "Homeless Cases") on the figure.

3.84 Database Management and Statistical Analysis

Demographic, clinical and bacteriologic data on all cases was entered, stored and analyzed using the Epi Info software package. Shelter registration data was downloaded from the shelter's computer into an ASCII datafile, then stored and analyzed using the PC-SAS (SAS Institute, Cary, NC) software package.

Tests of statistical significance used to test for differences between groups included the student's t-test, for continuous variables, and the chi-square test (or Fisher's exact test, where expected cell sizes were less than 5), for categorical variables. The chi-square test for linear trend was used to test the significance of an observed trend in risk of tuberculosis associated with increasing exposure to the shelter. All tests used a significance criterion of $p<.05$.

3.9 DNA Fingerprinting Methods

3.91 Selection of Isolates for DNA Fingerprinting

Two categories of isolates were selected for DNA fingerprinting: all available isolates from the shelter-associated total case pool, and a "background" comparison group selected from Manitoba tuberculosis cases not associated with the shelter. Isolates from all 68 culture- confirmed shelter-associated cases were submitted for analysis. Cost restrictions limited the number of "background" isolates which could be tested; it was decided to submit all available non-shelter associated Manitoba isolates from the entire 1992-93 period, to assess the background distribution of RFLP patterns for the period encompassing the peak of the outbreak. There were 143 background isolates submitted from the 1992-93 period. The isolates were prepared by the Health Sciences Centre Mycobacteriology Laboratory, and submitted to the laboratory of Dr. D. Kunimoto, Department of Medical Microbiology and Infectious Diseases, University of Alberta, Edmonton, for DNA fingerprinting.

3.92 Laboratory generation of RFLP patterns

The widely-accepted and internationally-standardized RFLP method (Van Embden et al, 1993) was used, in the following basic steps. First, the MTB DNA was extracted from each isolate by vortexing in the presence of siliconized glass beads followed by successive phenol/chlorm extractions and ethanol precipitation (Palittapongarnpim et al, 1993). The DNA was then digested with the restriction enzyme

PvuII and electrophoresed on a 0.8% agarose gel. Molecular weight markers and an arbitrarily chosen control strain were included in every gel to serve as a standard and ensure uniformity among gels. The separated DNA fragments were then transferred from the gels to nylon filters, cross-linked by UV light and probed with the IS*6110* insertion sequence probe (Thierry et al, 1990; Mazurek et al, 1991). Next, IS*6610* bands were visualized using a chemiluminescent method (Boehringer Mannheim) and autoradiography.

3.93 Matching of RFLP patterns

Identification of isolates with matching RFLP patterns also was performed by Dr. Dennis Kunimoto. The RFLP patterns for all isolates were digitized by the IMAGER video camera system (Appligene, Illkirch, France). Digitized gel images were then analyzed using *Gelcompare* computer software (Applied Maths, Kortrijk, Belgium). In this procedure, the gel lanes were first identified and any distortions corrected. Then the gel was normalized by standardizing a reference lane to a standard, in order that patterns from different gels could be compared to each other. Then, two rounds of comparison of RFLP patterns were performed. The first relied on computer chosen patterns and a Pearson correlation coefficient to all possible track pairs. RFLP patterns were designated as clustered using the unweighted pair group method using arithmetic averages (UPMGA). The second round of comparisons involved confirming and marking every band present in a lane, for every lane. A Dice coefficient was generated for each possible pair of patterns, and a dendrogram was generated by UPMGA. All isolates matched as

"identical" or "similar"(one band difference) by the computer software were manually confirmed, i.e. the lanes on the original autoradiographs were compared to each other visually (written communication, D.Kunimoto, June 1995).

3.94 Analysis of RFLP Data

Once the RFLP patterns of the submitted isolates were determined by Dr. D. Kunimoto, these data were then analyzed in this study in several steps. First, the clusters of matching isolates were labelled as RFLP Patterns A, B, C, and so on. Those isolates whose RFLP pattern did not match any other isolates were labelled as "U", for "unique". Next, the distribution by RFLP Pattern for all shelter-associated isolates and all background Manitoba isolates was tabulated. Finally, the time distribution of the RFLP patterns among the shelter associated cases was plotted. The time distribution of the two most frequently observed RFLP patterns among the shelter-associated isolates were plotted and analyzed separately.

3.95 Comparison of Conventional Epidemiologic Data With RFLP Data

The RFLP pattern label for each shelter-associated case isolate was added to the map of hypothesized transmission patterns previously generated using conventional epidemiologic methods. This allowed direct comparison of the two methods, to determine if the molecular epidemiologic method supported or refuted the transmission hypotheses generated by conventional methods. This comparison was summarised quantitatively in a two-by-two table, allowing calculation of the estimated sensitivity and

positive predictive value (PPV) of the conventionally-hypothesized transmission links, using RFLP-confirmed transmission links as the "gold standard". The distribution of clustered RFLP patterns for the shelter-associated cases was then compared to that of the non-shelter-associated Manitoba isolates from the 1992-93 period. Finally, the demographic characteristics associated with the cases categorized by RFLP pattern were determined.

4. Results of Conventional Epidemiological Methods

4.1 Characteristics of Cases

A total of 84 cases of active tuberculosis were determined to be epidemiologically associated with the shelter during the 1990-93 study period, based on the case definitions and ascertainment methods described in the previous section. This total case pool was comprised of **58 shelter user cases, 24 contact cases**, and **2 shelter staff cases**. Demographic and bacteriologic characteristics of the 84 shelter-associated cases are tabulated in **Table 1**. Males comprised 69% of cases, and females comprised 31% of cases. The female cases were younger, on average, than the male cases (female mean age 27 years, male mean age 41 years, $p<.001$). Treaty (as defined by the Indian Act) and Nontreaty Aboriginal persons comprised 66 (80%) of all cases, with the remaining 18 cases being classified as Caucasian or "Other". Thirty-eight (46%) of the cases lived in inner-city private housing, while 19 (23%) lived in one of the single room occupancy hotels along Main Street, 13 (16%) were homeless and 12 (14%) lived primarily on an Aboriginal reserve. Fifty-four (93%) of the male cases and 22 (85%) of the female cases had a recorded history of alcohol abuse.

Comparing the contact cases to the shelter user cases, the contact cases were younger (mean age 30.9 years, vs. 39.2 years in shelter users, $p<.05$), and included five children under age 12. Contact cases were also more likely to be female (50%, vs 24% of shelter users, $p<.05$). Contact cases had a similar race/ethnicity distribution as shelter user cases, with 79% classified as Treaty or Nontreaty Aboriginal, and 21% Caucasian or

"Other".

Overall, 80% of cases were culture-positive for tuberculosis, with 24 (41%) of the males and 8 (31%) of the females also being sputum smear-positive for acid fast bacilli (AFB) at the time of diagnosis.

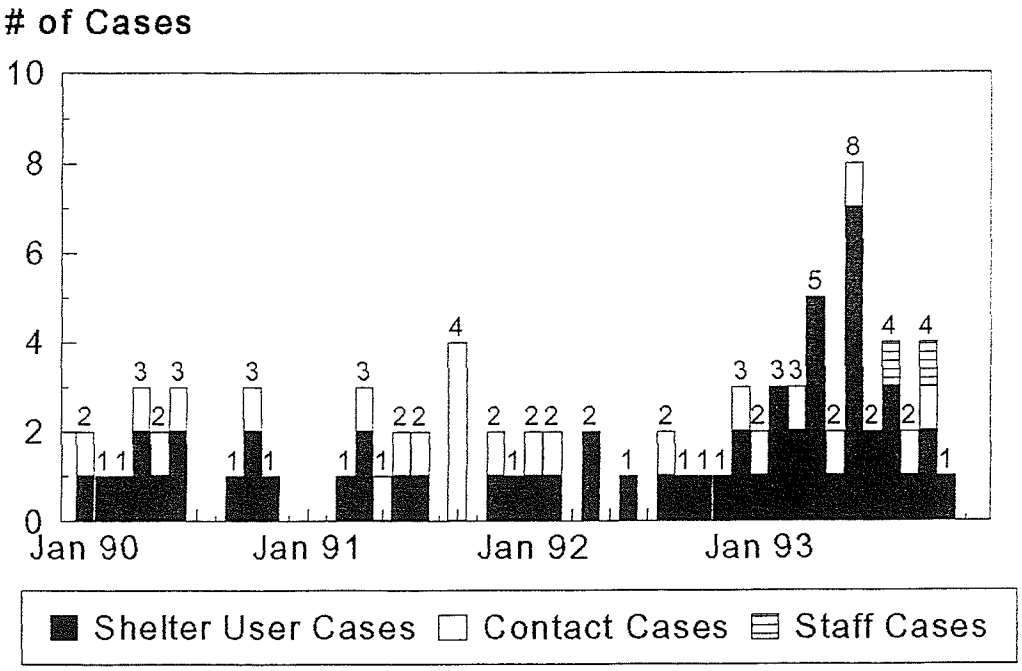
Table 1. Characteristics of 84 shelter-associated cases, 1990-93

	MALES		FEMALES		TOTAL	
	n	%	n	%	n	%
1. GENDER	58	100	26	100	84	100
2. AGE						
0-19 YR	2	3	5	19	7	8
20-39 YR	27	47	18	69	45	54
40-59 YR	21	36	3	12	24	28
>59 YR	8	14	0	0	8	9
3. ETHNICITY						
Treaty Aboriginal	32	55	20	77	52	63
Nontreaty Aboriginal	9	16	5	19	14	17
Caucasian/Other	17	29	1	4	18	20
4. RESIDENCE						
Homeless	8	15	5	19	13	16
Hotels	16	29	3	11	19	23
Inner City	24	43	14	52	38	46
Reserves	7	13	5	18	12	14
5. SPUTUM						
Smear Positive	24	41	8	31	32	38
Culture Positive	47	81	20	77	67	80
6. ALCOHOL ABUSE						
Positive History	54	93	22	85	76	91
7. CASE TYPE						
Shelter User	44	76	14	54	58	69
Shelter Staff	2	3	0	0	2	2
Contact Case	12	21	12	46	24	29

4.2 Time Distribution of Cases

The time distribution of the 84 shelter-associated cases over the four year period 1990-1993 inclusive is shown in **Figure 1.** There were 17 shelter-associated cases in 1990, 16 in 1991, 15 in 1992 and 36 in 1993.

Figure 1. Epidemic Curve of Shelter-Associated Cases, 1990-1993



As seen in **Figure 1**, there was an average of about one case of active TB per month among shelter users during 1990 to 1992, with a distinct increase beginning at the end of 1992, peaking at seven cases in the month of June 1993, then returning to the baseline by November 1993. There was a total of 28 cases among shelter users in 1993.

Figure 1 also illustrates that contact cases occurred at an average rate of less than one per month throughout the study period, with the exception of September 1991, when a spike of four contact cases occurred. The two shelter staff cases occurred towards the end of the 1993 peak, in August and October 1993, respectively.

4.3 Comparison of Shelter User Cases to General Shelter Using Population

To further examine the characteristics of the shelter user cases occurring in the 1992-93 outbreak period, comparisons were made with the general shelter-using population for the same period (**Table 2**).

Table 2. Characteristics of General Shelter-Using Population, 1992-93

	MALES		FEMALES		TOTAL	
	n	%	n	%	n	%
1. GENDER	5558	75	1827	25	7385	100
2. AGE						
0-19 YR	383	7	128	7	511	7
20-39 YR	3486	63	1218	67	4704	64
40-59 YR	1339	24	397	22	1736	24
> 59 YR	315	6	70	4	385	5
Unknown Age	-	-	-	-	56	1
3. ETHNICITY						
Treaty Aboriginal	1957	35	1008	55	2965	40
Nontreaty Aborig.	687	12	273	15	960	13
Caucasian/Other	2491	45	369	20	2860	39
Unknown	423	8	177	10	607	8

As seen in **Table 2,** the population of 7385 shelter users who stayed at the shelter on at least one day in 1992-93 consisted predominantly of males (75%) between 20-59 years of age (mean age 34.4 years). Treaty and Nontreaty Aboriginals made up 53% of the population, while Caucasion and "other" races made up the rest of those for whom race was recorded on admission to the shelter. By comparison, the 39 shelter-using TB cases in 1992-93 had a similar gender distribution (79% male), but were older (mean age 38.5 years, $p<.01$) and more likely to be Aboriginal (92%, $p<.001$) than the general shelter-using population.

4.4 Association of Frequency of Shelter Use With Risk of TB

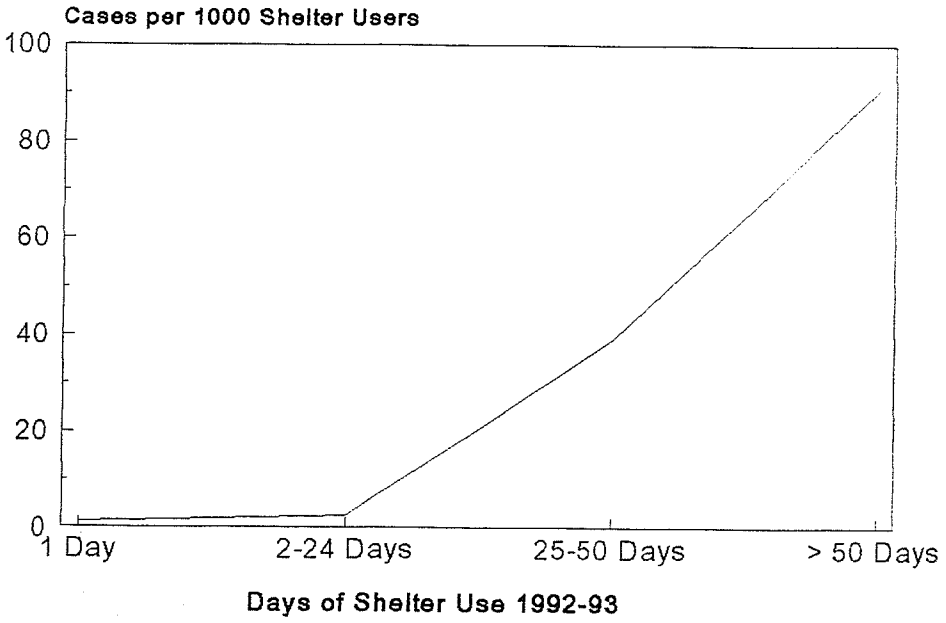
Another characteristic of shelter user cases compared to the general shelter using population for 1992-93 was the frequency of shelter use, i.e. exposure to the shelter. **Table 3** compares the distribution of shelter use frequency for all shelter users with that of the shelter-using TB cases diagnosed in 1992-93. This comparison allowed the calculation of the relative risk of TB for four categories of shelter use frequency: one day, 2-24 days, 25-50 days, and more than 50 days of shelter use over the two year period. A marked increase in risk of TB was observed with increasing frequency of shelter use, with those persons using the shelter on more than 50 days in the 1992-93 period having a relative risk of TB of 82 (95% C.I. 27-245) when compared to the reference category of those using the shelter on only one day. This trend of increased risk with increasing

exposure to the shelter was found to be significant ($p<.0001$, chi-square test for linear trend), and is graphically illustrated in Figure 2.

Table 3. Shelter Use Frequency, TB Cases Compared With All Shelter Users, 1992-93

Days Using Shelter	Total Shelter Users	TB Cases	Cases per 1000 Users	Rate Ratio (95% CI)
1 Day	3597	4	1.1	1.0
2-24 Days	3488	9	2.6	2.3 (0.7-7.5)
25-50 Days	153	6	39.2	35 (10-124)
> 50 Days	154	14	90.9	82 (27-245)

Figure 2. Risk of TB by Frequency of Shelter Use, 1992-1993



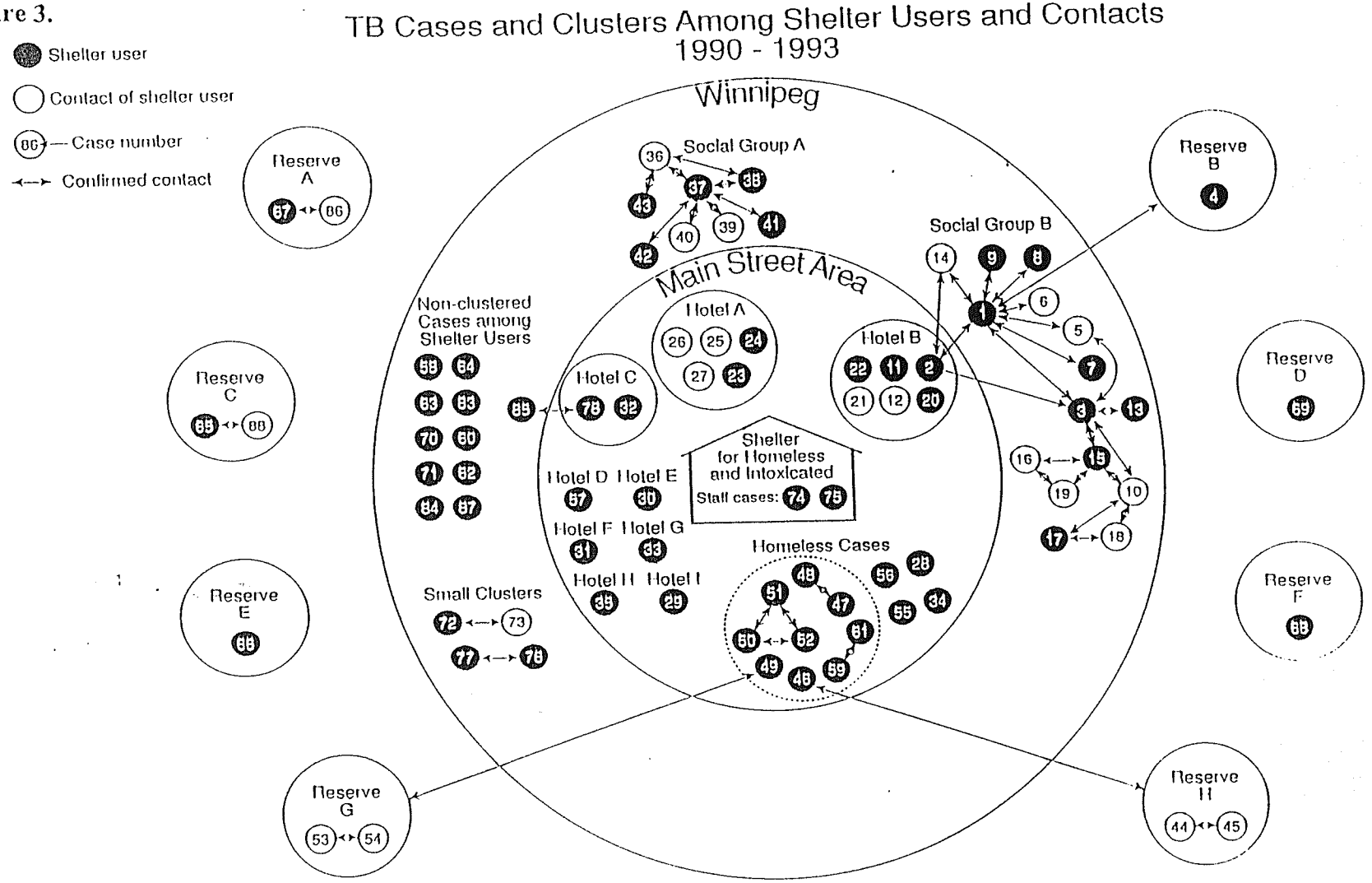
4.5 Proportion of Winnipeg and Manitoba TB Cases Associated With Shelter

Shelter-associated cases comprised a significant proportion of all cases of tuberculosis diagnosed in Winnipeg and Manitoba residents, respectively, during the study period, as illustrated in **Table 4**. This was especially so in 1993, when outbreak-associated cases comprised 45% of all Winnipeg TB cases and 33% of all Manitoba cases. Overall during the four year 1990-93 period, shelter-associated cases comprised 28% of all Winnipeg TB cases and 22% of all Manitoba TB cases.

Table 4. Proportion of Winnipeg and Manitoba TB Cases Associated with Shelter

YEAR	WINNIPEG			OUTSIDE WINNIPEG			MANITOBA		
	Total Cases	Shelter Assoc.	% of Total	Total Cases	Shelter Assoc.	% of Total	Total Cases	Shelter Assoc.	% of Total
1990	62	16	26	30	1	3	92	17	18
1991	65	14	22	36	2	6	101	16	16
1992	59	11	19	27	4	15	86	15	17
1993	69	31	45	39	5	13	108	36	33
Totals	255	72	28	132	12	9	387	84	22

Figure 3.


TB Cases and Clusters Among Shelter Users and Contacts
1990 - 1993
Shelter user
Contact of shelter user
Case number
Confirmed contact
Winnipeg
Main Street Area
Reserve A
Reserve B
Reserve C
Reserve D
Reserve E
Reserve F
Reserve G
Reserve H
Social Group A
Social Group B
Non-clustered Cases among Shelter Users
Hotel A
Hotel B
Hotel C
Hotel D
Hotel E
Hotel F
Hotel G
Hotel H
Hotel I
Shelter for Homeless and Intoxicated
Staff cases:
Homeless Cases
Small Clusters

4.6 Hypothesized Transmission Patterns of Shelter-Associated Cases

Figure 3 schematically depicts the distribution of the 84 shelter-associated cases by place of residence (or occupation, in the two shelter staff cases) as well as the hypothesized transmission patterns based on conventional epidemiologic evidence. Each small circle represents a case, with filled circles representing shelter user or staff cases, and unfilled circles representing contact cases. As previously summarized in **Table 1**, there were 13 cases in homeless people, 19 cases among residents of nine hotels in the Main Street Area, 38 cases living in residential housing of inner-city Winnipeg, and 12 cases living primarily on one of eight Aboriginal reserves remote from Winnipeg.

Epidemiologically-linked cases and clusters are schematically depicted in **Figure 3** with double-headed arrows. These arrows represent epidemiologic links identified from contact list information. Three large clusters of epidemiologically-linked cases were identified and labelled as "Social Group A" (a group of eight friends, including one married couple: case numbers 39 and 40), "Social Group B" (a group of eight friends plus eight additional family members, including children) and the "Homeless Cases" cluster (a group of nine homeless people who were in frequent close contact.) The Hotel A and Hotel B clusters represent hypothesized clusters of 5 and 6 cases, respectively. The epidemiologic link depicted with Reserve B represents frequent visits to Winnipeg by case number 4, to socialize with members of Social Group B. In contrast, the links depicted with Reserves G and H represent visits by homeless case numbers 49 and 46, respectively, back to their community of origin, where they were in contact with family

members. Finally, the hypothesized transmission on Reserves A and C represents situations where an adult reserve member who had stayed in the shelter while in Winnipeg subsequently developed active TB, and was in contact with a family member who also subsequently developed TB.

Together, all of the clusters of two or more cases depicted in **Figure 3** represent the hypothesized transmission patterns based on all conventional epidemiological evidence, to be compared subsequently to evidence obtained from RFLP typing.

5. Results of RFLP Analysis

5.1 Distribution of RFLP Pattern Types of Shelter-Associated Cases

Isolates from 68 of the 84 (81%) shelter-associated cases were available for RFLP typing. The remaining 16 cases were culture-negative for *M. tuberculosis*. The results of the RFLP typing are summarized in **Table 5.** Six different clusters of matching RFLP patterns were identified for the four year 1990-93 period, with cluster sizes ranging from 2 to 33 isolates. These six clusters were arbitrarily given the labels "Pattern A" to "F", with Pattern A representing 33 isolates, Pattern B representing 15 isolates, Pattern C representing 3 isolates, and Patterns D,E and F representing two isolates each. Four of the 33 isolates in the Pattern A cluster had an RFLP pattern which differed by only one band from the other 29 isolates, and thus were included in this cluster, as discussed in **Methods**. Similarly, the RFLP pattern of two of the 15 Pattern B isolates differed by only one band from the rest, and thus were included in this cluster. The isolates within the remaining four clusters were all identical to eachother.

As seen in **Table 5**, the Pattern A strain comprised nearly half (49%) of all available isolates for the entire 1990-93 study period, and comprised the majority (68%) of available isolates from the 1992-93 outbreak period. Also apparent in **Table 5** is that while the Pattern B strain comprised 22% of available isolates from the entire study period, it accounted for only 5% of isolates from the 1992-93 outbreak period, and thus was not strongly associated with the outbreak.

Unique RFLP pattern strains accounted for 12% of isolates from the 1992-93 period, and comprised 16% of isolates from the entire 1990-93 study period. This means that the remaining 88% of the shelter-associated isolates from the 1992-93 outbreak period, and the remaining 84% of the isolates from the entire study period, were clustered by RFLP type.

Table 5. RFLP Patterns of Shelter-Associated Isolates, 1990-93 and 1992-93

RFLP Pattern	1990 - 1993		1992 - 1993	
	Number	%	Number	%
A	33	49	28	68
B	15	22	2	5
C	3	4	2	5
D	2	3	0	0
E	2	3	2	5
F	2	3	2	5
Unique	11	16	5	12
Culture Neg.	16	N/A	10	N/A
TOTALS	84	100	51	100

5.2 Comparison of RFLP Pattern Distribution of Shelter-Associated Isolates With Manitoba Background RFLP Pattern Distribution

The distribution of the RFLP patterns of the 143 non-shelter-associated Manitoba isolates submitted for comparative purposes is tabulated in **Table 6**, along with the

shelter-associated RFLP patterns for the same period. Sixteen clusters of matching RFLP patterns were identified in the Manitoba group, with cluster sizes ranging from 2 to 15 isolates. Pattern A comprised the largest cluster of 15 isolates; however, since 10% of Manitoba isolates were Pattern A, whereas 68% of shelter-associated isolates were Pattern A, this confirmed the strong association of Pattern A with the shelter during the 1992-93 period ($p<.001$). Overall, 46% of the Manitoba background isolates were clustered by RFLP type (i.e. occurred in clusters of matching RFLP patterns).

Table 6. RFLP Patterns of Shelter-Associated Isolates and Background Manitoba Isolates, 1992-93

RFLP Pattern	Shelter-Associated		Manitoba Background	
	Number	%	Number	%
A	28	68	15	10
B	2	5	11	8
C	2	5	2	1
D	0	0	0	0
E	2	5	0	0
F	2	5	1	1
G - T	0	0	37*	26
Unique	5	12	77	54
TOTALS	41	100	143	100

***Includes 2 clusters of 6 isolates, 1of 4 isolates, 1 of 3 isolates and 9 of 2 isolates.**

5.3 Time Distribution of RFLP Patterns

The time distribution of RFLP Patterns A and B is depicted in **Figure 4**. This figure graphically illustrates that the strain represented by the 33 Pattern A isolates was the etiologic agent for 12 cases over the first three years of the study period and for a peak consisting of 21 cases in 1993. In contrast, the strain represented by the 15 Pattern B isolates was the etiologic agent for four cases in 1990 and nine cases in 1991 (with a spike of four cases in September 1991), and only two cases in 1992-93.

Figure 4. Time Distribution of RFLP Patterns A and B, 1990-93

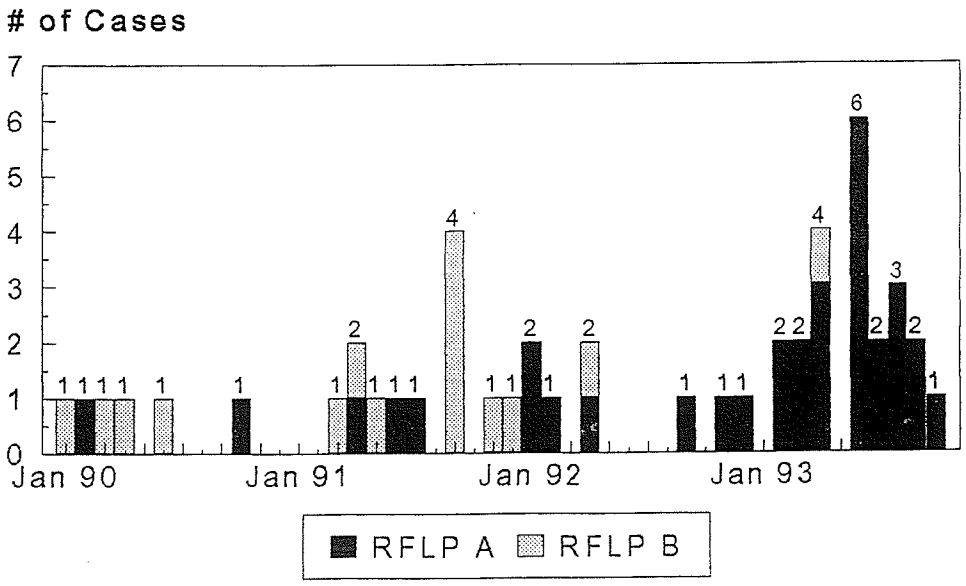
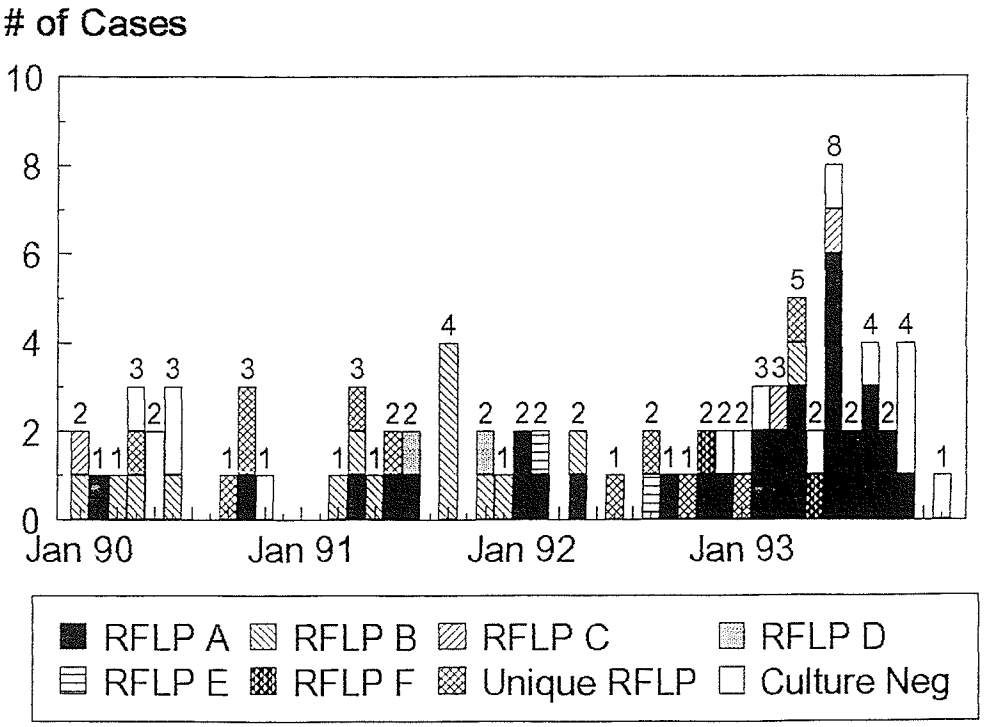


Figure 5 shows the time distribution of all of the RFLP patterns over the study period. The major contribution of the Pattern A strain to the outbreak peak in 1993 is clearly seen, as is the distinct small spike attributable to Pattern B in 1991. While the first of three cases attributible to the Pattern C strain occurred in January 1990, the other two cases were diagnosed within 3 months of eachother in 1993. The remaining three pairs of matching RFLP patterns represented by Patterns D, E and F also were related in time, with 6 months or less between the two matching strains. Finally, the strains with unique RFLP patterns were responsible for sporadic cases throughout the study period.

Figure 5. Time Distribution of All RFLP Patterns, 1990-93



6. Results of Comparison of Conventional Epidemiologic Data With RFLP Analysis Data

6.1 Comparison of Hypothesized Transmission Patterns to RFLP Evidence

In **Figure 6**, the RFLP patterns obtained from all available shelter-associated isolates for the entire 1990-93 study period are superimposed on the schematic map of hypothesized transmission patterns generated by conventional methods, allowing comparison of the two methods. The RFLP evidence in **Figure 6** supports the hypothesized transmission patterns in the majority of instances, while also refuting transmission in several instances.

The observation of the common strain, Pattern A, within the hypothesized homeless case cluster, the Hotel A cluster, and in most cases of the Social Group A cluster supports the hypothesized transmission within these clusters. The occurrence of Pattern A in the contact of a homeless shelter user case on Reserve G supports the hypothesized transmission in that instance. The occurrence of additional Pattern A cases among shelter users living in Hotels B, C and H, on Reserve D and in six additional shelter users living in the inner city supports the hypothesized association of these cases with the shelter.

The RFLP evidence of a common Pattern B strain within Social Group B and between this social group and one resident of Hotel B and Reserve B also is supportive of the hypothesized transmission pattern, albeit with a different strain than the Pattern A strain most strongly associated with the shelter. Several of the culture negative cases

within Social Group B were children with primary infection, who likely were also infected with the same Pattern B strain as their close contacts. It was this propagated cluster of cases associated with Social Group B which led to the small spike of cases in mid-1991 observed in the epidemic curve (**Figure 1**). The distinct RFLP pattern of this cluster makes it unlikely that it shared transmission sources with the subsequent extended cluster involving Social Group A, Hotel A, and the homeless shelter users.

Figure 6 also demonstrates supporting evidence for transmission within four pairs of cases, with strains other than the predominant Pattern A strain. These pairs include a married couple within Social Group A (Pattern C), an uncle and nephew in inner-city Winnipeg (Pattern E), an uncle and niece on Reserve A (Pattern F) and an uncle and nephew on Reserve C (Pattern D). The fact that these linked cases had different RFLP patterns than the predominant outbreak strain provides evidence that these small clusters were not part of the shelter-associated outbreak.

RFLP evidence refuting hypothesized transmission in several instances is also illustrated in **Figure 6**. The observation that 11 of the cases produced isolates with unique RFLP patterns refutes the hypothesized shelter-associated transmission in these cases; the cases must either represent reactivation of remote infection or recent infection with strains not responsible for any other shelter-associated cases. The sibling (with the unique RFLP pattern) on Reserve H of a member of the homeless case cluster is unlikely to have contracted her TB from her brother, as their TB isolates had different RFLP patterns. The married couple within Social Group A whose isolates were Pattern C rather than Pattern A were unlikely to have contracted their TB from the other members of

Social Group A, as hypothesized based on conventional epidemiologic data. Similarly, the occurrence of a third Pattern C case within the hypothesized Hotel B cluster refutes the hypothesis that this case acquired his infection from other members of this hypothesized cluster. In fact, although there were no recorded contacts between this man from Hotel B and the couple from Social Group A, all three originally came from reserves in Northwestern Ontario, and it is possible they were all infected with the Pattern C strain while in Ontario.

Finally, the RFLP data provided some evidence of transmission that had not been suspected based on conventional epidemiologic methods. The four additional single cases of RFLP Pattern B observed in residents of Hotel G, Hotel I and among the non-clustered Winnipeg cases provides some evidence that these cases were likely epidemiologically linked to the Social Group B cluster; however, contact tracing did not reveal these links. Similarly, the observation that out of the 143 Manitoba TB isolates from 1992 and 1993 that were not known to be associated with the shelter, 15 subsequently were found to be the Pattern A strain, suggests that some linked cases were not detected by contact tracing.

Figure 6.

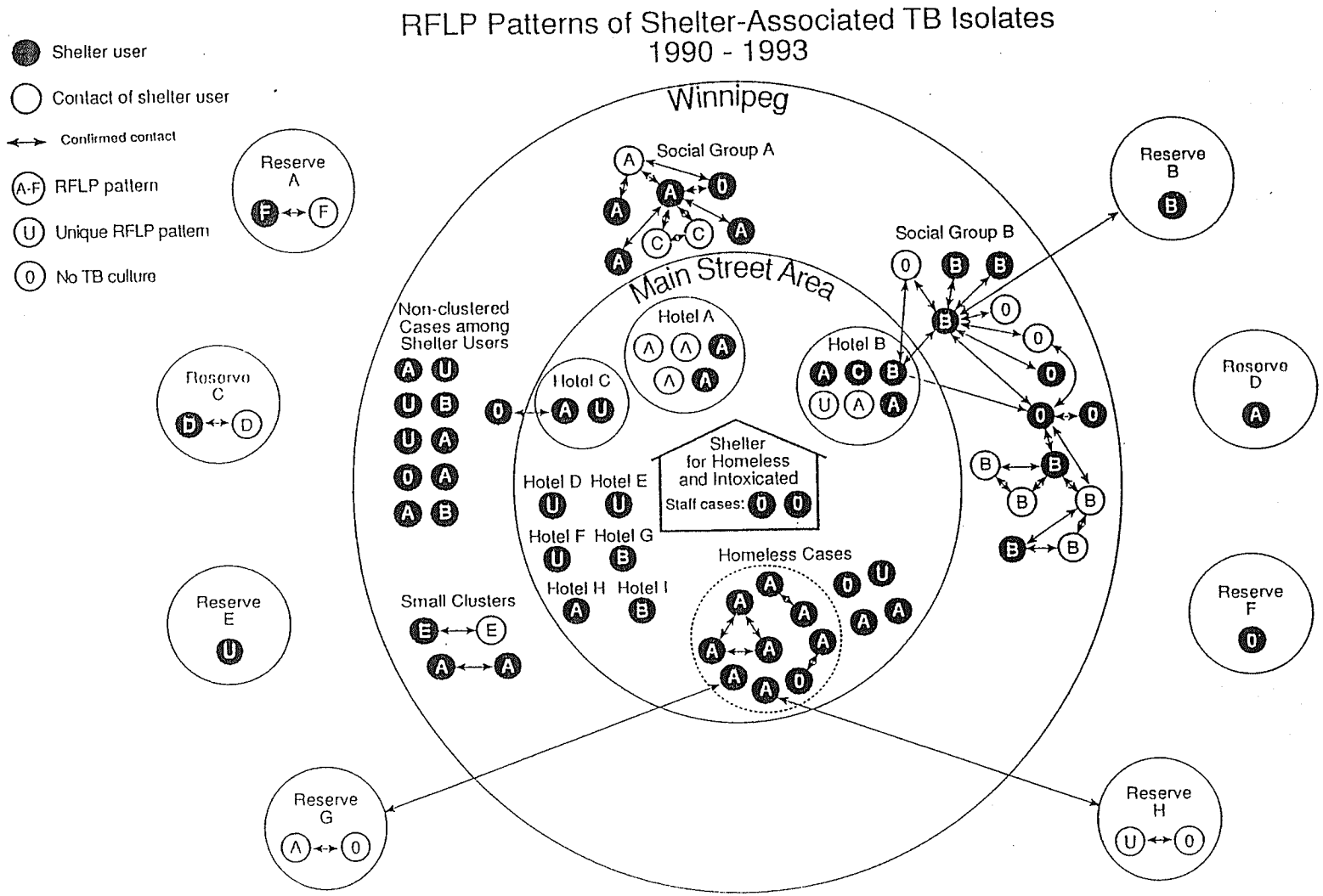
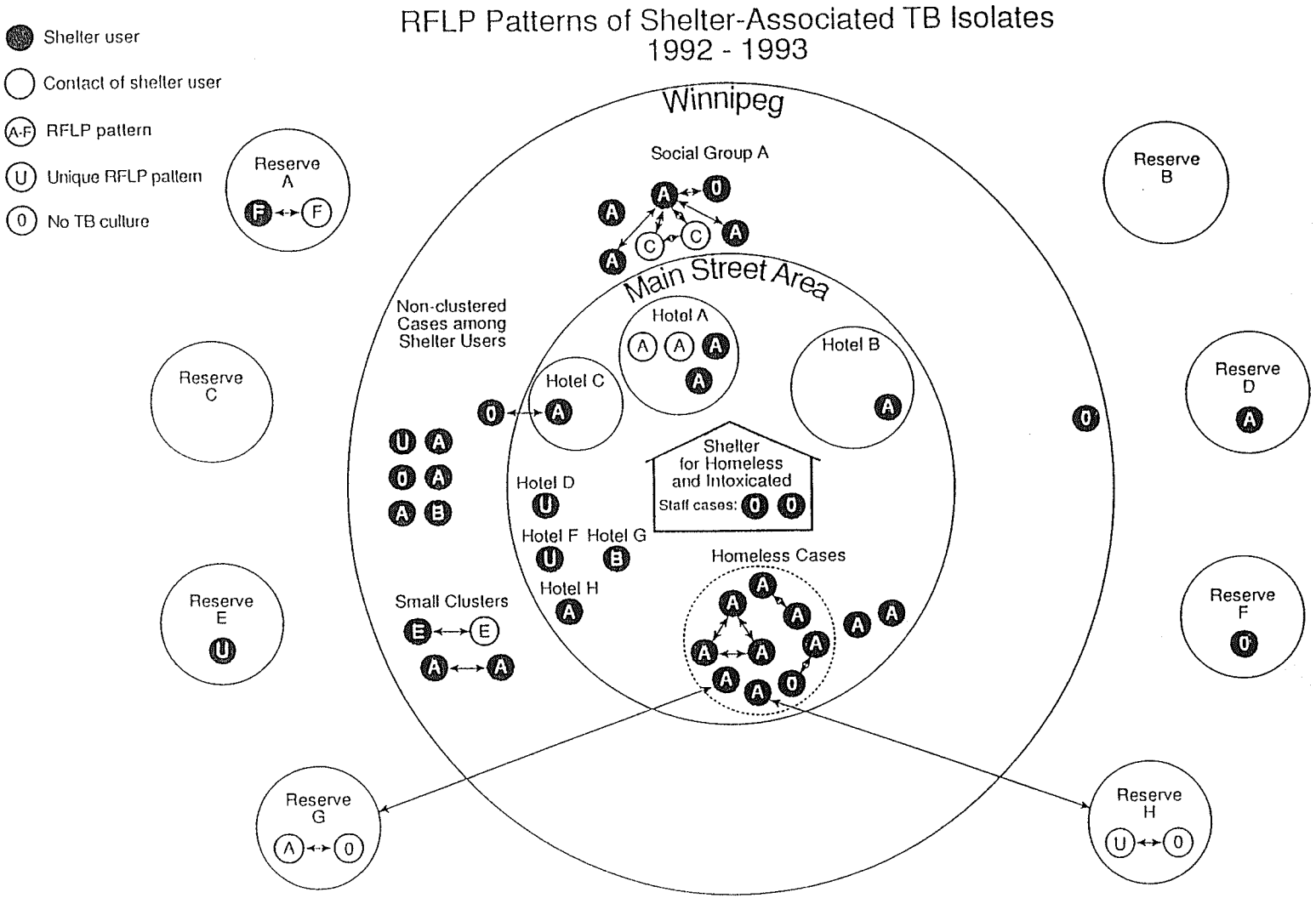

RFLP Patterns of Shelter-Associated TB Isolates
1990 - 1993
Shelter user
Contact of shelter user
Confirmed contact
A-F RFLP pattern
U Unique RFLP pattern
0 No TB culture
Winnipeg
Main Street Area
Social Group A
Social Group B
Non-clustered Cases among Shelter Users
Hotel A
Hotel B
Hotel C
Hotel D
Hotel E
Hotel F
Hotel G
Hotel H
Hotel I
Shelter for Homeless and Intoxicated
Staff cases:
Homeless Cases
Small Clusters
Reserve A
Reserve B
Reserve C
Reserve D
Reserve E
Reserve F
Reserve G
Reserve H

Figure 7.


RFLP Patterns of Shelter-Associated TB Isolates
1992 - 1993
Shelter user
Contact of shelter user
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Winnipeg
Social Group A
Main Street Area
Non-clustered Cases among Shelter Users
Hotel A
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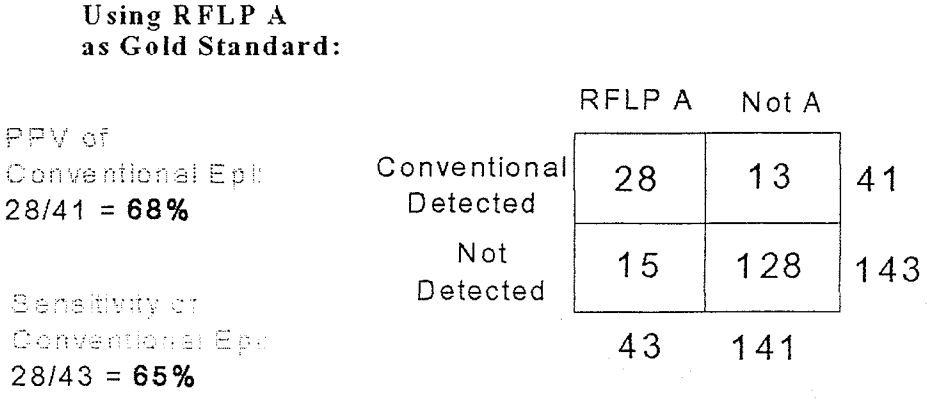
Hotel C
Hotel D
Hotel F
Hotel G
Hotel H
Shelter for Homeless and Intoxicated
Staff cases:
Homeless Cases
Small Clusters
Reserve A
Reserve B
Reserve C
Reserve D
Reserve E
Reserve F
Reserve G
Reserve H

Figure 7 displays the RFLP patterns superimposed on hypothesized transmission patterns only for those cases which occurred over the 1992-93 outbreak period. This figure further illustrates that RFLP evidence confirms the transmission of the common Pattern A strain in the majority of culture-positive cases occurring over the outbreak period. In addition, **Figure 7** illustrates the focus of the outbreak in the Homeless, Social Group A and Hotel A populations.

Figure 8 summarizes quantitatively the comparison between the conventionally-hypothesized transmission links and those supported by observation of the identical RFLP Pattern A over the 1992-93 outbreak period. Firstly, conventionally-hypothesized transmission was confirmed by RFLP in 28 out of 41 available shelter isolates over this period, for an estimated "positive predictive value" of contact tracing of 68%. In other words, RFLP typing refuted transmission in 13 (32%) of cases, helping to refine the delineation of the outbreak. Secondly, 28 out of the 43 1992-93 Manitoba TB cases found to have RFLP Pattern A had been identified as contacts by conventional contact tracing, allowing an estimation of the sensitivity of conventional methods at 65%. In other words, RFLP typing supported outbreak-associated transmission in an additional 15 cases over the 1992-93 period.

Figure 8. Quantitative Comparison of Conventional and RFLP Evidence for Transmission Over 1992-93 Outbreak Period



6.2 Demographic Characteristics of Cases by RFLP Pattern

Table 7 displays the mean age, gender distribution and ethnicity distribution of four categories of RFLP pattern cases: Pattern A, Pattern B, Patterns A to F (all RFLP clusters) and all unique patterns. The RFLP Pattern A cases, with a mean age of 42.2 years, were significantly older than the Pattern B cases ($p<.001$), but were younger than the cases with unique RFLP Patterns ($p<.05$). Together, all of the cases whose RFLP Patterns were clustered (Patterns A-F) were younger than those with unique RFLP patterns ($p<.01$).

There were no statistically significant gender distribution differences between the RFLP categories, although there was a tendency observed for RFLP-clustered cases to be more likely to be female than those with unique RFLP patterns ($p=.14$).

Finally, in comparison to those case with unique RFLP patterns, those with Pattern A ($p=.08$) or any clustered pattern (A-F, $p=.06$) were slightly more likely to be Aboriginal, although these differences did not reach significance at the $p<.05$ level.

Table 7. Demographic Characteristics of Cases, by RFLP Pattern Type, 1990-93

DEMOGRAPHIC VARIABLE	RFLP Pattern Type			
	A	B	A to F	Unique
AGE (mean years)	42.2	26.8	36.1	52.2
GENDER n (%)				
Males	24 (73)	9 (60)	40 (70)	10 (91)
Females	9 (27)	6 (40)	17 (30)	1 (9)
ETHNICITY n (%)				
Treaty Ab.	20 (61)	10 (67)	37 (65)	4 (36)
Nontreaty Ab.	7 (21)	1 (7)	9 (16)	2 (18)
Caucas./Oth.	6 (18)	4 (26)	11 (19)	5 (46)

7. Conclusions and Discussion

7.1 Conclusions of Conventional Epidemiologic Investigation

Based on the results of the conventional epidemiologic methods in the investigation, the following conclusions can be made. An outbreak of active tuberculosis occurred among the shelter users, their contacts and shelter staff over the 1992-93 period. The outbreak began in late 1992, peaked in June 1993, and resolved by November 1993. The outbreak mainly affected male (79%) Aboriginal (92%) shelter users between 20-59 years of age (mean age 38.5 years.) Compared to the shelter user TB cases, the contact cases were younger (including some children) and more likely to be female. Compared to the general population of shelter users, those affected by the outbreak were slightly older, more likely to be Aboriginal, and had used the shelter on a more frequent basis. A marked increase in risk of developing active tuberculosis was observed with increasing frequency of shelter use: those persons using the shelter on more than 50 days in the 1992-93 period were 82 times more likely to develop TB than those who used the shelter on only one day. This increased risk was observed across all frequencies of shelter use. Thus, we can conclude that exposure to the shelter was strongly associated with development of active TB over the 1992-93 period.

It can be concluded that shelter-associated TB cases comprised significant proportions of all Manitoba cases (22%) and all Winnipeg cases (28%) over the 1990-93 study period, and even larger proportions over the 1992-93 outbreak period (45% and 33%, respectively.) Thus, the public health burden of tuberculosis in Manitoba was

largely borne by this subpopulation over the study period.

Finally, conventional methods resulted in the hypothesized epidemiological links between cases as depicted in **Figure 3**. Based on contact tracing information, three large clusters and eleven small clusters of linked cases were hypothesized.

7.2 Conclusions of RFLP Analysis

RFLP typing of the 68 available shelter-associated isolates revealed that 57 of the isolates fell into one of six distinct RFLP pattern clusters, ranging in size from 2 to 33 isolates per cluster. The 33 isolates with RFLP Pattern A comprised 49% of all available shelter-associated isolates from the entire 1990-93 study period, and comprised the majority (68%) of available isolates from the 1992-93 period. As Pattern A comprised only 10% of the non-shelter-associated Manitoba isolates from the 1992-93 period, it is concluded that the Pattern A strain was strongly associated with the outbreak. Analysis of the time distribution of Pattern A cases over the 1990-93 period confirmed that this strain was responsible for sporadic cases from 1990 through 1992, while being responsible for 21 cases during the outbreak peak year, 1993.

The other large cluster of isolates, with the matching RFLP Pattern B, consisted of 15 isolates occurring mainly in 1990 and 1991. Thus it can be concluded that this strain was not associated with the observed outbreak in 1992-93.

The other four small RFLP clusters of two or three isolates each were dispersed over the four year study period and were not associated with the observed outbreak.

Within each cluster, however, isolates were linked in time, with 6 months or less between matching strains.

Overall, clustering of RFLP type was found for 84% of available isolates from 1990-93, and for 88% of isolates from 1992-93, confirming that a large proportion of cases in the study population resulted from recent transmission. Clustering was also observed in 46% of Manitoba background isolates, providing evidence of signicant recent transmission in the province as a whole.

7.3 Conclusions of Comparisons of Conventional Epidemiology Results With RFLP Analysis Results

The molecular RFLP evidence supported the transmission hypotheses generated by conventional methods in the majority of cases. For the 1992-93 outbreak period, the conventionally-hypothesized transmission of a common outbreak strain was confirmed by DNA fingerprinting in 68% of culture positive cases. This strain was observed in only 10% of non-shelter-associated Manitoba isolates for the same period.

In addition, the RFLP evidence further refined the delineation of the outbreak, by refuting transmission hypotheses in several instances, and by indicating transmission of a different strain than the outbreak strain, in other instances. This additional evidence helped further define the "core" groups involved in the outbreak (i.e. the homeless shelter users and their contacts in Social Group A and Hotel A), as well as further defining an earlier extended cluster in Social Group B which was not part of the 1992-93 outbreak.

Finally, the RFLP data provided some evidence of transmission that had not been

suspected based on conventional contact tracing information. Contact tracing had linked approximately 65% of all Manitoba Pattern A cases over the 1992-93 period. The additional cases with presumed unsuspected epidemiologic links could subsequently be further investigated.

The combination of the RFLP data with the data available from conventional epidemiologic investigation provided some insight which would not have been available with either method alone. For example, the observation of three cases with RFLP Pattern C (with epidemiologic links previously found between only two of them) caused a review of basic demographic information on these cases. It was then noticed that all three cases had moved to Winnipeg from the same area in northwestern Ontario, and it was thus concluded that they may all have been infected with the same strain in Ontario. Similarly, the re-analysis of the demographic characteristics of all cases when categorized by RFLP pattern provided some new insights, including the observation that RFLP-clustered cases on average were younger and more likely to be Aboriginal than cases with unique RFLP patterns. This observation supports the conclusion that risk for recent transmission of TB is higher in the younger, Aboriginal subpopulation associated with the shelter than in the older, non-Aboriginal subpopulation.

Finally, hypotheses of how the outbreak might have originated in association with the shelter can be formed by combining conventional with RFLP data. From the conventional investigation, it was learned that Case #51 (**Figure 3**) was a very frequent shelter user right up until he was diagnosed with laryngeal tuberculosis in November 1992, after several months of symptoms. Laryngeal tuberculosis has been demonstrated

in previous studies to be very infectious, with single cases being responsible for multiple secondary cases (Riley and Amundson, 1992; Braden, 1995). The confirmation that this individual was infected with the RFLP Pattern A strain supports the hypothesis that this individual may have been responsible for the observed excess of cases of the Pattern A strain over the subsequent months.

7.4 Discussion

There are several potential limitations of this study. One limitation is that only cases of active tuberculosis were considered in the conventional epidemiologic study of the outbreak, with no ascertainment of new infections with MTB (i.e. tuberculin converters). The decision to restrict analysis to cases of active TB only was based on the fact that little data was available on the occurrence of infection in the study population: tuberculin screening and followup was not logistically possible for many contacts in this population, and is not felt to be an efficient method of casefinding due to the high prevalence of tuberculin positivity.[38] Therefore casefinding consisted of radiographic screening for active disease. For the purposes of evaluating the contributions of RFLP analysis, however, limiting the study to cases of active TB was not a limitation, since RFLP typing can only performed on culture-positive cases.

A second potential study limitation is that demographic and shelter use frequency data for the general shelter-using population was available for comparison to cases for only the 1992-93 period. However, since the majority of shelter-associated cases, and

indeed the peak of the outbreak, occurred in this period, the comparisons should still be valid.

A third potential limitation of the study is that RFLP analysis was done on all non-shelter-associated Manitoba isolates only for the years 1992 and 1993, while the isolates from all four years of shelter-associated cases were RFLP typed. Again, as the shelter-associated outbreak was confined to the 1992-93 period, and all comparisons with the Manitoba RFLP "background" were limited to this period in the study analysis, the comparisons should still be valid.

This study is the first reported to explicitly compare and contrast transmission hypotheses first generated by conventional epidemiologic methods with molecular evidence subsequently derived from DNA fingerprinting of available MTB isolates. Previous applications of RFLP to outbreak investigations involved the confirmation of one outbreak strain implicated in the outbreak (Van Soolingen et al, 1991; Pearson et al, 1992; Daley et al, 1992; Dwyer et al, 1993). This study not only confirmed one outbreak strain responsible for the majority of cases, but it also illustrated how RFLP can help refine the understanding of an outbreak by refuting transmission in some cases, and detecting some previously unsuspected instances of transmission of the outbreak strain. Taken together, the conventional and molecular evidence provided evidence for how the shelter-associated outbreak may have evolved (as discussed in the previous section): one or more very infectious individuals who frequented the shelter prior to diagnosis may have infected numerous other shelter users and two staff members.

Previous studies (Small et al, 1994; Alland et al, 1994) in populations with a relatively high incidence of tuberculosis (San Francisco and inner-city New York) found clustering of RFLP patterns indicative of recent transmission in approximately 31% and 38% of culture-positive cases, respectively. The present study revealed that 84% of shelter-associated isolates from the 1990-93 study period were part of an RFLP cluster. While the population of this study was defined by association with the shelter (rather than representing a geographically-based population), the very high observed degree of RFLP clustering indicates a very high proportion of recently transmitted cases of TB in the shelter-associated population. This observation is contrary to previously-held beliefs that most active tuberculosis in the inner-city, alcoholic population results from reactivation of latent tuberculosis. In addition, the observation that 46% of **non-shelter-associated** Manitoba isolates from 1992-93 were clustered suggests that there was also a high background level of TB transmission during these years in Manitoba.

Application of RFLP analysis to provide an estimate of the "accuracy" of tuberculosis contact tracing has previously been performed by Small et al (1994) in San Francisco, where the authors observed that only 10% of RFLP-clustered cases had been linked by contact tracing. The authors speculated that the low "accuracy" (or sensitivity) could be explained by the overrepresentation in clusters of unemployed and homeless persons, who have multiple transient contacts which are difficult to reconstruct by routine tracing techniques. The finding in the present study that contact tracing identified approximately 65% of the cases in the Pattern A cluster suggests that contact tracing was much more efficacious in this Manitoba study population, yet still did not identify

approximately 35% of linked cases.

In summary, the DNA fingerprinting technique of RFLP typing of MTB isolates can provide a powerful tool for the epidemiologic study of tuberculosis transmission. In outbreak investigations, this method can be used in conjunction with conventional epidemiologic methods to confirm and refute transmission and further refine our understanding of the transmission patterns of the outbreak. RFLP typing can help in the evaluation of the accuracy of contact tracing and can point to previously undetected transmission, leading to further followup (Dobkin et al, 1993). It can be used to evaluate tuberculosis control programs, by defining the extent and populations affected by recent transmission. Tracing and followup of multi-drug resistant TB and nosocomial outbreaks can be facilitated by RFLP typing. Several large-scale population-based studies of RFLP types are underway to map the geographic origins and transmission routes of particular strains (Gomez-Marin et al, 1995). The classification of therapeutic failure as either true relapse versus exogenous reinfection can be accurately performed using RFLP typing. Finally, fundamental research questions that have been long-unanswered-- such as the proportion of cases resulting from reactivation versus new infection, and the precise role of brief contact in transmitting infection--can being addressed using this new method.

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