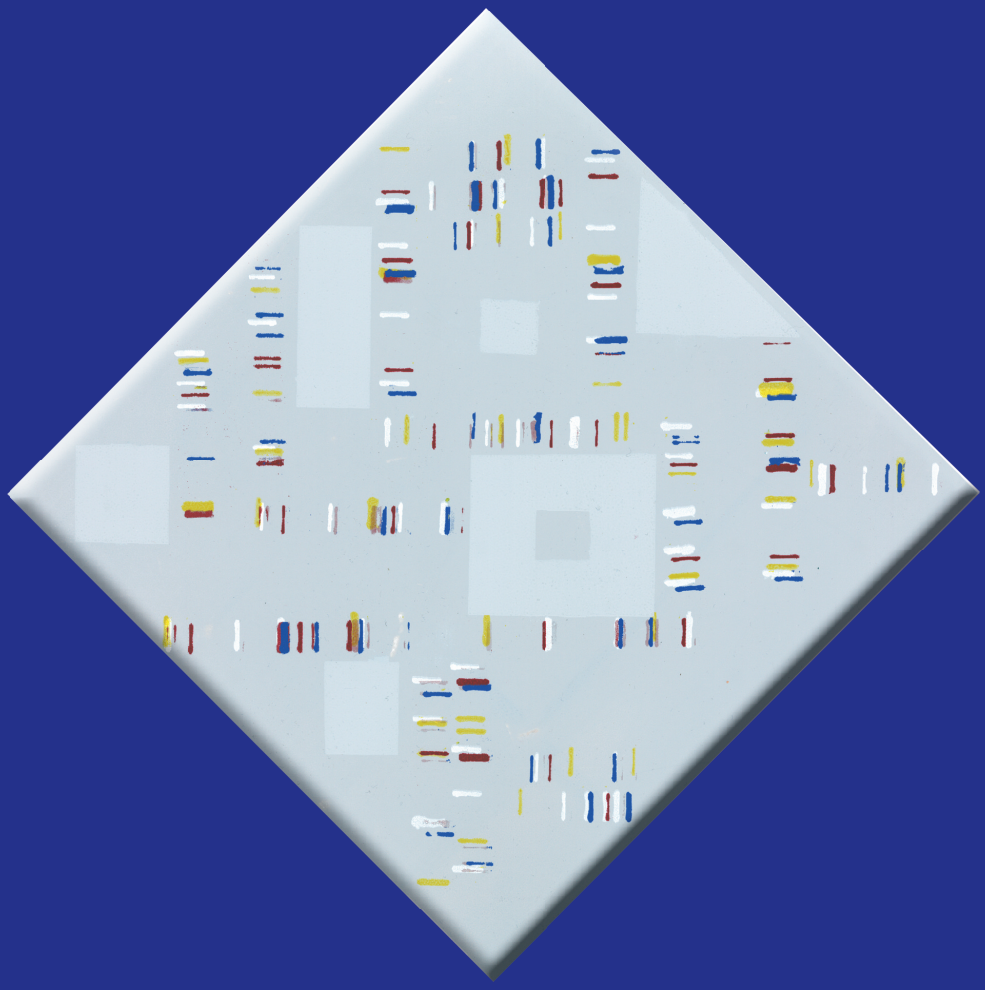

DNA Fingerprinting for Tuberculosis Control in a Metropolitan Area

Gerard de Vries

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DNA Fingerprinting for Tuberculosis Control in a Metropolitan Area

DNA fingerprinting voor de tuberculosebestrijding in een grootstedelijk gebied

Proefschrift
ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
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Voor Florence,
Douwe en Koen

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Abbreviations

AAD	area address density
BCG	bacille Calmette-Guérin
DNA	deoxyribonucleic acid
DOT	directly observed therapy
DR	direct region
EU	European Union
HCW	health care worker
HIV	human immunodeficiency virus
IGRA	interferon-gamma release assays
IS6110	insertion sequence 6110
KNCV	Koninklijke Nederlandse Centrale Vereniging tot bestrijding der tuberculose (Royal Netherlands Tuberculosis Association)
LTBI	latent tuberculosis infection
MDR	multidrug-resistant
MIRU	mycobacterial interspersed repetitive unit
MPHS	Municipal Public Health Services
NTR	Netherlands Tuberculosis Register
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism
RIVM	Rijksinstituut voor Volksgezondheid en Milieu (National Institute for Public Health and the Environment)
TB	tuberculosis
TST	tuberculin skin test
VNTR	variable number of tandem repeats
WHO	World Health Organisation
XDR	extensively drug-resistant

1

Introduction

1.1 Tuberculosis & control

Tuberculosis (TB) is an infectious disease that caused 9.2 million new cases and 1.7 million deaths in the world in 2006 (1). Over one-third of the world population is infected with *Mycobacterium tuberculosis*, the causative organism of TB. In the Netherlands, TB was the leading cause of death in the first decades of the last century, but improved socio-economic circumstances, infection-control measures and the introduction of effective chemotherapy have reduced TB to a relatively uncommon disease. TB mortality rates in the Netherlands declined a thousand fold in one century and incidence was the lowest ever recorded in 2006 (2). Prospects are yet less good in other parts of the world. The human immunodeficiency virus (HIV) pandemic multiplied the TB caseload in many sub-Saharan countries and drug resistance is on the increase in several countries with high rates of multidrug-resistant (MDR) strains. Recently, extensively drug-resistance (XDR) was described and XDR-TB is an emerging public health probem in a number of countries (3).

1.1.1 Infection and disease

A person with infectious pulmonary TB expels aerosol droplets (0.5 to 5 µm in diameter) containing *M. tuberculosis* bacteria by coughing, sneezing, singing or talking. People with prolonged and frequent contact have a high risk to inhale the bacteria and become infected. The asymptomatic stage, without clinical or radiological symptoms, is referred to as latent TB infection (LTBI). Persons with LTBI have approximately a 10% lifetime risk to progress to TB. The risk of TB increases in HIV-infected persons with low CD4 count, patients receiving immune-suppressive therapy, and certain medical conditions, such as end-stage kidney disease and diabetes mellitus. Persons coinfected with *M. tuberculosis* and HIV, have an annual risk of 5-8% to develop TB (4, 5). The diagnosis of LTBI is by tuberculin skin test (TST), also called the Mantouxtest. Interferon-gamma release assays (IGRAs) have recently been introduced as another, or supplementary, diagnostic tool for LTBI.

The incubation period of TB varies between some months to several decades, although most frequently infection progresses to disease within two years of infection. TB can affect almost any part of the human body, though the lungs are involved in two-third of cases. Symptoms related to pulmonary TB are productive and prolonged cough, haemoptoe, fever, night sweats, weight loss and fatigue. Other organs commonly involved are pleura, lymph nodes, urogenital system, nervous system, bones and joints. The diagnosis of TB depends on the organs involved. In pulmonary TB, a chest X-ray may show a typical radiographic picture with cavitation, although it can be normal as more frequently occurs in HIV-infected TB cases. Infectious pulmonary TB is highly suspected when acid fast bacilli are visible on direct microscopy of sputum by Ziehl-Neelsen or auramine-rhodamine staining methods. Other forms of TB may need more specific

diagnostic procedures such as bronchoscopy and biopsy of affected organs. The diagnosis is confirmed by a positive culture of body material for *M. tuberculosis* complex. Strain typing, drug sensitivity testing and molecular analysis of isolates complete the microbiological steps of the diagnosis of TB (see chapter 1.2).

In the Netherlands, LTBI is generally treated with six months isoniazid, which has an efficacy between 70 and 90% (6). LTBI can also be treated with four months rifampicin (7), e.g. if cases are infected with isoniazid-resistant strains, or if serious side effects of isoniazid complicate therapy. Treatment of TB requires daily administration of a combination of four drugs: isoniazid, rifampicin, pyrazinamide and ethambutol (8, 9). The World Health Organisation (WHO) policy is to directly observe the treatment (DOT) of TB. The six-month treatment regime of fully-sensitive TB has high cure rates with only 1-2% relapses. The treatment schedule can be adapted to the drug sensitivity of the strain. MDR strains are by definition resistant to isoniazid and rifampicin; XDR strains have additional resistance against quinolones and aminoglycosides. Treatment of MDR-TB and XDR-TB is less effective, complex and usually exceeds 18 months chemotherapy with five or more drugs. Large scale treatment results of XDR-TB are not yet available, although one case study reported devastating outcomes (3).

1.1.2 Epidemiology

TB incidence or incidence rate has been estimated by dividing the case notification per 100,000 persons in a year with the estimated proportion of cases detected (10). Thus, notified cases are the actual number of reported cases and incident cases the true number of new cases in a population in a specific time period. Estimated TB incidences vary considerably around the world from 363 per 100,000 population in the African region to 49 per 100,000 in the European region and 39 per 100,000 in the Americas (Figure 1.1) (1). In 2006, the country with the highest estimated incidence was Swaziland, with 1,155 cases per 100,000 population. India had the highest number of new cases in the world with an estimated 1.9 million cases (incidence 168 per 100,000). China had an estimated 1.3 million cases (incidence 99 per 100,000). In the United States incidence declined to 4.3 per 100,000 population. In the European Union (EU) incidence was highest in Romania (128 per 100,000) and Lithuania (62 per 100,000), and lowest in the Scandinavian countries (5-6 per 100,000)[1]. Since 2000 incidence has declined in all EU states except the United Kingdom, the three Baltic states and Romania (1).

[1] Excluding incidences in some smaller EU countries such as Cyprus and Malta with even lower incidences.

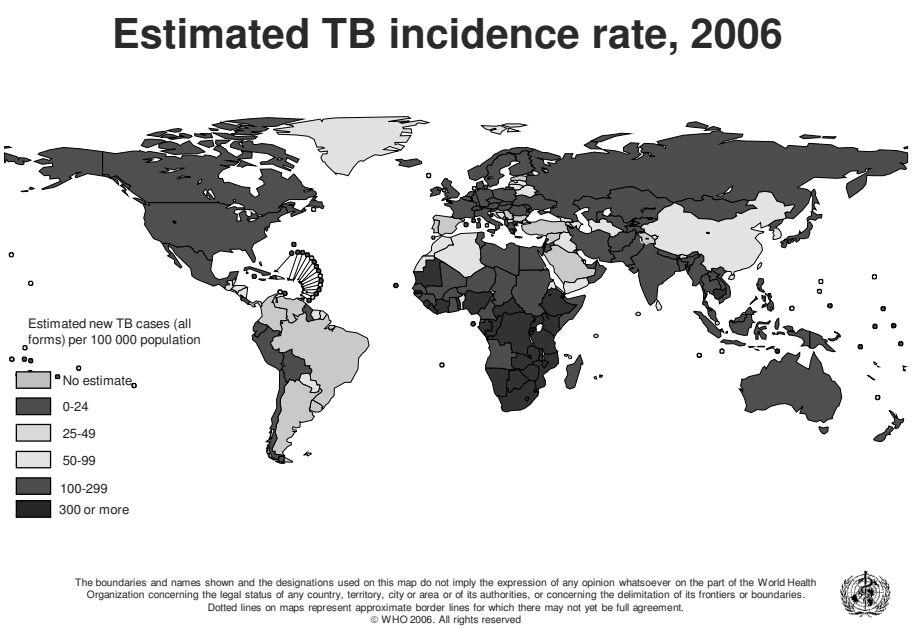

Figure 1.1. Estimated TB incidence by country in 2006 (1).

In the Netherlands, TB disease notification started in 1951 and was based on hospital admissions. For countries with a good health care and information system, such as the Netherlands, incidence and notification are usually considered identical. In 1951, incidence was nearly 150 per 100,000. In 1972, when mandatory notification of all cases to the Dutch Health Care Inspectorate started, incidence had already dropped to 18.4 per 100,000 (Figure 1.2). Incidence continued to decline steadily until the mid-eighties when a high influx of immigrants caused an increase of TB incidence. In the last ten years, incidence in the Netherlands declined again and reached the lowest ever recorded rate of 6.2 per 100,000 in 2006 (2).

The Municipal Public Health Service (MPHS) Rotterdam-Rijnmond carries out the TB control activities for the municipality Rotterdam and 27 surrounding municipalities. In figure 1.2, TB incidences in Rotterdam and these other municipalities are depicted. The Rotterdam TB incidence steadily increased from 1988 on, and doubled over a 15-year period to 29.2 per 100,000 in 2003. In that year, the Rotterdam City Council requested the director of the MPHS Rotterdam-Rijnmond to investigate the causes of the high TB caseload in Rotterdam.

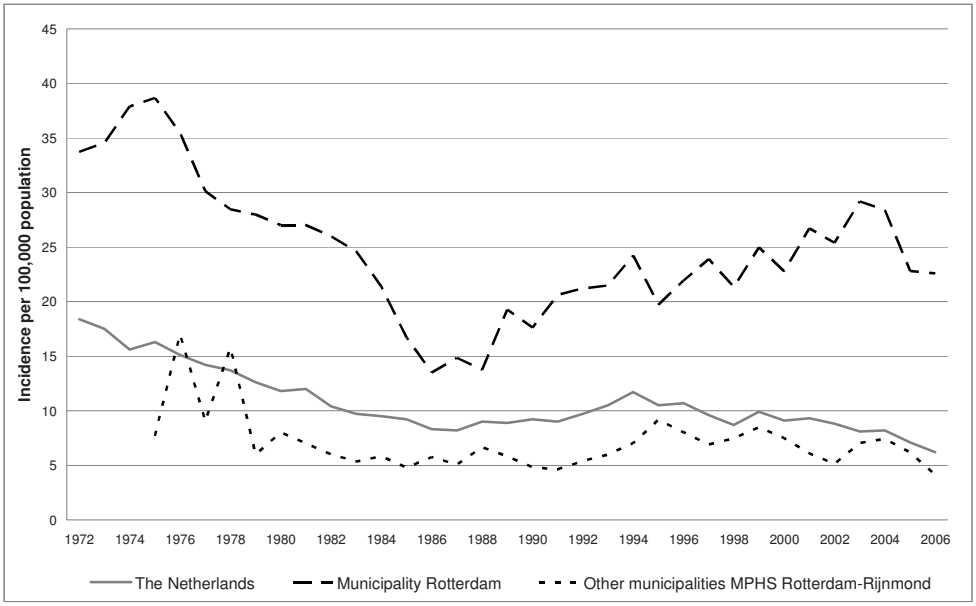

Figure 1.2. Tuberculosis incidence in the Netherlands, the municipality Rotterdam and 27 other municipalities of the Municipal Public Health Service Rotterdam-Rijnmond (2).

Fifty-six percent of the catchment population of the Department of TB Control of the MPHS Rotterdam-Rijnmond lives in three very highly urbanised (>2500 addresses/km^2) municipalities (Rotterdam, Schiedam and Vlaardingen). They form the metropolitan area in this thesis.

The main characteristics of TB patients in the Netherlands is summarised in the annual surveillance reports that are compiled by KNCV Tuberculosis Foundation (2). In 2006, 1,021 cases were notified; 644 (63%) were foreign-born, 66% had pulmonary TB, 7% had a previous episode of TB, 69% were culture-confirmed, 5.7% of isolates were isoniazid-resistant and 0.9% MDR-resistant, 4.0% of cases were HIV-coinfected. Twenty-five percent of cases were actively traced and 82% of cases diagnosed in 2005 completed treatment; 7% of the cases died before or during treatment.

1.1.3 Organisation of TB control in the Netherlands

The basic principles of TB control are prompt TB diagnosis of symptomatic persons (passive case finding), completion of TB treatment (case holding) and identification of asymptomatic TB and LTBI cases (active case finding). Isolation of cases during the infectious stage of the disease is essential to reduce transmission. Mandatory isolation of patients

noncompliant with isolation procedures should have a legal basis. Institutional infection-control measures should be in place to prevent spread e.g. in health care facilities (11). Vaccination with bacille Calmette-Guérin (BCG) protects young children from severe childhood TB, but does not prevent disease in adults.

In the Netherlands, TB is a notifiable disease and should be reported to the MPHS within 24 hours after diagnosis according to the Infectious Diseases Act. Nurses of the Departments of TB Control guide patients throughout their treatment and sometimes supervise medication intake by applying DOT. TB and LTBI cases are actively searched for by contact tracing, and by risk and contact group screening. National guidelines have been developed for these interventions (12). Contact tracing is carried out according to the stone-in-the-pond approach (13). Risk groups have been classified as well-defined groups with a TB incidence of more than 50 per 100,000 population. Immigrants, asylum seekers, prisoners, seamen are considered nationwide risk groups, while illicit drug users and homeless persons are locally defined risk groups depending on the epidemiological context. Professional contacts of risk groups are so-called contact groups, e.g. health care workers and social workers involved in care for homeless persons. Persons belonging to risk groups or contact groups are or may be periodically screened for TB or LTBI, either by chest X-ray or by TST.

Municipalities are responsible for public health in the Netherlands and have formed Municipal Public Health Services (MPHS), as outlined in the Public Health Act. The Departments of TB Control of these MPHSs carry out the TB control activities. They receive the reports of new cases and report nowadays to the national Centre of Infectious Diseases. The Departments of TB Control have a low threshold for symptomatic clients, provide free care for TB cases, conduct contact investigations, screen risk and contact groups, are responsible for local surveillance and outbreak response, and implement selected BCG vaccination of high-risk children.

The National Institute for Public Health and the Environment (RIVM) receives all *M. tuberculosis* isolates for strain typing, drug sensitivity testing and molecular analysis. The Committee for Practical TB Control, organised by KNCV Tuberculosis Foundation, is responsible for national policy and guideline development. KNCV Tuberculosis Foundation is also responsible for national surveillance and holds the Netherlands Tuberculosis Register (NTR). The low TB incidence in the Netherlands has made a reorganisation of TB Control necessary, which is now increasingly organised at a regional level. At this moment, the country is divided into 7 regions with 8 back offices with the South-Holland region having two back offices, i.e. Rotterdam and The Hague. Furthermore, a strategic programme is currently being developed to move towards the elimination of TB, aiming to reduce the incidence to 1 case per million population (14).

1.2 DNA fingerprinting methods

The complete genome of the *M. tuberculosis* laboratory strain H37Rv consisting of 4,411,529 base pairs (bp) was sequenced and published in 1998 (15). Since then other *M. tuberculosis* strains and a strain of *M. bovis* have been fully sequenced (16). Although the *M. tuberculosis* genome is genetically highly conserved, insertion sequences, repetitive elements, genomic deletions and single nucleotide polymorphisms cause genetic polymorphisms which can be identified by various molecular DNA typing techniques, also called DNA fingerprinting (17).

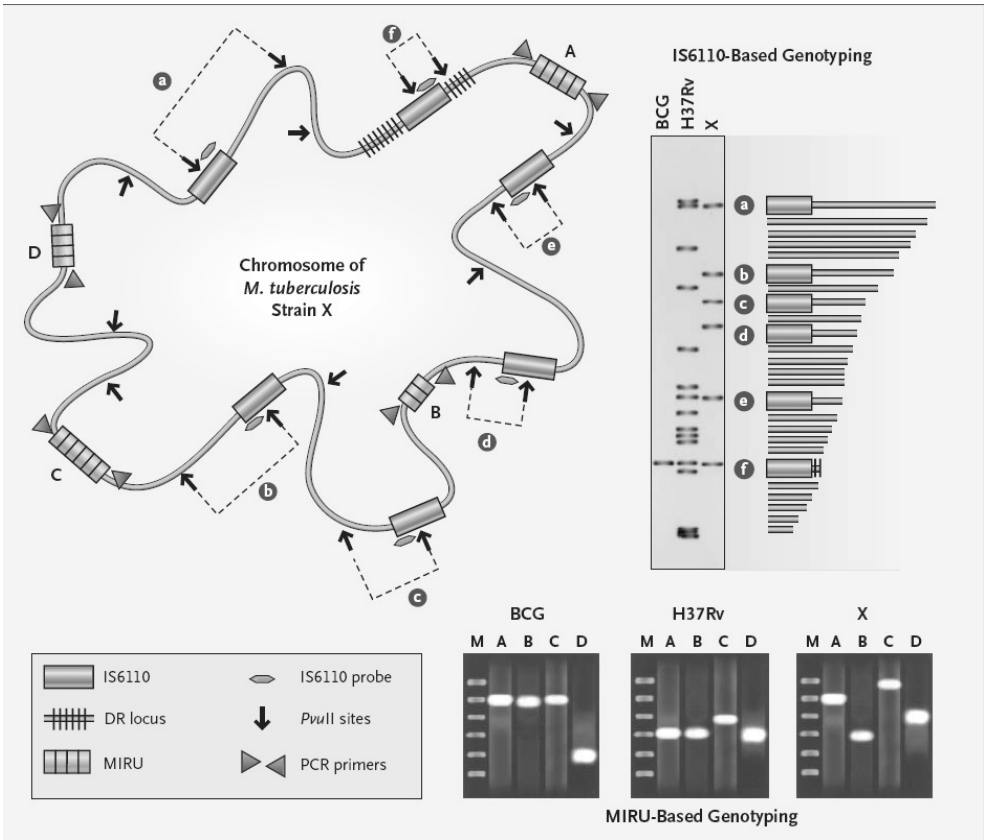

Figure 1.3. Chromosome of *Mycobacterium tuberculosis* hypothetical strain X and genotyping of *M. bovis* BCG, *M. tuberculosis* strain H37Rv, and strain X on the basis of IS*6110* insertion sequences and mycobacterial interspersed repetitive units (MIRUs) (18).

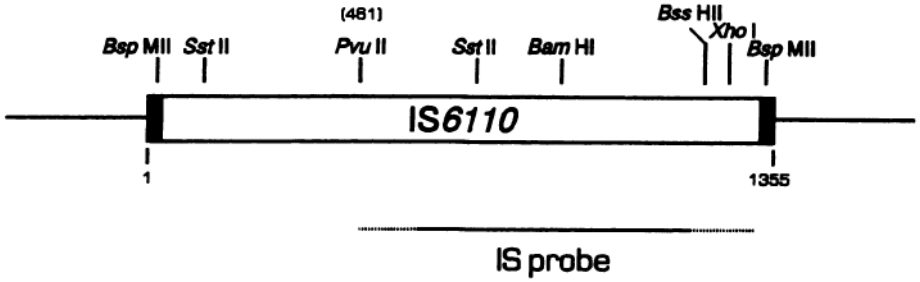

Figure 1.4. Physical map of the 1.35-kb *M. tuberculosis* insertion element IS*6110*. The cleavage sites of several restriction enzymes are depicted. *PvuII* cleaves the element at base pair 461 (19).

IS6110-based RFLP typing. The most widely applied genotyping method for *M. tuberculosis* is restriction fragment length polymorphism (RFLP) typing. The technique is based on identifying insertion sequences (IS) that are common in bacterial genomes. One of these elements, IS*6110,* was first reported by Thierry *et al.* (20). IS*6110* occurs specifically in the genome of *M. tuberculosis* complex strains and is 1,355 bp in length (Figure 1.3). In 1993, van Embden *et al*. proposed a standardised method for strain identification using IS*6110*-based fingerprinting (19). Basically, a restriction endonuclease (*PvuII*) cuts the circular chromosome of *M. tuberculosis* at specific sites, including bp 461 in the IS*6110* segment (Figure 1.4). The result is that the *M. tuberculosis* chromosome is divided into thousands of DNA fragments of varying size. These negatively charged DNA fragments are separated by algarose gel eletrophoresis and transferred to a nylon membrane (Southern blotting). The chemi-luminiscence-labelled probe specific to the right side of IS*6110* hybridises to the DNA fragments containing the right-hand part of IS*6110*. The banding pattern, visualised after chemiluminiscence, is detected on a light-sensitive film. Computer-assisted analysis using a dedicated software application facilitates storage and comparison of the DNA fingerprints. Standardised molecular weight markers, i.e. between 0.6-kb (kilo bp) and 23.1-kb, enable comparison of DNA fingerprints within and between laboratories. Figure 1.5 shows a number of fingerprints of *M. tuberculosis* isolates.

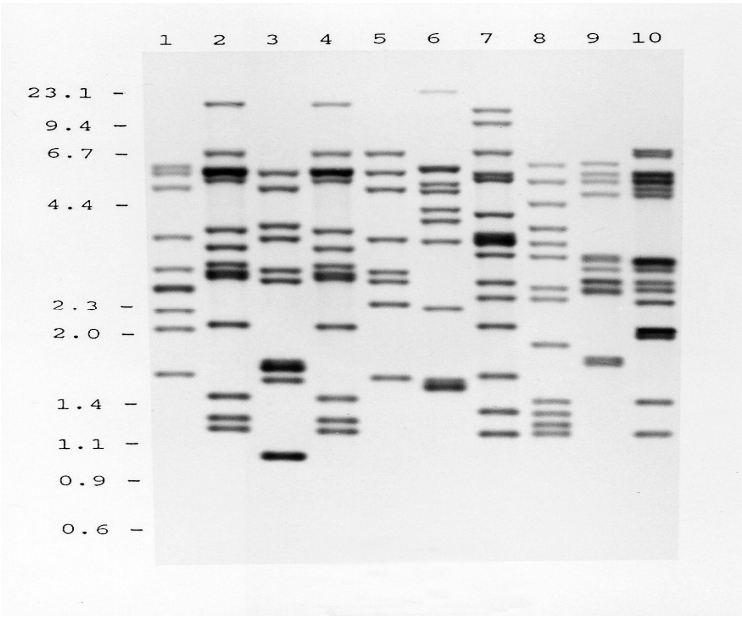

Figure 1.5. DNA fingerprinting: IS*6110* restriction fragment length polymorphism patterns of 10 isolates of *Mycobacterium tuberculosis*. Lanes 2 and 4 show identical patterns suggesting epidemiological links between cases of whom these isolates were obtained.
Source: National Institute for Public Health and the Environment (RIVM)

Reproduced with permission from the National Mycobacteria Reference Laboratory, National Institute of Public Health and the Environment (RIVM), Bilthoven.

The result of IS6110-based genotyping differs by strain because of variability in the number and sizes of the IS6110-hybridizing DNA fragments. *M. tuberculosis* strains have 0 to 25 copies of IS*6110* (21), *M. bovis* strains usually contain one to five copies, and *M. bovis BCG* strains one or two (22). Isolates with fully identical RFLP patterns form molecular clusters (Figure 1.5), although some studies accepted a one or two band difference between fingerprints. Isolates with four or less (the Netherlands), five or less, or six or less bands are defined as low-copy strains. For these low-copy and zero-copy strains, IS*6110*-based typing lacks specificity (16, 23) and additional or alternative typing methods are required, such as the polymorphic GC-rich repetitive sequence (PGRS) RFLP typing, as applied in the Netherlands (24).

Strains need to change fast enough that unlinked cases harbour different isolates and slowly enough that isolates from related cases are identical (21). The half-life of RFLP genotypes has been estimated to vary between 3 and 10 years (25-27) and this interval is suitable for distinguishing epidemiologically related and unrelated isolates (21).

RFLP typing has been the method of choice because of its high reproducibility, good discriminatory power and high specificity (28). However, apart from the limitations for low-copy strains, the RFLP typing procedure is also laborious, costly, and can only be performed on viable isolates. The turn-around time is long because the required amount of DNA (2 µg) is only reached after several weeks of culture (21). Furthermore, exchange of IS*6110* data between laboratories is difficult because of the photographic type of result of RFLP typing.

Other genotyping methods. Spacer oligonucleotide typing (spoligotyping) is a widely used polymerase chain reaction (PCR)-based technique to simultaneously detect and type *M. tuberculosis*. The *M. tuberculosis* genome usually contains a distinct chromosomal region, the so-called direct repeat (DR) region. This region consists of 36-bp DRs separated by spacer DNA sequences of 37 to 41 bp. Forty-three known spacer sequences are identified by spoligotyping. The result of this typing method is binary data derived from the presence or absence of these spacer sequences. Another relatively new DNA typing method is the mycobacterial interspersed repetitive unit (MIRU) or variable-number tandem repeats (VNTR) genotyping. This method determines the number of repeats in various independent repeat loci in the genome. Similarly to spoligotyping, this technique has the advantage of being PCR-based, and thus does not require extended cultivation of the *M. tuberculosis* strains before typing. The results from VNTR typing can also be represented numerically, allowing easy storage, comparison and exchange of data. Recently, a standardised VNTR typing method was proposed to include 15 VNTR loci for routine epidemiological studies and 24 for phylogenetic studies (29). The discriminatory power of the 15-loci VNTR typing was found to be equal to that of IS*6110* RFLP typing for an international collection of *M. tuberculosis* strains. At the RIVM in the Netherlands, where RFLP typing has been applied on a routine basis since 1993, all *M. tuberculosis* isolates of 2004 to 2008 have been retyped with VNTR typing (personal communication Kristin Kremer), and since 1 January 2009 VNTR typing replaced IS*6110*-based RFLP typing

1.3 Molecular epidemiology of tuberculosis

Molecular epidemiology can be defined as "the science that focuses on the contribution of potential genetic and environmental risk factors, identified at the molecular level, to the etiology, distribution and prevention of disease within families and across populations" (http://www.pitt.edu/~kkr/molepi.html). This chapter discusses the relevance of genotyping for understanding TB transmission and its use for control activities.

The European Concerted Action on Molecular Epidemiology and Control of Tuberculosis outlined factors that should be considered by those conducting and interpreting studies of *M. tuberculosis* in populations (30). They stated that these studies should be conducted in conjunction with conventional epidemiological investigations of contacts, should involve a high proportion of all cases in a population and should provide specific information on population and patients. A number of review articles described current insights and applications of DNA fingerprinting in TB control (16-18, 31, 32).

1.3.1 Contribution of molecular epidemiology to understand TB transmission

Two population-based studies from San Francisco and New York, both published in 1994, combined for the first time molecular and conventional epidemiological methods to investigate TB transmission in a large number of patients (33, 34). The assumption that 90% of incident cases in the United States resulted from reactivation of latent, remote TB infection (35) was challenged in these studies, because they found that about one-third of urban cases had clustered strains and were presumably recently infected. Both studies were considered landmark for the application of DNA fingerprinting (22). Since that time, many other studies confirmed that a considerable proportion of disease in low-prevalence countries was caused by recent transmission, in particular in urban areas. High clustering rates in rural areas without documented outbreaks may however result from infections that occurred many years or decades before by strains circulating at that time and having survived without molecular change (23). Non-matching, i.e. unique fingerprints, suggest that these patients are not involved in recent chains of transmission in the area under study and result from an infection that was acquired several years in the past (reactivation) or outside the area under study (importation).

Molecular epidemiological studies identified risk factors for clustering, which varied according to the specific epidemiological situation (33, 34, 36, 37). Common risk factors in these studies were sex, younger age, nonimmigrants, AIDS patients or HIV-coinfection, alcohol abuse, injection drug use and homelessness (31). Also, certain subpopulations or

ethnic groups had a higher risk of clustering such as Hispanics and the black population in a number of American studies and black Caribbean ethnicity in London (33, 34, 38). High rates of clustering among persons and risk groups reflect primarily the risk of infection, although e.g. HIV-infected persons also have a higher probability for disease development. Some studies showed that drug resistance was a risk factor for clustering indicating transmission of these strains in communities (33). However, van Soolingen *et al*. found that drug-resistant strains were less transmissible (39). The clustering proportion usually reaches a plateau after 3-4 years of universal genotyping (30).

Molecular epidemiological studies have confirmed high-risk sites for TB transmission. Cronin *et al*. defined traditional settings for TB transmission as households or contacts with close relatives and friends, and all other settings as non-traditional (40). Homelessness is an independent risk factor for clustering in almost all molecular population-based studies, and thus transmission most likely occurs in places common to these persons, such as shelters (41). Other non-traditional settings, such as the workplace, hospitals, bars, schools, prisons and churches have been described in outbreak reports (42-44).

A number of genotyping studies focused on the concept of recurrent TB, i.e. disease reoccurring after a first episode (45-49). A second episode can either be caused by the same strain (endogenous reactivation or relapse) or result from an infection with a new strain. Relapse TB due to reactivation or to reinfection are clinically indistinguishable. Genotyping has shown the ability to differentiate the different pathogenesis of recurrent TB. In low-incidence countries, the proportion of recurrent TB caused by reinfection was much higher than previously believed. In high-endemic countries, reinfection rates are markedly high due to the high risk of infection in these communities (48, 49).

Prospective population-based studies using genotyping showed that some epidemiological links of clustered cases could only be established after meticulously interviewing clustered patients (50, 51). A considerable proportion of TB transmission thus occurs during casual contact, e.g. in local shops and public transport, with few opportunities to target these contacts for enhanced contact tracing (16, 18, 39, 40, 44, 50). Clustered cases that are not epidemiologically linked may also result from remote transmission or transmission in the country of origin of immigrants (51, 52). Van Deutekom *et al*. reinvestigated a subset of seven RFLP clusters without epidemiological links and found that five were subdivided by MIRU-VNTR typing, indicating false clustering of TB patients by IS*6110* typing (53).

1.3.2 Applications of molecular epidemiology for TB control

Molecular typing has been used to identify and confirm outbreaks, as described in many studies (42, 54-59). Common in these studies is that they report on a single strain or a limited number of strains causing the

outbreak, often in a high-risk group. In the beginning of the 1990s, DNA fingerprinting revealed that nosocomial transmission in hospitals with drug-resistant strains contributed to the resurgence of TB in the United States which affected HIV-infected persons and health care workers (60, 61). This alerted public health officials and revived the national TB programme (56).

Molecular epidemiology also showed that recently infected cases were mostly not identified by conventional contact tracing (18). In the Netherlands, genotyping results only led to the extension of 1% of contact investigations, and relatively few additional persons with TB or LTBI were diagnosed (51). The use of DNA fingerprinting in conventional contact tracing activities is further limited because of the long latency period of TB and the slow turn-around time of RFLP results.

Population-based molecular epidemiological studies investigated transmission dynamics and clustering rates in several cities, states and countries, most of them in low-incidence countries. In a recent meta-analysis including 36 studies in 17 countries the TB clustering proportion varied greatly between 7.0 and 72.3% (31). Clustering proportions are usually higher in urban areas. Table 1.1 provides an overview of clustering rates in selected cities of low-endemic countries.

Transmission dynamics between immigrants and nonimmigrants in low-incidence countries has been researched in a number of studies (62-64). DNA fingerprinting also can help local public health authorities to identify high-risk populations and TB transmission sites (18, 41). Fingerprinting has been used as a tool to monitor trends, evaluate interventions and programmes (37). In San Francisco, the clustering rate declined from 51.2 to 29.8 per 100.000 population after TB control activities were intensified (65). In Denver, the proportion of clustering strains among homeless persons decreased from 49 to 14 after 4 years of screening for LTBI and TB (66). The overall decline of TB incidence in the Netherlands and in Arkansas was caused by a decrease of nonclustered case rates among persons aged 65 years or more, which is mainly the result of a cohort effect (67, 68). In Texas, genotyping was combined with geographical information systems to identify high-risk groups for targeted TB testing programmes (69).

Genotyping also highlighted the occurence and significance of laboratory cross-contamination (70). In general, contamination is suspected when identical fingerprints are cultured from different patients in the same laboratory within a seven days period (21, 71). In the Netherlands, laboratory cross-contamination was approximately 3 percent and reduced after quality enhancing procedures, such as sending multiple samples and critically comparing laboratory results with clinical findings, were installed (26).

Table 1.1. Overview of clustered tuberculosis rates, confirmed epidemiological links and main findings of selected population-based molecular studies in cities of low-endemic countries.

First author	Year of publication	City	Study year	Cases (*n*)	Sample coverage (%) *	Clustered (%)	EPI links (%)†	Main findings
Genewein (72)	1993	Berne	1991-1992	163	61	28		- In the native population transmission was largely between young adults, and the complex network pattern of transmission was not identifiable by conventional contact tracing.
Alland (33)	1994	New York – Bronx hospital	1990-1992	130	83	38		- Recently transmitted TB more common than previously thought. - Risk factors for recent transmission: younger age, Hispanic ethnicity, HIV-infection, and infection with drug-resistant organisms.
Small (34)	1994	San Francisco	1991-1992	585	85	40	(10)	- A poorly compliant patient had substantial adverse effect on TB control. - Ongoing transmission of a few strains of *M. tuberculosis* in specific subgroups of the population. - Risk factors for recent transmission: younger age, AIDS, birth in the United States. - Conventional contact tracing identified only 10 percent of the patients in clusters.
Van Deutekom (73)	1997	Amsterdam	1992-1995	510	90	47	(6)	- Conventional contact tracing identified only 5.6 percent of the patients in clusters. - Hard-drug use was one of the significant risk factors for clustering.
Gutierrez (74)	1998	Paris	1995	326		35		- Homeless people play an important role in the transmission of TB. - About 30% of the patients older than 60 years had clustered isolates, showing that new infection may also be common in elderly people.

* Sample coverage is the proportion of culture-confirmed cases included in the studies.
† Figures between brackets represent cases identified by contract tracing, the other figures are retrospectively confirmed proportion of epidemiological links.

Table 1.1 (continued). Overview of clustered tuberculosis rates, confirmed epidemiological links and main findings of selected population-based molecular studies in cities of low-endemic countries.

First author	Year of publication	City	Study year	Cases (*n*)	Sample coverage (%) *	Clustered (%)	EPI links (%)†	Main findings
Barnes (41)	1999	Los Angeles	1994-1996	289	86	55	58	- Homeless shelters were sites of TB transmission. - HIV-infected TB patients did not play a major role in the spread of TB.
Jasmer (65)	1999	San Francisco	1991-1997	1761	85	19 §		- Clustered case incidence decreased after intensification of control measures, from 10.4 per 100,000 persons in 1992 to 3.8 in 1997.
Solsona (75)	2001	Barcelona - Ciutat Vella district	1997-1998	180	92	46	27	- Risk factors for clustering: intravenous drugs use, having a positive sputum smear, pulmonary TB. - Recent immigration was protective for clustering; HIV not a significant factor for clustering. - DNA fingerprinting revealed that 39% of cases identified through contact investigation did not acquire the infection from the putative source; all of the discordant results were among immigrants.
Maguire (38)	2002	London	1995-1997	2490	77	23	14	- Largely, reactivation or importation of infection in recent immigrants - Newly acquired infection common among people with recognised risk factors.
Geng (76)	2002	New York Manhattan neighbour-hood	1990-1999	745	73	48		- The proportion clustered cases decreased from 63.2% in 1993 to 31.4 in 1999. - TB was unlikely to result from recent transmission in foreign-born persons because relatively few such persons had TB with clustering fingerprints.

* Sample coverage is the proportion of culture-confirmed cases included in the studies.
† Figures between brackets represent cases identified by contract tracing, the other figures are retrospectively confirmed proportion of epidemiological links.
§ Clustering defined as occurrence of the same isolate within 1 year.

Table 1.1 (continued). Overview of clustered tuberculosis rates, confirmed epidemiological links and main findings of selected population-based molecular studies in cities of low-endemic countries.

First author	Year of publication	City	Study year	Cases (*n*)	Sample coverage (%) *	Clustered (%)	EPI links (%)†	Main findings
Diel (52)	2002	Hamburg	1997-1999	549	77	34	64	- Alcohol abuse strongest predictor for recent transmission. - High rates of transmission presumably in a particular bar in the red-light district and in a hostel for men. - Contact tracing insufficient to detect recent transmission chains (only 28% of epidemiologically linked cases found by contact tracing).
Catta-manchi (77)	2006	San Francisco	1991-2003	2675	89	19 §		- Since 1999-2000 no further decrease in overall case rates or rates of clustered cases. - Overall case rates will not decline further unless more effective control measures, such as identification and treatment of LTBI, are applied.
Inigo (78)	2007	Madrid	1991-2003	887	70	36	22	- Extensive transmission between Spanish- and foreign-born populations mainly by autochthonous *M. tuberculosis* strains. - Risk factors for clustering: homelessness, birth in Spain.
Ohkado (79)	2008	Shinjuku City, Tokyo	2002-2006	445	87	38		- Transmission more frequently among the homeless than in non-homeless persons - Also transmission by casual contact between the homeless and the general population was shown.

* Sample coverage is the proportion of culture-confirmed cases included in the studies.
† Figures between brackets represent cases identified by contract tracing, the other figures are retrospectively confirmed proportion of epidemiological links.
§ Clustering defined as occurrence of the same isolate within 1 year.

1.4 Aim of the thesis and research questions

The aim of this thesis is to investigate the role of DNA fingerprinting for TB control. Based on current knowledge of the use of DNA fingerprinting in other cities and countries, the following specific research questions have been formulated:

1) What has been the added value of DNA fingerprinting for TB control in a metropolitan area?

2) What are the causes of the high TB incidence in a metropolitan area, as revealed by DNA fingerprinting?

3) What do our studies add to the knowledge of TB transmission?

1.5 Outline of the thesis

The first research question is addressed in the chapters 2 and 3. **Chapter 2** describes a transmission classification model to determine place and time of infection of all TB cases. The extent of misclassification was assessed for cases with a DNA fingerprint if genotyping was not combined with epidemiological information. In **Chapter 3** an illustrative cluster is presented graphically to show epidemiological links between cases and transmission places. The specific cluster findings are used to discuss relevant issues in TB control.

The second research question is addressed by describing several applications of DNA fingerprinting in a metropolitan area. In **Chapter 4** demographic and disease-related factors of tuberculosis cases in a highly urbanised area are compared with cases of less-urbanised municipalities. In **Chapter 5** an extensive contact investigation among illicit drug users and homeless persons in Rotterdam is described. This investigation was carried out in 2001 and initiated a screening programme among illicit drug users and homeless persons. In **Chapter 6** the results from an evaluation of this screening programme, using DNA fingerprinting, are discussed.

Results from the methodological studies in **chapters 2 and 3**, and the different studies on metropolitan tuberculosis in **chapters 4, 5 and 6** also address the third research question, as well as the study described in **Chapter 7**, concerning the use of DNA fingerprinting to determine which health care workers were nosocomially infected in the Netherlands in the period 1995 to 1999. The general discussion (**Chapter 8**) reviews the research questions and the results of the studies in the context of the literature, it discusses the implications for future TB control in the Netherlands, and lists the conclusions and recommendations.

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2

Transmission classification model to determine place and time of infection of tuberculosis cases in an urban area

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Abstract

We conducted a population-based study in the Rotterdam region of The Netherlands to determine the place and time of infection of tuberculosis (TB) cases using conventional epidemiological and genotyping information. In particular, we focused on the extent of misclassification if genotyping was not combined with epidemiological information.

Cases were divided into those with a unique mycobacterial DNA fingerprint, a clustering fingerprint, and an unknown fingerprint. We developed transmission classification trees for each category to determine whether patients were infected in a foreign country or recently (<2 years) or remotely (>2 years) infected in The Netherlands.

Of all TB cases during the 12-year study period, 38% were infected in a foreign country, 36% resulted from recent transmission in The Netherlands, and 18% resulted from remote infection in The Netherlands, while in the remaining cases (9%) either the time or place of infection could not be determined. The conventional epidemiological data suggested that at least 29% of clustered cases were not part of recent chains of transmission. Cases with unknown fingerprints, almost all culture negative, relatively frequently had confirmed epidemiological links with a recent pulmonary TB case in The Netherlands and were more often identified by contact tracing.

Our findings highlight the idea that genotyping should be combined with conventional epidemiological investigation to establish the place and time of infection of TB cases as accurately as possible. A standardized way of classifying TB into recently, remotely, and foreign-acquired disease provides indicators for surveillance and TB control programme performance that can be used to decide on interventions and allocation of resources.

Introduction

The steady decline of tuberculosis (TB) incidence, especially since the introduction of chemotherapy in the 1950s, was reversed in the late 1980s in many developed countries due to immigration, concurrent human immunodeficiency virus (HIV) infections, and inadequate TB control practices (1). In the last 10 years, however, the general downward trend has resumed in many of these countries, necessitating review of current TB control strategies. Several countries where the incidence of TB is low are currently developing TB elimination plans in order to reach the goal of less than 1 case per million population per year (2, 3).

For TB control, it is relevant to know where and when patients were infected, because recently infected patients represent ongoing transmission, those with remotely acquired infection are a result of TB transmission in the past, and patients infected in a foreign country are an expression of the particular TB situation in that country. The absolute TB incidence and the relative contributions of recently, remotely, and foreign-acquired disease influence the choice of TB control strategies (4-6). The proportion of recent transmission is also an important indicator for surveillance and TB control programme performance (7, 8).

DNA fingerprinting of *Mycobacterium tuberculosis* isolates provides a tool to disentangle the different transmission pathways (6, 9, 10). The percentage of clustered cases in an area indicates the amount of recent transmission in a community, but clustering is not identical with recent transmission (11-13). Furthermore, fingerprinting studies alone ignore culturenegative cases, although their contribution to the TB caseload and to recent transmission may be substantial. Thus, a combined use of conventional epidemiological and genotyping data will ascertain more accurately where and when patients were infected (7, 8, 14). A standardized way of classifying transmission will help to monitor and compare TB control programmes.

We developed and applied a transmission classification model to determine the place and time of infection of all TB cases in a highly urbanized area by using information from conventional epidemiological investigation and molecular typing. In addition, we assessed the extent of misclassification for cases with a DNA fingerprint if genotyping was not combined with epidemiological information.

Materials and Methods

Study area and study population. The study was conducted in a highly urbanized area with 1.9 million inhabitants in the southern part of the province of South-Holland, The Netherlands. Reported cases diagnosed between 1 January 1995 and 31 December 2006 were included in the study, excluding 41 sailors with a residential address outside The Netherlands and 12 inmates of a deportation centre for illegal immigrants transferred from other regions of the country. *M. tuberculosis* complex

strains were identified by using an Accuprobe culture confirmation test (Genprobe, Inc., San Diego, CA) or, since January 2004, with a GenoType MTBC assay (Hain Lifescience GmbH, Nehren, Germany). Species within the M. tuberculosis complex were distinguished by a combination of biochemical tests and DNA fingerprint methods, as described in detail by van Klingeren *et al*. (15). M*ycobacterium africanum* isolates were not distinguished from *M. tuberculosis* and are therefore included among the isolates identified as *M. tuberculosis*. All 34 patients infected with *Mycobacterium bovis*, the 2 patients with *Mycobacterium canettii* strains, and the single patient with *Mycobacterium bovis* bacillus Calmette-Guérin were excluded from our study. In culture-negative cases, diagnosis was based upon clinical, radiographic, histopathological, and/or epidemiological grounds.

The national TB disease register provided data for reported cases after approval by its data protection committee. Cases that resulted from laboratory cross-contamination had been withdrawn from the register. The data were completed and validated using local registers, patient records, and the DNA fingerprinting register of the National Mycobacteria Reference Laboratory. The Medical Ethics Committee of Erasmus MC, University Medical Center Rotterdam, Rotterdam, approved the study protocol.

Recurrent TB was defined as disease reoccurring more than 1 year after the start of a previous episode. Twenty-five patients had two episodes during the study period, all with a culture-confirmed first episode. Of these recurrent cases, 14 were considered relapses, because 6 were not bacteriologically confirmed in the second episode, 7 had the same fingerprint as the first episode and their clusters had no pulmonary case since the first episode, and in 1 case, the *M. tuberculosis* isolate was erroneously not forwarded for restriction fragment length polymorphism (RFLP) typing. These 14 second-episode cases were excluded from the study. The other 11 recurrent cases were considered reinfections and both episodes were included in the study. Five of these cases had different fingerprints in both episodes, and 6 had identical fingerprints, but these cases shared epidemiological features with 1 or more pulmonary TB cases that were added to the cluster since the first episode.

DNA fingerprints. Since 1 January 1993, all *M. tuberculosis* isolates in The Netherlands have been subject to standardized IS*6110*-based RFLP typing, also called DNA fingerprinting (16). Clusters are defined as groups of patients having isolates with fully identical RFLP patterns or, if mycobacterial strains harbour fewer than 5 IS*6110* copies, isolates with identical subtyping in assays using the polymorphic GC-rich sequence (PGRS) probe (17). The first case in each cluster was classified as unique. In one cluster, the first and second positions were changed because a 4-month-old child was the first case in the cluster but she was unquestionably infected by her mother, who was diagnosed with urogenital TB 2 months later (18).

Transmission classification model. Cases were grouped into three main categories: (i) cases with a unique fingerprint, (ii) cases with a clustering fingerprint, and (iii) cases with an unknown DNA fingerprint. The category with unique fingerprints was subdivided into cases in immigrant and nonimmigrant patients. Transmission classification trees were developed for each category (see Table 2.1 and the supplemental material 2.1 to 2.5) and discussed during three consensus meetings with TB public health specialists.

The outcome of the classification process was, first of all, a likelihood scale of place of transmission, i.e., confirmed infected in a foreign country, probably infected in a foreign country, indeterminate, probably infected in The Netherlands, and confirmed infected in The Netherlands. The questions leading to this outcome were related to the date and time period of residence in The Netherlands for cases in immigrants; documented contact with a pulmonary TB case; documented history of frequent travel to countries where TB is endemic, i.e., more than 3 months cumulative during the 5 years prior to diagnosis; and the time difference with the last preceding pulmonary case in the cluster for clustered cases. In addition, the results of entrance screening were used to determine the most likely place of infection in immigrant patients without a DNA-fingerprinted isolate.

For patients infected in The Netherlands, the time of infection was classified as recently infected (≤2 years), remotely infected (>2 years), and unknown time of infection. Decisions were based on information routinely collected by TB public health nurses on the relationship between clustered cases and their assessment of whether an epidemiological link was confirmed, i.e., the patient knew a person in the cluster by name or was at the same place at the same time with a clustered pulmonary case, or the link was possible, i.e., the patient shared behavioural patterns (such as homelessness, illicit drug use, and pub visiting) with other patients in the cluster (7, 19). For confirmed epidemiologically linked clustered cases, the time difference between the dates of sample collection determined whether a secondary case had a recent or remote infection. Clustered cases without a confirmed epidemiological link were considered infected by the last preceding pulmonary case in the cluster. In cases without a fingerprint, decisions on the time of infection were based on a documented contact with a pulmonary TB case and a history of previous TB.

Results

Table 2.1 shows selected demographic and disease characteristics of the 2,636 cases included in the analysis. Of the 2,027 cases (77%) with a known DNA fingerprint, 919 (45%) were unique, of which 135 were the first case in a national cluster and 1,108 (55%) were clustered cases which were not the first case. Of the 609 cases (23%) with an unknown DNA fingerprint, 13 were culture confirmed but not RFLP typed and 596 were culture negative.

Table 2.1. Selected demographic and disease-related factors of 2,636 TB cases in the Rotterdam region, 1995 to 2006.

Patient characteristics	No. or % of TB cases with indicated type of fingerprint					
	Unique		Clustering		Unknown	
	No.	%	No.	%	No.	%
Total no.	919		1,108		609	
Male	485	52.8	738	66.6	322	52.9
Age (yr)						
0-14	15	1.6	32	2.9	109	17.9
15-29	258	28.1	386	34.8	145	23.8
30-44	276	30.0	400	36.1	157	25.8
45-64	164	17.8	229	20.7	121	19.9
≥ 65	206	22.4	61	5.5	77	12.6
Born in the Netherlands	219	23.8	338	30.5	273	44.8
Previous history of TB	68	7.4	58	5.2	35	5.7
HIV infection	51	5.5	50	4.5	13	2.1
Illicit drug user or homeless person	25	2.7	157	14.2	19	3.1
Pulmonary TB	576	62.7	811	73.2	271	44.5
Active case found by contact investigation	11	1.2	107	9.7	121	19.9
Active case found by screening	99	10.8	108	9.7	57	9.4

Patients older than 64 years more often had an infection with a unique fingerprint rather than a clustering or unknown fingerprint, patients between 15 and 44 years old more frequently had an infection with a clustering fingerprint rather than a unique or unknown fingerprint, and patients less than 15 years old more often had an infection with an unknown fingerprint rather than a known fingerprint. Patients with clustered cases were more often male, illicit drug users, or homeless than patients with infections with a unique fingerprint. Patients with isolates with a known fingerprint had pulmonary TB or an HIV coinfection more frequently than patients with isolates with an unknown fingerprint, while patients with isolates with an unknown fingerprint were more frequently born in The Netherlands or were identified in a contact investigation.

Classification of place of transmission. Epidemiological information suggested in 19 of 700 (3%) cases in immigrants with an isolate with a unique fingerprint that they were probably infected in The Netherlands (Table 2.2). Travel history suggested in 32 of 219 (15%) cases in nonimmigrants with an isolate with a unique fingerprint that they were probably infected in a foreign country. Sixty-two clustered cases were not

preceded by a pulmonary case in the cluster (52 were the second, 8 the third, 1 the sixth, and 1 the seventh case in a cluster) and followed the classification tree for unique fingerprints. Altogether, 114 (10%) clustered cases were classified as infected in a foreign country, while the remaining 994 (90%) were probably or confirmed infected in The Netherlands. Of the cases with an unknown fingerprint, 318 (52%) were classified as probably or confirmed infected in The Netherlands and 221 (36%) as probably or confirmed infected in a foreign country, and for the remaining 70 (12%), no decision could be made on possible place of infection.

Classification of time of transmission for patients infected in The Netherlands. All cases with a unique fingerprint with a probable or confirmed infection in The Netherlands were classified as remotely infected in The Netherlands, with the exception of two patients who had a documented contact with a visiting person diagnosed with pulmonary TB shortly after leaving The Netherlands (Table 2.2). Figure 2.1 shows the classification of time of infection for clustered cases infected in The Netherlands, indicated in Table 2.2 by boldface. Of these 994 cases, 369 patients (2 + 256 + 111) (37%) were confirmed to be recently infected, 414 (42%) were possibly recently infected, and 211 (10 + 160 + 41) (21%) were confirmed remotely infected. Of all cases with an unknown fingerprint, 154 (25%) were recently infected in The Netherlands. The majority of these cases (121 of 154) were identified by tracing contacts, while 33 cases had a documented contact with a recent pulmonary TB case in The Netherlands.

Epidemiological links. An epidemiological link was confirmed in 260 (23%) clustered cases, with 197 patients being recently infected and 63 remotely infected. In 190 (17%) clustered cases an epidemiological link was likely, because these patients shared the same behavioural characteristics. The other cases did not fulfil our criteria for a confirmed or possible epidemiological link, although patients often had a similar ethnic background and lived in the same geographical area. Epidemiological links were significantly more frequently confirmed in RFLP clusters with at least 5 bands (254 of 1,031; 25%) than in low-copy-number RFLP clusters which required additional PGRS typing (6 of 77; 8%) (P value of <0.01). Cases with an unknown fingerprint (154 of 609; 25%) had a confirmed recent epidemiological link more often than cases with a clustering strain (197 of 1,108; 18%) (P value of <0.01).

Table 2.2 summarizes data showing that of all culture-confirmed and culture-negative TB cases, 38% were probably or confirmed infected in a foreign country, 36% were probably or confirmed recently infected in The Netherlands, and 18% were probably or confirmed remotely infected in The Netherlands. In 4% of all cases, a decision on the time of infection in The Netherlands could not be reached, while in 5% of all cases, both the place and time of infection remained indeterminate in the classification process.

Table 2.2. Classification of place and time period of transmission of TB cases in the Rotterdam region, 1995 to 2006.

Criterion[a]	Place of infection					Time of infection for patients infected in The Netherlands[e]		
	Infected in a foreign country		Indeterminate[b]	Infected in The Netherlands		≤ 2 yrs	> 2 yrs	Unknown
	Confirmed	Probably		Probably	Confirmed			
Cases with a unique fingerprint (*n*=919)								
Cases in immigrants (*n*=700)								
Arrived in The Netherlands after 1/1/1993	376							
In residence before 1/1/1993 and had documented contact with PTB case in The Netherlands				18	1	1[c]	18	
In residence before 1/1/1993 and had documented history of frequent travel to countries where TB is endemic		79						
In residence before 1/1/1993 and lived more yrs in foreign country than in The Netherlands in the pregenotyping period		170						
In residence before 1/1/1993 without identified risks			56					
Cases in nonimmigrants (*n*=219)								
Documented contact with PTB case in The Netherlands					39	1[c]	38	
Documented history of frequent travel to countries where TB is endemic		32						
No documented risks				148			148	
Cases with a clustering fingerprint (*n*=1,108)								
No preceding PTB in the cluster[d] (*n*=62)	29	21		**12**		**2[c]**	**10**	
Diagnosis within 3 mo of arrival	32							
Not in residence in The Netherlands with a clustered PTB case	32							
Cases with a confirmed epidemiological link					**258**	**195**	**63**	
Cases with a possible epidemiological link					**190**	**185**	**5**	
Cases without an established epidemiological link				**534**		**401**	**133**	

Cases without a DNA fingerprint (*n*=609)								
Documented contact with PTB case in The Netherlands					192	154	38	
Documented contact with PTB case in a foreign country	13							
Chest X-ray abnormalities or positive TST at entrance screening	43							
Cases in immigrants who lived more yrs in a foreign country than in The Netherlands		151						
Cases in immigrants without other documented risks			70[b]					
Cases in nonimmigrant cases with documented history of frequent travel to countries where TB is endemic		14						
Cases of recurrent TB in nonimmigrants				13			13	
Cases in nonimmigrants without documented risks				113				113
Totals (*n* = 2,636)	525	467	126	838	680	939	466	113
%	19.9	17.7	4.8	31.8	25.8	35.6	17.7	4.3

[a] Dates are given as month/day/year. PTB, pulmonary tuberculosis; TST, tuberculin skin test.
[b] Twenty-two of 56 and 21 of 70 cases had unknown dates of arrival in The Netherlands.
[c] Patients were infected by a visiting person later diagnosed with infectious TB shortly after leaving The Netherlands.
[d] These clustered cases without a preceding PTB were classified according to the trees for unique fingerprints.
[e] The classification of time of infection for 994 clustered cases infected in The Netherlands, as outlined and presented in boldface in this table, is explained in Figure 2.1.

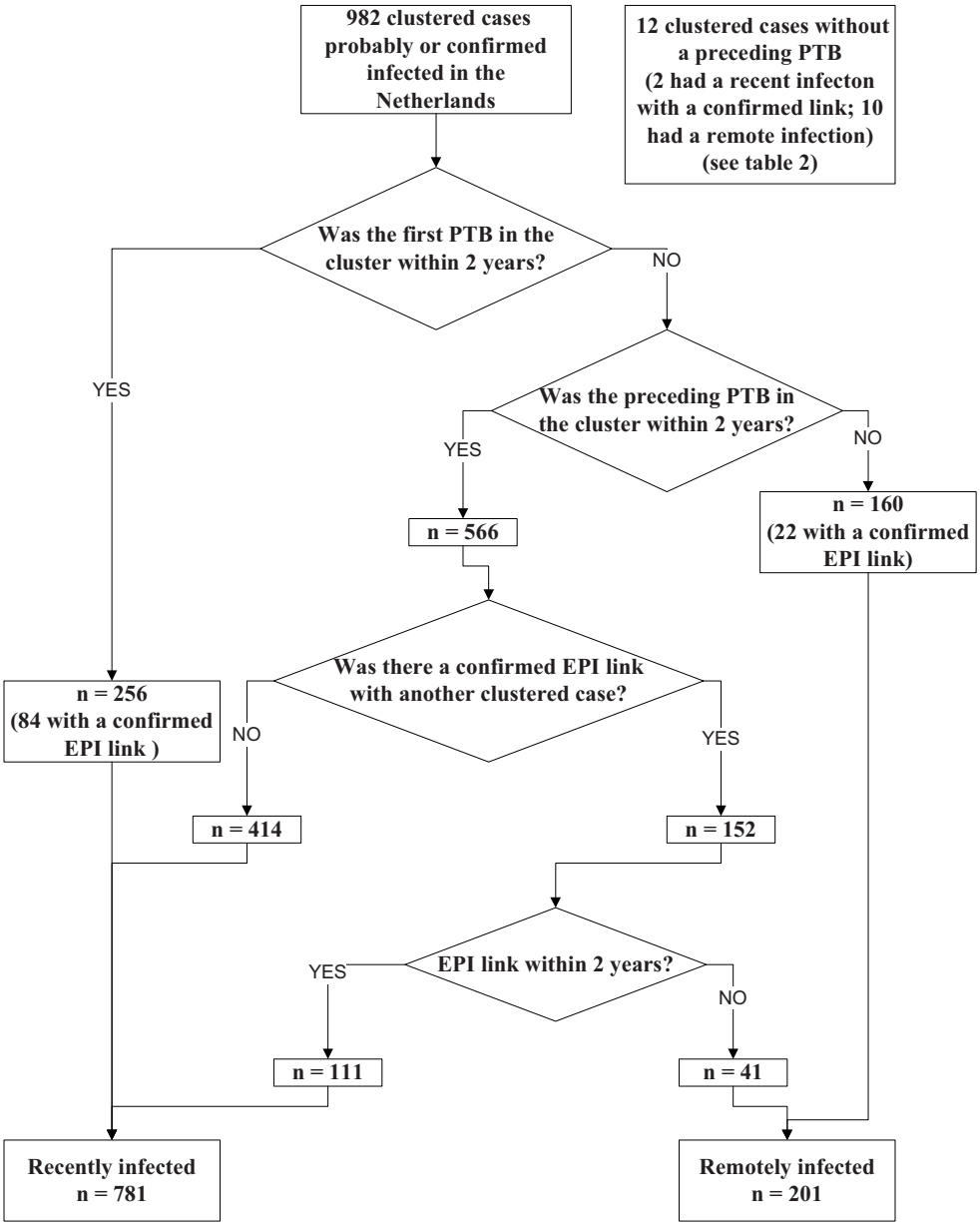

Figure 2.1. Classification of time of infection for clustered cases from the Rotterdam area that were probably or confirmed infected in The Netherlands, 1995 to 2006 (n=994). PTB, pulmonary tuberculosis; EPI link, epidemiological link.

Cluster size. The 1,243 clustering bacteria strains, including 135 cases which were the first case in a national cluster, were part of 452 national clusters. The cases in the study contributed with 1 case to 263 national clusters, with 2 cases to 98 clusters, with 3 to 10 cases to 74 clusters, and with more than 10 cases to 17 clusters. The largest cluster in The Netherlands, with 160 cases nationwide, contained 132 cases from the study area. The proportion of clustered cases recently infected in The Netherlands increased with the sequential number in a cluster (Table 2.3). Thirty-eight percent of cases that had the second rank in a cluster were recently infected in The Netherlands, while this proportion increased to more than 75% when the person was more than the 10th case in a cluster.

Table 2.3. Contribution of to cluster size in The Netherlands of clustered cases from the Rotterdam region that were not the first in the cluster and their classification of transmission, 1995 to 2006.

Patient's sequential no. in national clusters	Total no. of regional cases	No. (%) of patient that were:		
		Infected in a foreign country	Recently infected in The Netherlands	Remotely infected in The Netherlands
2	184	55 (29.9)	70 (38.0)	59 (32.1)
3	98	18 (18.4)	49 (50.0)	31 (31.6)
4-5	121	10 (8.3)	76 (62.8)	35 (28.9)
6-10	195	18 (9.2)	144 (73.8)	33 (16.9)
11-20	168	8 (4.8)	130 (77.4)	30 (17.9)
21-50	197	4 (2.0)	178 (90.4)	15 (7.6)
≥ 51	145	1 (0.7)	135 (93.8)	8 (5.5)
Total	1,108	114 (10.3)	783 (70.7)	211 (19.0)

Discussion

We used classification trees and combined molecular and conventional epidemiological data to ascertain the place and time of infection of all TB cases in our study population. Although most studies divide transmission into recent and remote infections, we added a separate category of infections acquired in a foreign country and limited the recent and remote categories to those infections acquired in The Netherlands. Of all TB cases during the 12-year study period, 38% were infected in a foreign country, 36% resulted from recent transmission in The Netherlands, and 18% resulted from remote infections in The Netherlands, while in the remaining cases (9%), either the time or place of infection could not be determined. The conventional epidemiological data suggested that at least 29% of

clustered cases were not recently infected in The Netherlands. Cases with unknown fingerprints, almost all culture negative, relatively frequently had confirmed epidemiological links with a pulmonary TB case and were more often identified by contact tracing and, therefore, presumably were recently infected in The Netherlands.

Molecular studies often use the n – 1 method, in which the first case in a cluster is considered unique (12, 20). The proportion of clustering, however, strongly depends on the time period of the study, the geographical area, and the proportion of cases included in a fingerprinting programme (12, 14). In The Netherlands, genotyping has been performed for nearly all *M. tuberculosis* isolates for 14 years and clustering is confined to the national borders. If we had restricted the n – 1 method to the Rotterdam study region, 452 instead of 135 cases that were the first in a cluster would be considered unique and the clustering proportion would decrease from 55 to 39%, which underscores the influence of the geographical area on clustering.

In most studies, recently transmitted TB is defined as disease occurring within 2 years of infection (4, 21-23), although some studies have limited the latency period to 1 year (6, 24, 25) or extended it to 5 years after infection (26). There is a need to decide on the latency period of recent disease development so that outcomes, such as programme performances, can be compared. We propose to use the 2-year latency period, as applied in our study.

Cases with unique strains are rarely the result of recent transmission in a country if universal genotyping has been applied for more than 2 years. There are some rare exceptions that we also encountered, such as transmission by a visiting person who is diagnosed with TB after leaving the country. Recently transmitted bacteria may also be reported as unique strains if the *M. tuberculosis* genotype has changed over time (8). The half-life of RFLP genotypes is unclear but has been estimated to vary between 3 and 10 years in certain situations (27, 28). In our study, five initially unique fingerprints of epidemiologically linked cases were reinvestigated by the National Mycobacteria Reference Laboratory and showed a 1-band difference with the expected clusters, and these strains were therefore placed in the respective RFLP clusters. We recommend that fingerprinting programmes should assess epidemiological links if RFLP patterns differ by 1 band, to identify these molecular changes.

In our study, 71% (783 of 1,108) of clustered cases were confirmed or possibly recently infected in The Netherlands, 211 (19%) were remotely infected in The Netherlands, and 114 (10%) were infected in a foreign country. The proportion of clustered cases due to recent transmission in our study is an overestimation because all cases without known epidemiological links and with a preceding pulmonary case in the cluster within 2 years were classified as recently infected in The Netherlands. In particular in circumstances of high transmission with large clusters, it is more difficult to ascertain the source case and time of infection (29).

The results of our study also showed that patients with low rank numbers in their clusters were frequently infected in a foreign country or remotely infected in The Netherlands. There are basically three explanations for clustered cases not representing recent transmission (9, 11, 30). Immigrant patients may have been infected in their countries of origin with a genetically homogenous strain also present in the national fingerprinting database. Nonimmigrant patients may have been infected several years or decades before with a strain circulating at that time in the country under study. And last, RFLP typing may be unable to differentiate two nonidentical strains. We recommend that additional genotyping, such as direct repeat sequence or mycobacterial interspersed repetitive units and variable-number tandem repeat analysis, is considered if epidemiological links are not confirmed in small-sized clusters, as was done in other studies (31, 32). Cases in PGRS clusters with low-copy-number RFLP strains had a relatively low percentage of confirmed epidemiological links in our study, confirming the lack of discriminatory power of additional PGRS typing (33, 34).

In most developed countries, the diagnosis of TB is confirmed by culture in 80% of all cases at the most (35, 36). Thus, transmission studies that rely exclusively on genotyping overlook the contribution of culture-negative cases to recent transmission. In our study, cases without a fingerprint had a confirmed recent epidemiological link significantly more often than cases with a clustering strain, indicating the importance of culture-negative TB as a result of recent transmission.

In our classification model, we used a number of questions leading to decisions of place and time of infection with a certain probability. Positive answers to questions about recent residence in The Netherlands in cases with a unique fingerprint and documented contact with a pulmonary TB case clearly provide stronger evidence for the outcome than, e.g., questions about frequent travel to countries where TB is endemic. We applied the questions stepwise, and in this way, we believe that we have made optimal use of relevant and available information. In the future, with improved interviewing skills of TB public health nurses and the use of a more-systematic approach to investigate and confirm links between cases, classification can become more accurate. Furthermore, the model should be evaluated in other transmission settings to assess its application in these circumstances.

Our findings underline the consensus that clustering should not be considered identical with recent transmission and that genotyping should be combined with conventional epidemiological investigation. A standardized way of classifying TB into recently, remotely, and foreign-acquired disease provides indicators for surveillance and programme performance that can be used to decide on interventions and allocate resources. Programmes with predominately recent transmission may focus on a package of targeted activities for active case finding, while those with a high proportion of imported strains should consider screening for TB and latent TB infection in immigrants and those with mainly reactivated cases can shift attention to the elimination of TB.

Acknowledgement

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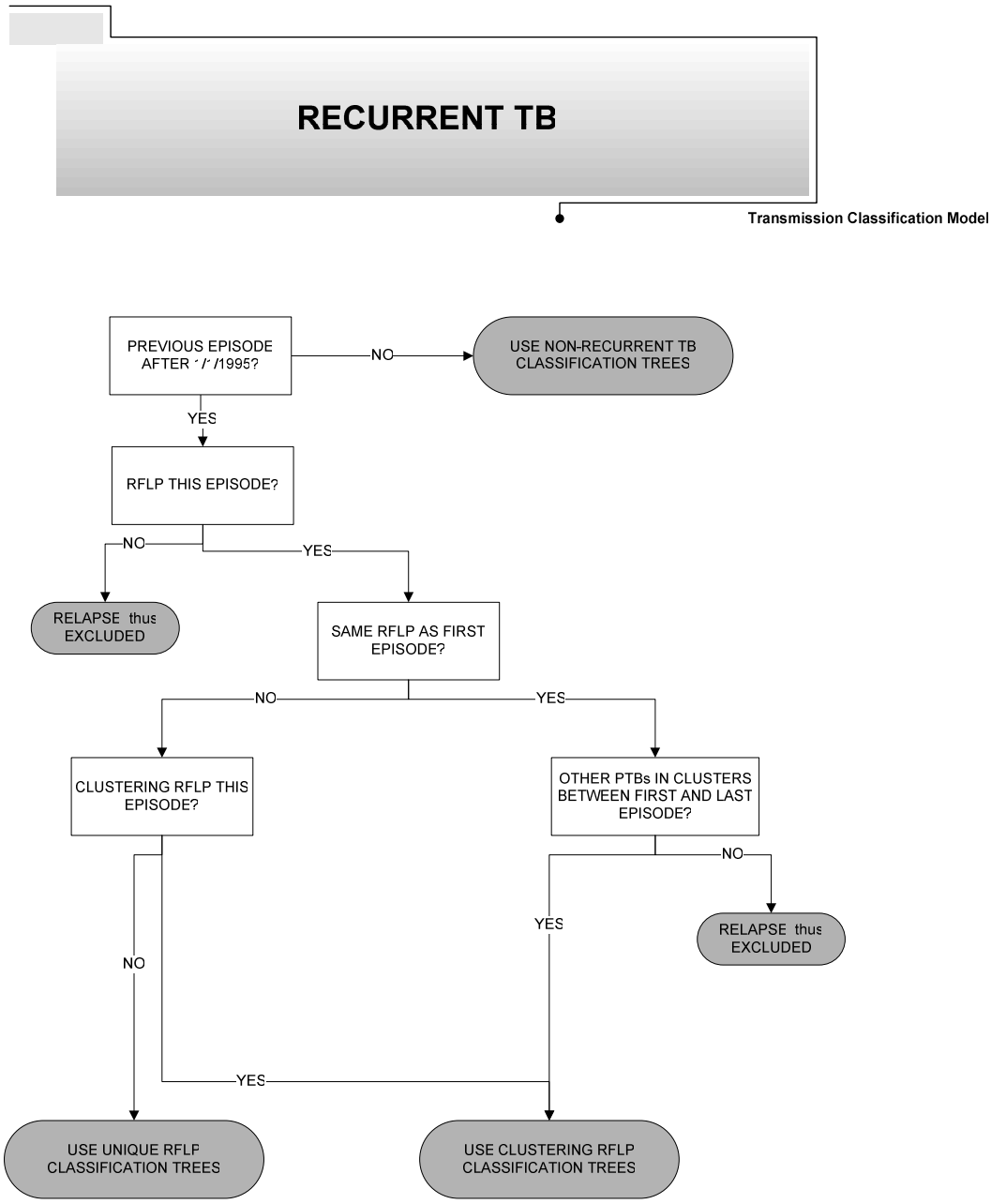
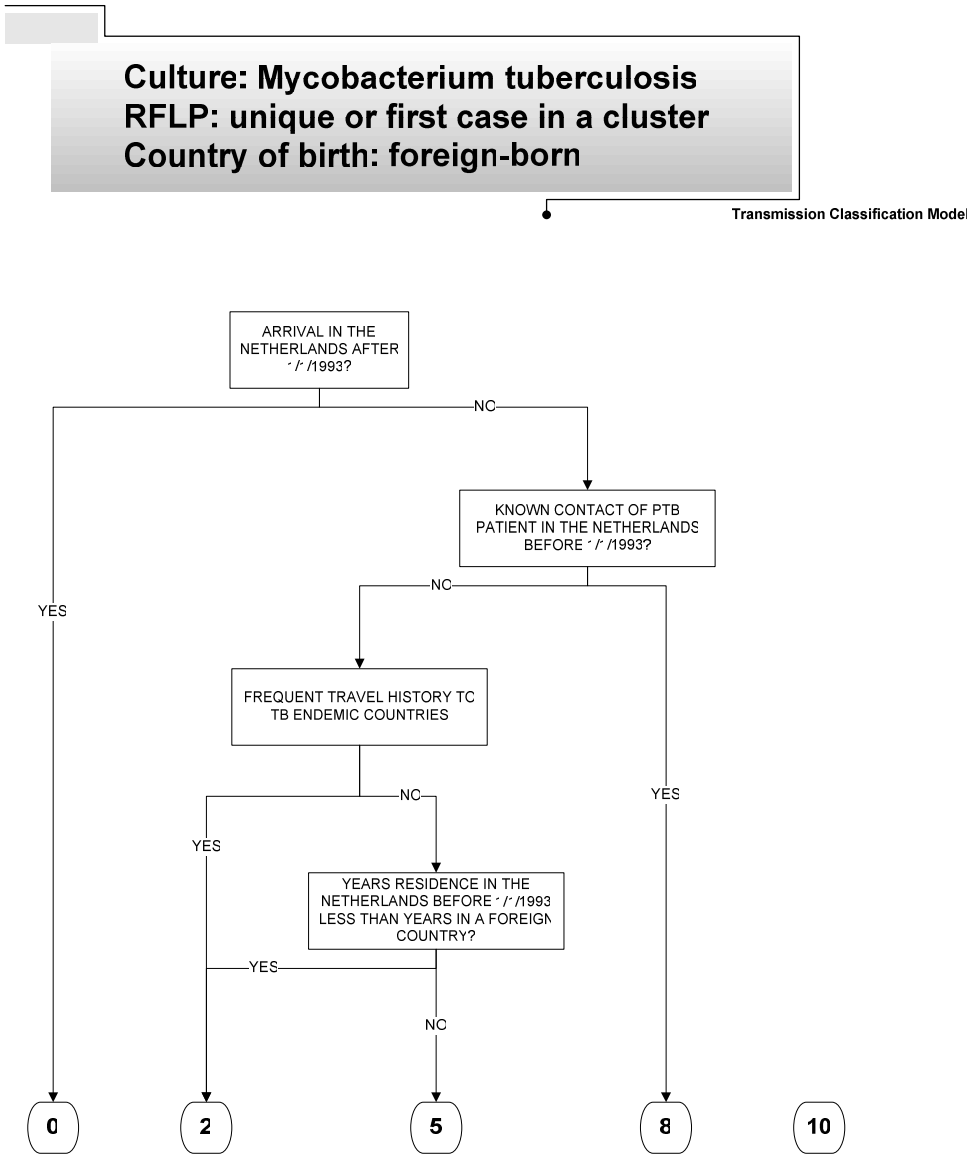
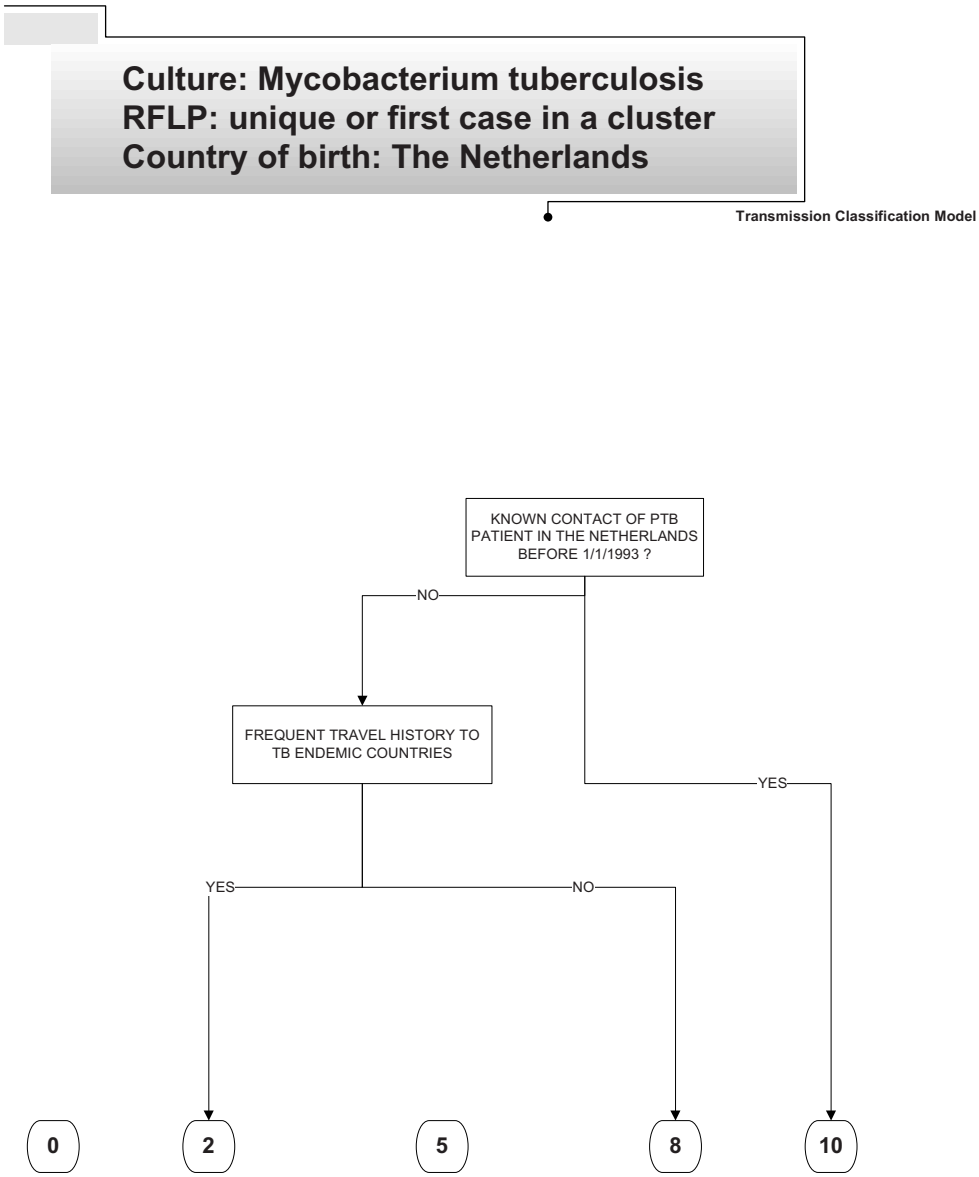


Supplement 2.1. Classification of recurrent TB cases that also had a first episode during study period.

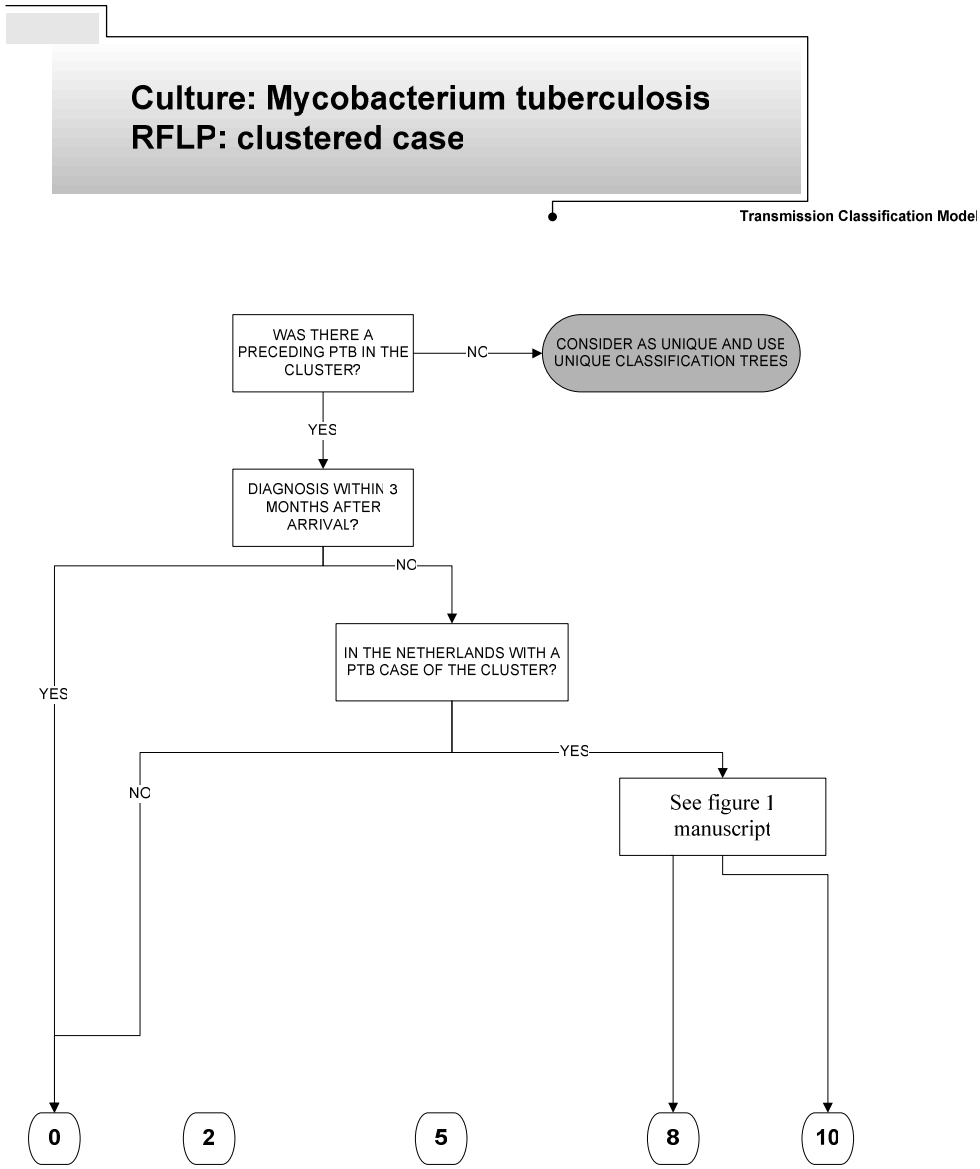


Supplement 2.2. Classification tree for cases with a unique fingerprint (immigrant cases).

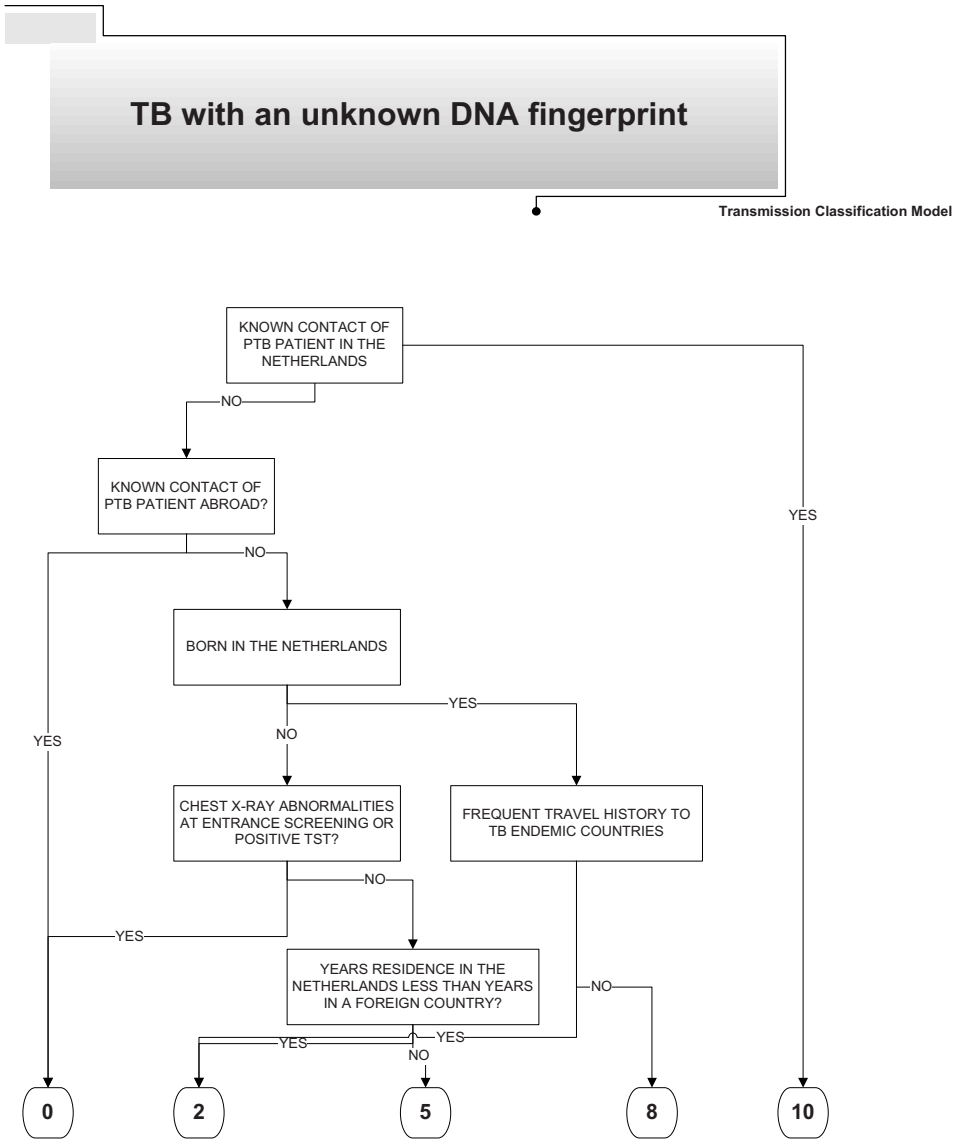


Probability scale of place of infection. 0 = confirmed infected in a foreign country; 2 = probably infected in a foreign country; 5 = indeterminate; 8 = probably infected in the Netherlands and 10 = confirmed infected in the Netherlands

Supplement 2.3. Classification tree for cases with a unique fingerprint (nonimmigrant cases).



Supplement 2.4. Classification tree for cases with a clustering fingerprint.



Probability scale of place of infection. 0 = confirmed infected in a foreign country; 2 = probably infected in a foreign country; 5 = indeterminate; 8 = probably infected in the Netherlands and 10 = confirmed infected in the Netherlands

Supplement 2.5. Classification tree for cases with an unknown fingerprint.

3

A *Mycobacterium tuberculosis* cluster demonstrating the use of genotyping in urban tuberculosis control

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Abstract

DNA fingerprinting of *Mycobacterium tuberculosis* isolates offers better opportunities to study links between tuberculosis (TB) cases and can highlight relevant issues in urban TB control in low-endemic countries.

An outbreak with 28 cases with identical DNA fingerprints and 4 epidemiologically linked culture-negative cases was used for the development of a visual presentation of epidemiologic links between cases.

Of 32 cases, 17 (53%) were linked to the index case, and 11 (34%) to a secondary case. The remaining four (13%) could not be linked and were classified as possibly caused by the index patient. Of the 21 cases related to the index case, TB developed within one year of the index diagnosis in 11 patients (52%), within one to two years in four patients (19%), and within two to five years in six patients (29%).

Cluster analysis underscored several issues for TB control in an urban setting, such as the recognition of the outbreak, the importance of reinfections, the impact of delayed diagnosis, the contribution of pub-related transmissions and its value for decision-making to extend contact investigations. Visualising cases in a cluster diagram was particularly useful in finding transmission locations and the similarities and links between patients.

Introduction

In descriptive epidemiology, the distribution and transmission of infectious diseases is usually described in terms of place, person, and time. This approach is appropriate for diseases with a short incubation period but far less so for chronic diseases such as tuberculosis (TB), which progresses from latent infection to active disease over a period ranging from months to many years. In TB epidemiology, graphic presentation is often limited to trend description over years in a defined region, with sociodemographic characteristics of the patients (1, 2). However, in outbreak management, one would like to know which secondary cases are caused by an index patient. Presentation in a time line may help to detect epidemics, plan interventions, and evaluate these efforts.

DNA fingerprinting of *Mycobacterium tuberculosis* isolates has improved the understanding of TB transmission and helped to identify outbreaks, high-risk groups, and laboratory cross-contaminations (3, 4). To allow adequate interpretation and comparison, these molecular studies should involve a high proportion of cases in a population, be combined with conventional epidemiological investigations, and specify patient characteristics and study length (4, 5). In the Netherlands, the proportion of culture-confirmed cases is high (1); nearly all *M. tuberculosis* isolates have been genotyped for more than 14 years, and results are available at Municipal Public Health Services (MPHS) (6, 7).

We describe a cluster of cases in the Rotterdam area, introducing a novel cluster diagram that shows epidemiologic links between cases. The cluster analysis is used to discuss relevant issues in urban TB control in low-endemic countries.

Methods

Since 1993, all *M. tuberculosis* isolates in the Netherlands are subject to standardised insertion sequence (IS)*6110*-based restriction fragment length polymorphism (RFLP) genotyping**,** referred to as DNA fingerprinting (8). Clusters are defined as groups of patients having isolates with fully identical RFLP patterns or, if strains have fewer than five IS*6110* copies, with identical sub-typing determined by the Polymorphic GC-Rich Sequence probe (9). TB control nurses of MPHSs explore possible links between clustered cases during visits to patients.

The MPHS Rotterdam-Rijnmond covers the municipality Rotterdam (about 600,000 inhabitants) and 27 other municipalities, serving a total population of 1.3 million. Between 1993 and 2006, 2,562 TB cases were reported, resulting in an average annual incidence rate of 23.3 per 100,000 for Rotterdam municipality and 6.6 per 100,000 for other municipalities. Of the 2,562 cases, *M. tuberculosis* was cultured in 1,969 (76.9%), of which 1,939 (98.5%) were RFLP-typed. Of these, 881 (45.4%) were unique or initially unique first cases in a cluster, and 1,058 (54.6%) were non-first cases in a cluster. Of the RFLP-clustered cases,

497 (47.0%) were the 1-5th case in a national cluster, 325 (30.7%) the 6-50th case, while 236 cases (22.3%) were 51st or subsequent case in three large national, predominantly Rotterdam, clusters.

A medium-sized molecular cluster of 28 cases with considerable transmission in and around Rotterdam was used to develop a cluster diagram with Microsoft Visio® software including a time-period bar, zones suggesting locations of transmission, and symbols representing the ethnic background, disease site, and infectious status of patients, and arrows representing documented epidemiological links between cases. The causative *M. tuberculosis* strain was susceptible to all first-line TB drugs. Four culture-negative epidemiological linked cases were included in the cluster diagram.

Contact investigation was executed according to Dutch guidelines, i.e. close contacts of smear-positive TB patients are examined in two rounds with a tuberculin skin test (TST) performed immediately after diagnosis of the index patient and two months after their last contact. Cases identified with a latent TB infection are generally treated with six months isoniazid. Contacts ineligible for TST (born before 1 January 1945, vaccinated with bacille Calmette-Guérin (BCG), or having positive TST or TB in the past) are examined twice with a chest radiograph: immediately after diagnosis of the index and three months after the last contact with the index. An investigation is extended from close to casual contacts on finding high rates of recent TB infections or TB cases. All those investigated are linked to the index patient in the client information system of the Dept of Tuberculosis Control. Using this data, we analyzed the results of TSTs and radiographs of contacts linked to patients in our cluster.

The Medical Ethics Committee of Erasmus MC, University Medical Center Rotterdam, Rotterdam approved the study protocol.

Results

Description of the outbreak. The first strain identified in our cluster (Figure 3.1) was from a Surinamese person with pleural TB (case 1). One month later, pulmonary TB was diagnosed in a Bosnian woman (case 2), who four months earlier had reported to the Dept of Tuberculosis Control that a Bosnian man was her houseguest for several weeks and had subsequently entered a German hospital with smear-positive pulmonary TB (case X). Due to prior BCG vaccination, the woman had a chest X-ray at presentation and three months after her last contact with patient X. Her second radiograph showed discrete upper lobe infiltrative lesions. One month later, her sputum culture became positive for *M. tuberculosis*; at her next consultation, radiographic changes were more pronounced and sputum smears were positive.

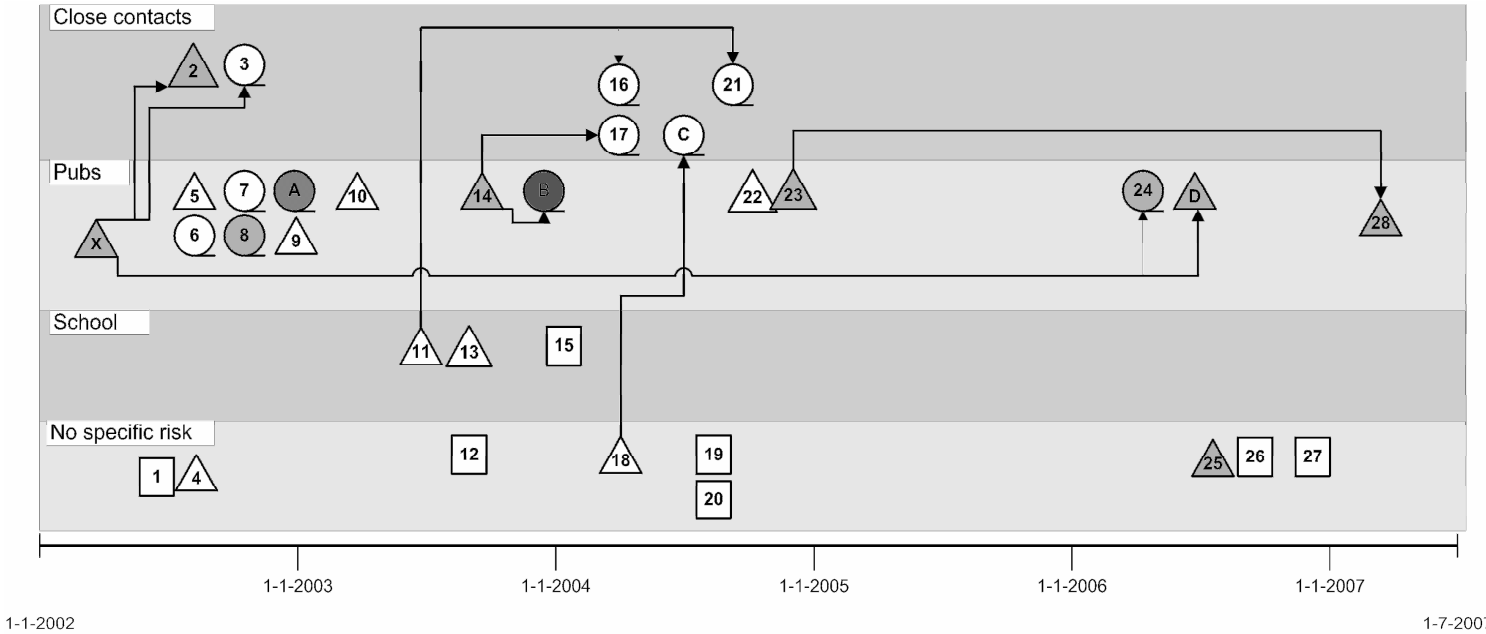

Figure 3.1. Diagram of clustered and epidemiologically linked cases in a tuberculosis outbreak.
Each symbol represents a patient. Triangles denote patients with smear-positive pulmonary tuberculosis (TB), squares patients with smear-negative pulmonary TB, and circles patients with extrapulmonary TB. Grey coloured symbols indicate patients with Yugoslavian ethnicity, and for simplicity, all cases with other background have uncoloured symbols. (Origins included Netherlands in 4 persons, Surinam or Netherlands Antilles in 7, Morocco in 5, Indonesia in 3, India in 1, and Turkey in 1). Cases with a number are culture-confirmed; cases with a letter are culture-negative cases epidemiologically linked to the cluster. Lines between cases indicate that cases know each other (see also Results).
Note: The diagram can be made with different coloured symbols for ethnic background. Instead of symbols, bars could be used to show the duration of symptoms of infectious TB patients.

Within a few months after clustering of cases 1 and 2, another eight cases were diagnosed with TB caused by a mycobacterial strain having an identical fingerprint. Almost all these patients frequented a certain pub in Rotterdam, and the TB control nurses found that patient X had been ill for several months and also visited this pub. Case 8 was a five-year-old child who had a positive TST and hilar lymphadenopathy on the chest X-ray. TB was diagnosed by positive culture for *M. tuberculosis* of the gastric lavage fluids. His brother (case A) likewise had a positive TST and hilar lymphadenopathy, and started TB treatment without further diagnostic investigation. Both children often accompanied their grandfather to the above-mentioned pub, which was owned by relatives.

Several months after case 10, smear-positive pulmonary TB was diagnosed in an 18-year old student of a vocational training school (case 11). He had been coughing for five months and visited his general practitioner several times. Contact tracing revealed latent TB infections (LTBI) in all his seven family members, in classmates, and persons at his vocational training site, including five persons with TST conversions. After two months, a second student from the school was diagnosed with smear-positive pulmonary TB (case 13). He did not share ethnic background or classes with case 11. His family, friends, and classmates were examined, but extension of the contact investigation was actually decided on the basis of DNA fingerprint results. When they confirmed clustering, the entire school was examined and yielded one case of pleural TB (case 15) – confirmed by culture of a pleural biopsy – and 40 LTBI cases. Disease developed in a brother of case 11 (case 16) despite treatment with isoniazid; he later admitted taking only three weeks of medication despite attending all medical check-ups. The disease developed in another relative of case 11 (case 21), who had been treated for TB twenty years earlier.

Five other patients (cases 14, 22-24, and D) had a link with the pub and were most likely infected by patient X. Patient D, one of the pub owners, had a chest X-ray in a general hospital four months before her TB diagnosis; it showed bilateral opacities and she was treated for pneumonia. Despite repeated visits to her general practitioner, smear-positive pulmonary TB was diagnosed only when she travelled to her native country. In the interim, she had stayed with her disabled child several months in a hospital, and had infected a nurse (case 27) who seven years earlier had completed LTBI treatment after converting the TST.

Patient characteristics. The 32 patients in this outbreak (excluding case X) were aged 5 to 67 years (average 33 years); 81% were male; 25 (78%) had pulmonary TB, of which 14 were smear-positive. None were found coinfected with HIV. All 32 patients completed TB treatment. Of the 32 patients, 21 lived in Rotterdam, seven in two nearby towns, and one (case 22) resided in a village 50 km from Rotterdam. After genotyping, this last patient was reinterviewed and revealed regular visits to the Rotterdam pub up to one year before diagnosis. The remaining three (cases 4, 18

and 27) lived outside the Rotterdam area but had links to the city or to other patients in the cluster.

Table 3.1. Parameters (time, person and place) by which cases in the outbreak were linked.

Tuberculosis case in cluster	Linked to source patient	Time	Person	Place
2, 3	Case X	Yes	Yes	Yes
1, 4, 11	Case X	Yes	No	No
5, 6, 7, 8, 9, 10, A	Case X	Yes	No	Yes
14, 22, 23	Case X	No	No	Yes
24, D	Case X	No	Yes	Yes
12, 13, 15	Case 11	Yes	No	Yes
16, 21	Case 11	Yes	Yes	Yes
B, 17	Case 14	Yes	Yes	Yes
C	Case 18	Yes	Yes	Yes
26, 27	Case D	Yes	No	Yes
28	Case 23	No	Yes	Yes
18, 19, 20, 25	No link established	No	No	No

Cases with a number are culture-confirmed; cases with a letter are culture-negative cases epidemiologically linked to the cluster.

Second and third generation cases in the outbreak. Of 32 cases, 17 (53%) were epidemiologically linked to case X (i.e. secondary cases) based on time intervals, personal contacts, or meeting places (Table 3.1), and 11 cases (34%) were certainly or possibly linked to a secondary case (i.e. tertiary cases). The remaining four clustered patients (13%) could not be linked and were classified as possibly infected by case X (i.e. secondary cases).

Figure 3.2 shows the interval between the month of case X diagnosis and the month specimens were collected from the 21 persons who were certainly or possibly infected by him. Of these, TB developed within a year of the index diagnosis in 11 (52%), within one to two years in four (19%), and within two to five years in six patients (29%).

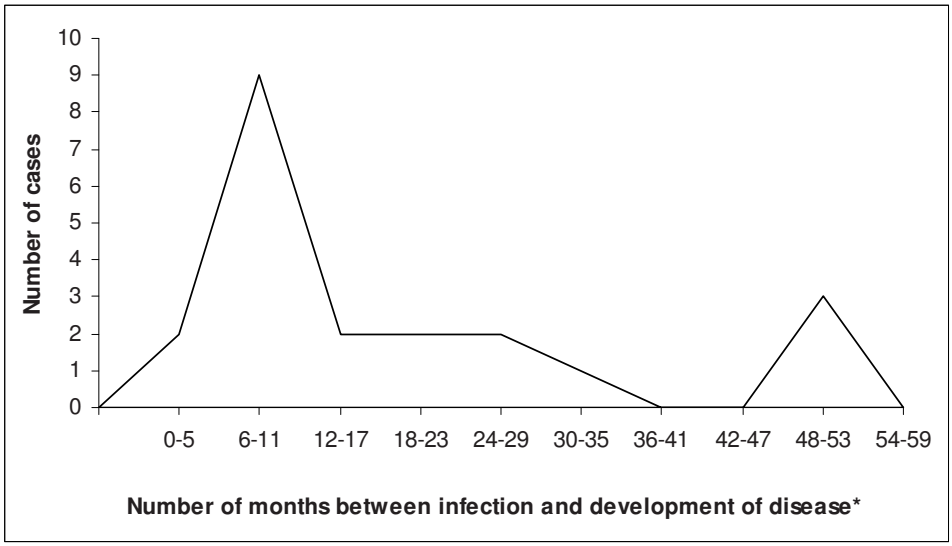

Figure 3.2. Curve of the time between tuberculosis infection and development of disease in 21 secondary cases. * The date of development of disease was assumed as the date of specimen collection and latest time of infection was defined as the diagnosis date of the index patient.

Contact investigation. Contact tracing was mainly limited to close contacts of pulmonary TB cases (Table 3.2). Three investigations were extended to many casual contacts after DNA fingerprinting confirmed that transmission had occurred, respectively, in the above-mentioned pub, vocational school and hospital. Only a few pub visitors outside the known contacts could be tested due to non-cooperation of the primary pub owner.

Table 3.2. Contact tracing results of smear-positive pulmonary and other tuberculosis patients of the cluster.

	Number of contacts examined	Number of contacts examined with TST	Number of tuberculosis infections	Proportion of tuberculosis infections among those tested	Tuberculosis cases
Mr. X	3	1	1	100	Case 2
Case 2	23	14	1	7	Case 3
Case 4	0	0	0	0	
Case 9	24	5	0	0	
Case 10	50	41	1	2	
Case 11	194	154	24	16	(Case 16)
Case 13	45	33	0	0	
School	1,730	964	40	4	Case 15
Case 14	1	0	0	0	Case B
Case 18	11	4	0	0	Case C
Case 22	31	15	1	7	
Case 23	4	4	1	25	
Case D	3	3	3	100	
Case 25	10	2	0	0	
Hospital	100	92	1	0	
Case 28	32	8	3	38	
Other	67	32	3	9	Case A
Total	2,328	1,372	79	5.8	6

Cases with a number are culture-confirmed; cases with a letter are culture-negative cases epidemiologically linked to the cluster.

Discussion

In retrospect, the index patient of the cluster was highly infectious, causing 11 secondary TB cases within one year after exposure. Contact investigation after detailed interviewing of an infectious patient normally identifies contacts for screening and preventive treatment. In this case, however, the index patient was initially unknown and the natural history of the disease was unimpeded. Five years after the index diagnosis, 28 culture-confirmed cases with identical fingerprints had been identified, forming a new molecular cluster. The cluster diagram highlighted several issues relevant to urban TB control in low-incidence countries.

Natural history of tuberculosis. The classification of patients in the cluster allowed us to draw a curve of secondary cases. Of these cases, TB developed within one year of infection in 11 (52%), within two years in 15 (71%), and between two to five years in six patients (29%). The curve recalls results of previous tuberculin conversion studies, which estimated

that TB develops within five years in 14% of adults infected, with 60% of cases presenting within one year, 85% within two years, and 15% within two to five years (10). Some of our late-secondary cases may have been misclassified as caused by the index patient, raising the proportion of secondary cases that occurred within two years.

The number of secondary cases reported in the first year allows one to estimate the total number of persons infected by the index patient and to predict the number to be expected in the next few years. As illustrated by our cluster, secondary cases can generate tertiary cases, and together with late-secondary cases they can protract an TB outbreak over many years (3, 11-14).

Our study included four culture-negative TB cases and 75 LTBI cases, indicating that conventional epidemiological investigation and molecular typing should be combined (5). Studies using only genotyping data exclude such cases and may thus underestimate the true extent of an outbreak and the value of active case finding.

Reinfection. Cluster analysis and conventional epidemiological investigation revealed two reinfections (cases 21 and 27). Molecular studies have demonstrated that the extent of exogenous reinfection in recurrent TB depends largely on the epidemiological context (4, 15). In populations at low risk, most recurrence results from reactivation, although the proportion due to reinfection in some low-endemic countries may exceed expectations, ranging between 16% and 44% (16-18). Our two reinfection cases illustrate that a contact investigation must also look carefully at previously infected persons.

Geographical clustering and urban spread. The outbreak was mainly limited to the Rotterdam area; patients outside the area were epidemiologically linked with a clustered case. Our experience with DNA cluster analysis is that most clustered patients in the Netherlands are residents of the same geographical region.

Genotyping can be used to monitor the magnitude of the problem of TB transmission. Several studies have demonstrated that the proportion of clustered cases in urban areas comprise 20 to more than 50 percent of all local TB cases, indicating recent transmission (3, 4, 19-23). A recent outbreak of an isoniazid-resistant *M. tuberculosis* strain with more than 132 cases in London and a molecular cluster of 150 in the Netherlands (of which 85% are in Rotterdam), underscore the need in large cities for interventions tailored to specific risk groups (12, 13, 24).

Tuberculosis among foreign-born persons. Typically, foreign-born residents account for more than 50% of TB cases reported in low-endemic countries (2). Although most are probably infected in their native countries (25), recent transmission in the country of residence can contribute significantly to high incidence in the foreign-born. In our cluster, the index patient directly and indirectly caused disease in 32 persons, of whom 10 (31%) shared his ethnic background and 22 (69%)

did not. In general, transmission between foreign nationalities and to the indigenous nationality varies widely in low-endemic countries, probably reflecting different social mixing patterns (25-28).

Transmission in high-risk settings. Our cluster diagram shows that half of the cases were infected in the same pub. Bars are known for high transmission rates due to crowding and poor ventilation (11, 29, 30). Smoking may also increase the risk of *M. tuberculosis* infection (31). Contact investigation is often hampered by pub owners' reluctance to assist in informing the clientele, difficulties in finding contacts, and unwillingness of infected persons to start a potentially hepatotoxic preventive treatment that precludes the use of alcohol (11).

Our cluster analysis also confirmed TB transmission at a school and in a hospital. The increased risk for health care workers is well-documented and is usually related to delayed diagnosis of TB patients (32, 33). In our study, a patient's relative was the source case of TB transmission to a health care worker, so the nosocomial setting was incidental.

Promoting early case detection and maintaining clinical expertise. Delays in seeking medical attention or receiving diagnosis facilitate TB transmission (4). Our index patient severely delayed seeking care despite the urging of his friends. On the other hand, two secondary patients repeatedly visited their general practitioners before being diagnosed with TB, and one of these was diagnosed abroad. Delays in these three cases led to several LTBIs and TB cases. In many low-incidence countries, TB expertise is declining in general practitioners, clinical specialists, and public health professionals, but resulting delays in diagnosis can be reduced by continuing education (34). Delays in seeking care can be reduced by public health education and low-threshold access to TB diagnostic and therapeutic services.

Outbreak management and contact investigation. In our outbreak, 6 TB cases and 75 persons with LTBI were traced. Most LTBI cases were diagnosed in the school investigation, and treatment controlled the situation because no more cases were identified in three years of follow-up. The importance of preventive therapy was highlighted by a noncompliant patient with LTBI who subsequently developed TB.

Failure to identify and examine eligible contacts, as occurred in the pub investigation, is a major reason for disease development (14). Our diagram shows that even detailed interviewing failed to disclose all links between patients and potential locations of transmission, a problem also encountered by others (3, 4, 7, 11, 14, 35). However, our study clearly demonstrates that clustering of cases in time and place, with similar sociodemographic patterns, resulted from recent transmission in the Netherlands.

Conclusions

In an urban area in a low-endemic country with ongoing TB transmission, cluster analysis was a useful complement to conventional epidemiological investigation. It revealed that foreign-born patients were infected in this low-endemic country and not their native countries, as might have been assumed. Visualising cases in a cluster diagram was particularly useful in finding transmission locations and the similarities and links between patients. It also helped to monitor the cluster and outbreak over time and to estimate the number and timing of future cases.

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4

Causes of high tuberculosis case rate in a metropolitan area

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Abstract

Rationale: The overall decline of tuberculosis (TB) incidence in most developed countries has made differences between urban and rural communities more prominent.
Objectives: To clarify the causes of the high TB case rate in a metropolitan area.
Methods: Comparing characteristics of patients and mycobacteria in urban and suburban municipalities of the Rotterdam region in the Netherlands between 1995 and 2006.
Main Results: The TB case rate in the urban municipalities was 3.8 times higher than in suburban municipalities. After stratification for country of birth, case rates were 1.7 times higher for immigrants and 2.8 times higher for nonimmigrants in the urban municipalities. At least 47% of urban immigrant TB cases had foreign-acquired disease against 62% of immigrant cases in the suburban municipalities. Recent transmission caused 40% of the case rate in the urban and 28% in suburban municipalities, translating into a 5.5 times higher recent transmission case rate in urban municipalities. Illicit drug users and homeless persons accounted for 10.3% of all urban cases.
Conclusions: The high urban TB case rate was related to the high proportion of urban immigrants who frequently reactivate a foreign-acquired infection. Besides that, recent transmission caused a substantial part of the TB caseload in urban municipalities, both among urban immigrants and nonimmigrants. Illicit drug users and homeless persons in urban municipalities were particularly at risk for TB due to recent infection. Metropolitan TB control should address these different causes of the high urban TB case rates by a package of targeted and tailored interventions.

Introduction

Over the past century tuberculosis (TB) incidence in developed countries gradually declined, especially after effective chemotherapeutic treatment regimens were introduced since the 1950s. An upsurge of TB occurred in the 1980s in many low-incidence countries due to increased immigration, concurrent human immunodeficiency virus (HIV) infections and inadequate TB control practices (1), but the general downward trend has resumed in many of these countries (2). In the Netherlands, the TB incidence of 2006 was the lowest ever recorded with 6.2 cases per 100,000 persons (3).

The overall decline has made differences of TB incidences in urban and rural areas more prominent (4, 5). In France, 43% of all cases lived in the Paris region, which contained 19% of the French population (5). In England and Wales, 41% of cases lived in London, although only 14% of the population resided in this city. The incidence in London increased to 44.8 per 100,000 in 2006 (personal communication Jonathan Croft). In 2006, the average TB incidence in the four largest cities in the Netherlands (Amsterdam, Rotterdam, The Hague and Utrecht) was 21.7 per 100,000 population (3). They notified 34% of all TB cases in the Netherlands, although only 13% of the Dutch population lived in these four cities. TB case rates in urbanized and rural areas probably reflect different dynamics. It is thought that in rural areas TB more often results from reactivation of remote infections, while in urban areas imported disease and ongoing transmission, more specifically among certain urban risk groups such as illicit drug users and homeless persons, account for higher cases rates (6).

DNA fingerprinting of *Mycobacterium tuberculosis* isolates provides a tool to disentangle the different transmission pathways (7, 8). Although the percentage of clustered cases in an area indicates the amount of recent transmission in a community, clustering is not identical with recent transmission (9-11). Combined use of conventional epidemiological and genotyping data, using standardized algorithms, ascertains more accurately recently and remotely infected cases (12-15).

The objective of this study is to clarify the causes of the high TB case rate in a very highly urbanized area by comparing the characteristics of patients and mycobacteria in these areas with those in less-urbanized municipalities. Based on the findings, we propose a package of targeted and tailored active case finding and case holding activities for metropolitan TB control.

Methods

Study area. The study was conducted in the city of Rotterdam (about 600,000 inhabitants) and 54 neighboring municipalities (about 1,300,000 inhabitants) located in the southern part of the province of South-Holland, the Netherlands. Population characteristics by municipality, age group and country of birth were obtained from the Office for National Statistic, Statistics Netherlands (http://www.cbs.nl). Statistics Netherlands divides every municipality in the Netherlands into one of the following five Area Address Density (AAD) categories: (i) very highly urbanized (>2500 addresses/km^2); (ii) highly urbanized (1500–2500 addresses/km^2); (iii) moderately urbanized (1000–1500 addresses/km^2); (iv) low urbanized (500–1000 addresses/km^2); and (v) rural (<500 addresses/km^2). We compared AAD1 with AAD2-5 municipalities, because of the expected high TB case rate in the very highly urbanized areas and our specific interest in its causes. In this study, we refer to AAD1 as urban and AAD2-5 as suburban; foreign-born persons are defined as immigrants and people born in the Netherlands as nonimmigrants. The Medical Ethics Committee of Erasmus MC, University Medical Center Rotterdam, Rotterdam approved the study protocol.

Study cases. The Netherlands TB register provided data of notified cases in the Rotterdam region after approval by its data protection committee. These data were completed and validated with local registers, patient records and the DNA fingerprinting register of the National Mycobacteria Reference Laboratory. Notified TB cases diagnosed between January 1, 1995 and December 31, 2006, were included in the study, excluding 41 sailors with a residential address outside the Netherlands, 12 inmates of a deportation center for illegal immigrants transferred from other regions of the country, 65 cases staying in asylum seekers centers, 14 recurrent cases with a relapse during the study period, 34 cases infected with *Mycobacterium bovis*, two cases infected with *Mycobacterium canetti* and one case infected with *Mycobacterium bovis* bacille Calmette-Guérin (BCG). Cases that resulted from laboratory cross-contamination were already withdrawn from the Netherlands TB register.

Mycobacteria and DNA fingerprints. Since 1993, all *M. tuberculosis* isolates in the Netherlands are subject to standardized insertion sequence (IS)*6110*-based restriction fragment length polymorphism (RFLP) typing, also called DNA fingerprinting (16). Clusters are defined as groups of patients having isolates with fully identical RFLP patterns and, if strains harbor less than 5 IS*6110* copies, with identical sub-typing by use of the Polymorphic GC-Rich Sequence probe (17).

Classification of transmission. We used a transmission classification model, combining conventional epidemiological information with DNA fingerprinting data, to classify cases into recently (≤2 years) or remotely (≥2 years) infected in the Netherlands or infected in a foreign country, as previously described (15). Briefly, clustered cases with a documented

contact with a pulmonary TB case within two years before diagnosis were classified as recently infected, and if the link was more than two years as remotely infected. Clustered cases without an epidemiological link were classified according to the last preceding pulmonary case in the cluster, i.e. recently if less than two years and remotely if more than two years. Cases with a unique fingerprint were classified as remotely infected in the Netherlands or infected in a foreign country depending on the time of residence in the Netherlands and travel history to countries where TB is endemic. Cases with unknown fingerprints, almost all culture-negative, were classified according to a documented contact with a pulmonary TB case, time of residence in the Netherlands, travel history to countries where TB is endemic, and for immigrant cases relevant findings at entrance screening.

Statistical Methods. We used univariate logistic regression to assess the importance of demographic and disease-related variables for TB cases. The dependent variable was residence in urban or suburban municipalities. All independent variables with a significance level of ≤0.05 were included in a multivariate logistic regression model. Data were analyzed by SPSS version 15 (SPSS, Inc., Chicago, IL).

Results

Demographic and disease characteristics of patients. Table 4.1 compares demographic and disease-related characteristics of all 1,817 TB cases in the three urban municipalities (Rotterdam, Schiedam and Vlaardingen) and all 755 cases in 52 suburban municipalities. After adjustment, cases in the urban municipalities were more often male, foreign-born, illicit drug user or homeless. Cases in suburban municipalities were more often older than 64 years. The *M. tuberculosis* strains of cases in urban municipalities clustered more frequently than strains from cases in suburban municipalities. Table 4.2 provides absolute number and proportion of cases by classification of transmission in both areas. Recent transmission caused 40% of the urban and 27% of the suburban TB caseload ($p<0.001$).

Table 4.1. Demographic and disease-related factors for tuberculosis cases in urban and suburban municipalities, 1995-2006.

Characteristics	Urban TB cases		Suburban TB cases					
	n	%	n	%	Crude OR (95% CI)	p Value	Adjusted OR (95% CI)*	p Value
TB Cases	1,817		755					
Average number of inhabitants	743,635		1,158,757					
Average proportion immigrants		22.6		7.7				
Average notification rate (per 100,000)	20.4		5.4					
Male sex	1,111	*61.1*	387	*51.3*	*1.5 (1.3-1.8)*	< 0.001	1.4 (1.1-1.6)	*0.01*
Age*						< 0.001		*< 0.001*
0-14 yr	108	*5.9*	44	*5.8*	*1.1 (0.8-1.5)*		1.6 (1.2-2.3)	
15-29 yr	556	*30.6*	200	*26.5*	*1.3 (1.1-1.5)*		1.0 (0.9-1.3)	
30-44 yr	616	*33.9*	196	*26.0*	*1.4 (1.2-1.7)*		1.0 (0.9-1.2)	
45-64 yr	370	*20.4*	141	*18.7*	*1.2 (1.0-1.4)*		1.0 (0.8-1.2)	
≥ 65 yr	167	*9.2*	174	*23.0*	*0.4 (0.4-0.5)*		0.6 (0.5-0.7)	
Immigrant	1317	*72.5*	425	*56.3*	*2.0 (1.7-2.4)*	< 0.001	2.1 (1.7-2.5)	*< 0.001*
Previous history of TB	97	*5.3*	57	*7.5*	*0.7 (0.5-1.0)*	0.03	1.0 (0.7-1.5)	*0.09*
HIV infection	86	*4.7*	22	*2.9*	*1.7 (1.0-2.7)*	0.04	1.1 (0.7-1.8)	*0.76*
Illicit drug user or homeless	188	*10.3*	12	*1.6*	*7.1 (4.0-12.9)*	< 0.001	6.0 (3.2-11.0)	*< 0.001*
Pulmonary TB	1,180	*64.9*	436	*57.7*	*1.4 (1.1-1.6)*	0.001	1.2 (1.0-1.5)	*0.06*
Positive sputum or bronchoalveolar lavage fluid smears†	608	*51.5*	222	*50.9*	*1.0 (0.8-1.3)*	0.83	-	-
Culture positive	1,439	*79.2*	553	*73.2*	*1.4 (1.1-1.7)*	0.001	1.0 (0.8-1.3)	*0.93*
Drug resistance against isoniazid or rifampicin (against both drugs, i.e. multidrug resistance)‡	83 (9)	*5.8(0.6)*	43 (5)	*7.8(0.8)*	*0.7 (0.5-1.0)*	*0.08*	-	-
Clustering mycobacteria (excluding first cases in a cluster)§	840	*58.7*	247	*44.9*	*1.7 (1.4-2.0)*	*< 0.001*	*1.4 (1.1-1.7)*	*0.006*
Active case finding (identified by contact investigation or screening)	357	*19.6*	116	*15.4*	*1.4 (1.1-1.7)*	*0.01*	*1.0 (0.8-1.3)*	*0.94*

Definition of abbreviations: CI = confidence interval; HIV = human immunodeficiency virus; OR = odds ratio; TB = tuberculosis
* Odds ratios for age were scaled to have a (geometrical) mean of one. This allows better comparing between crude and adjusted odds ratios.
† In 524 urban cases smears were positive for acid-fast bacilli and 84 had positive bronchoalveolar lavage fluid smears for acid-fast bacilli. For suburban cases, 167 had positive smears and 55 positive bronchoalveolar lavage fluid smears for acid-fast bacilli.
‡ In one urban and four suburban cases drug sensitivity results were unknown.
§ DNA fingerprinting was not performed in eight culture-confirmed urban cases and in one culture-confirmed suburban case.

Table 4.2. Classification of place and time period of transmission of tuberculosis cases by DNA fingerprint and urban and suburban municipalities, 1995-2006.

	Urban municipalities					Suburban municipalities				
	No finger-print*	Unique fingerprint	Clustering fingerprint	Total	%	No finger-print*	Unique fingerprint	Clustering fingerprint	Total	%
Recently (≤2 yrs) infected in The Netherlands	107	1	621	729	40	47	1	153	201	27
Remotely (>2 yrs) infected in The Netherlands	25	104	152	281	15	26	100	59	185	25
Infected in a foreign country	138	451	67	656	36	65	181	35	281	37
Unknown place or time of infection	116	35	0	151	8	67	21	0	88	12
Total	386	591	840	1,817		205	303	247	755	

* No bacteriological confirmation or no DNA fingerprinting performed.

Case rate by municipality. For the 12-year study period, average TB case rates for each municipality are shown in Figure 4.1. Case rates were 3.8 times higher in urban municipalities (20.4 per 100,000 population) than in suburban municipalities combined (5.4 per 100,000 population). Case rates decreased with declining urbanization grade (AAD1: 20.4, AAD2: 6.7, AAD3: 5.0, AAD4: 4.0 and AAD5: 3.1 per 100,000 population; χ^2 test for trend, $p<0.001$). Case rates also differed considerably between postal code areas within the Rotterdam municipality, with rates above 40 per 100,000 population in two inner boroughs of the city, both with an address density of more than 5,000 per km^2.

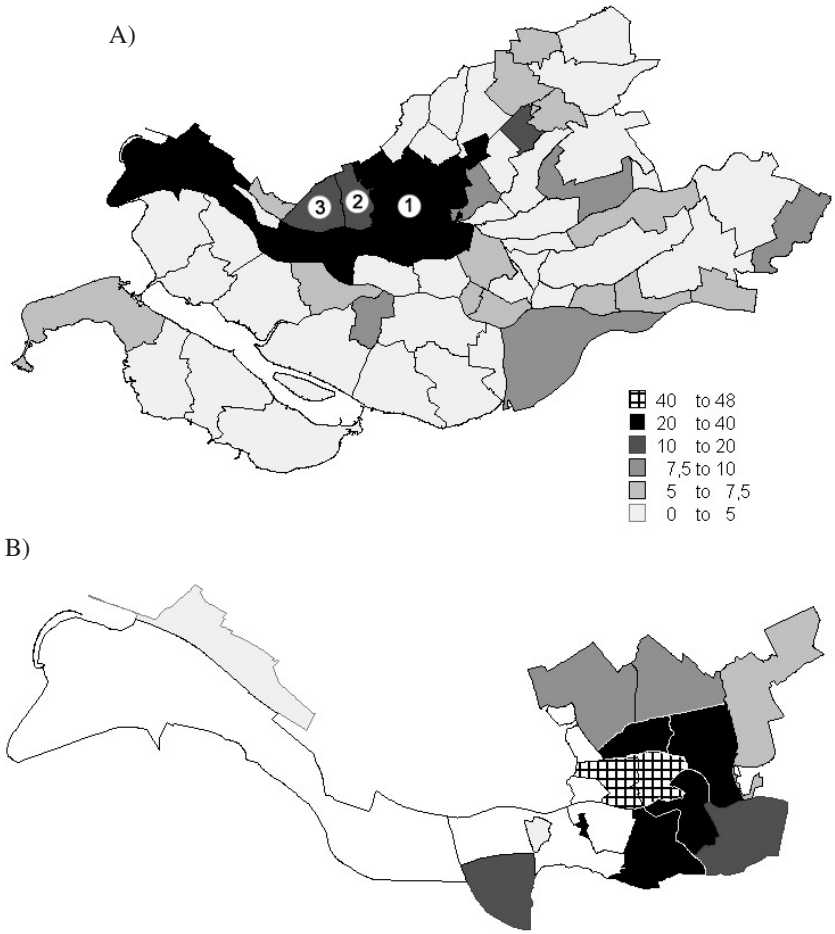

Figure 4.1. Average tuberculosis case rate (per 100,000 population) A) for Rotterdam and 54 surrounding municipalities (above) and B) for Rotterdam divided by postal code areas (below), 1995-2006.
1 (Rotterdam), 2 (Schiedam) and 3 (Vlaardingen) are very highly urbanized municipalities with an area address density of more than >2500 addresses/km^2).

Case rates by age group, country of birth and RFLP typing. All age-specific case rates (per 100,000 population) for immigrants and nonimmigrants were higher in urban municipalities than in suburban municipalities (Table 4.3). Immigrants in urban municipalities had a 1.7 times higher case rate than immigrants in suburban municipalities (65.2 versus 39.5; $p<0.001$). The highest case rates were found in immigrants between 15 and 29 years, both in urban and suburban municipalities (78.2 and 67.2, respectively) with no significant difference between these rates ($p = 0.1$). Case rates in immigrant age groups between 30 and 44 years and between 45 and 64 years differed significantly between urban and suburban municipalities ($p<0.001$ for both). Nonimmigrants in urban municipalities had a 2.8 times higher case rate than nonimmigrants in suburban municipalities (7.2 versus 2.6; $p<0.001$). The difference was consistent for all nonimmigrant age groups, except for the group older than 64 years, in which the rate ratio was nonsignificant.

Case rates related to a unique RFLP in urban municipalities were 1.3 times higher than those in suburban municipalities for immigrants (23.6 versus 18.5; $p=0.004$) and 2.1 times higher for nonimmigrants (1.7 versus 0.8; $p<0.001$). The clustered case rate for immigrants in urban municipalities was 2.2 times higher than in suburban municipalities (29.8 versus 13.7; $p<0.001$). For nonimmigrants, the clustered case rate was 4.4 times higher in urban municipalities compared to suburban municipalities (3.4 versus 0.8; $p<0.001$).

Case rate by classification of transmission. The recent transmission case rate for all inhabitants of urban municipalities was 5.5 times higher than for those in suburban municipalities (8.2 versus 1.5; $p<0.001$) [Data not shown]. Stratified for country of birth (Table 4.4), the recent transmission case rate for immigrants in urban municipalities was 2.6 times higher than in suburban municipalities (23.1 versus 8.8; $p<0.001$) and for all nonimmigrants 4.6 times higher in urban municipalities (3.8 versus 0.8; $p<0.001$). Further stratification by age group showed that for immigrants, these recent transmission case rates were 1.7 times higher in age group 15 to 29 years ($p=0.002$), 2.4 times higher in age group 30 to 44 years ($p<0.001$) and 5.0 times higher in age group 45 to 64 years ($p<0.001$). The rate ratios of these case rates increased significantly by age groups (χ^2 test for trend, $p<0.001$). In nonimmigrants, these rate ratios were 4.4 for the age group between 0 and 14 years ($p<0.001$); 5.7 in the 15 to 29 years age group ($p<0.001$); 5.0 in the 30 to 44 years age group ($p<0.001$); and 6.0 in the age group 45 and 64 years ($p<0.001$).

Table 4.3. Number of tuberculosis cases and case rate (per 100,000 population) by age group, DNA fingerprint and recent transmission for immigrant (A) and nonimmigrant (B) inhabitants of urban and suburban municipalities, 1995-2006.

A) Immigrants

	Urban municipalities					Suburban municipalities				
		Case rate					Case rate			
Age group (yrs)	n	Total	No finger-print*	Unique fingerprint	Clustering fingerprint	n	Total	No finger-print*	Unique fingerprint	Clustering fingerprint
0-14	23	18.8	12.2	4.9	1.6	13	14.0	4.3	6.4	3.2
15-29	435	78.2	13.5	27.3	37.4	161	67.2	12.5	28.8	25.9
30-44	514	70.2	12.3	24.2	33.8	139	39.2	7.6	17.5	14.1
45-64	278	58.0	11.1	20.5	26.5	71	24.1	4.1	11.9	8.1
≥ 65	67	52.1	4.7	33.4	14.0	41	43.9	6.4	28.9	8.6
Total	1,317	65.2	11.8	23.6	29.8	425	39.5	7.3	18.5	13.7

B) Nonimmigrants

	Urban municipalities					Suburban municipalities				
		Case rate					Case rate			
Age group (yrs)	n	Total	No finger-print*	Unique fingerprint	Clustering fingerprint	n	Total	No finger-print*	Unique fingerprint	Clustering fingerprint
0-14	85	6.0	4.3	0.1	1.6	31	1.2	1.0	0.0	0.2
15-29	121	8.8	1.5	1.3	6.0	39	1.7	0.5	0.2	0.9
30-44	102	7.4	1.3	1.4	4.7	57	1.9	0.6	0.3	1.0
45-64	92	6.1	1.5	1.1	3.5	70	2.2	1.0	0.4	0.8
≥ 65	100	8.0	2.0	4.7	1.3	133	7.8	2.3	4.4	1.1
Total	500	7.2	2.1	1.7	3.4	330	2.6	1.0	0.8	0.8

* No bacteriological confirmation or no DNA fingerprinting performed.

Table 4.4. Tuberculosis case rate (per 100,000 population), recent transmission case rate and proportion recent transmission by age group for immigrant and nonimmigrant inhabitants of urban and suburban municipalities, 1995-2006.

	Urban municipalities						Suburban municipalities					
	Immigrants			Nonimmigrants			Immigrants			Nonimmigrants		
Age group (yrs)	Total case rate	Recent transmission case rate	%	Total case rate	Recent transmission case rate	%	Total case rate	Recent transmission case rate	%	Total case rate	Recent transmission case rate	%
0-14	18.8	4.9	26	6.0	4.7	79	14.0	2.1	15	1.2	1.1	90
15-29	78.2	29.5	38	8.8	5.8	65	67.2	17.1	25	1.7	1.0	62
30-44	70.2	24.6	35	7.4	4.1	55	39.2	10.2	26	1.9	0.8	42
45-64	58.0	21.9	38	6.1	3.5	58	24.1	4.4	18	2.2	0.6	27
≥ 65	52.1	8.6	16	8.0	0.6	8	43.9	3.2	7	7.8	0.6	8
Total	65.2	23.1	35	7.2	3.8	53	39.5	8.8	22	2.6	0.8	32

Figure 4.2 shows case rates for immigrants and nonimmigrants in urban and suburban areas by recent or remote infection in the Netherlands, foreign-acquired infection and unknown place or time of infection. Case rates due to recent transmission were higher for urban immigrants than for urban nonimmigrants (23.1 versus 3.8; p<0.001) and higher for suburban immigrants than suburban nonimmigrants (8.8 versus 0.8; p<0.001). TB caused by foreign-acquired infections contributed with 30.9 cases per 100,000 population (47%) to the TB case rate of urban immigrants and with 24.4 per 100,000 population (62%) to TB among suburban immigrants.

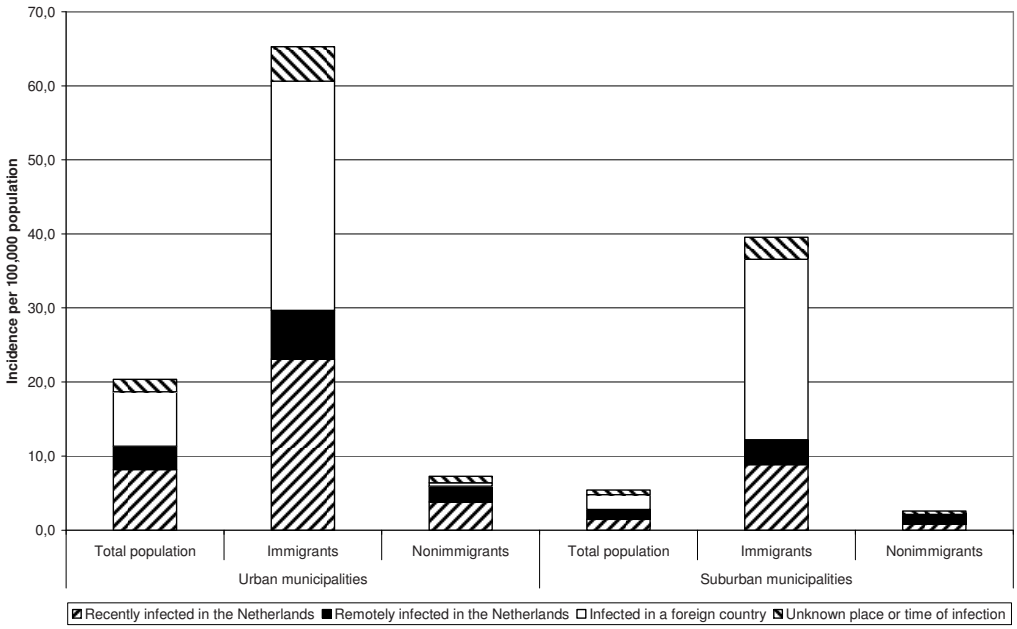

Figure 4.2. Case rate by classification of transmission for immigrants, nonimmigrants and total population in urban and suburban municipalities, 1995-2006.

Discussion

In our study, the TB case rate in three urban municipalities was 3.8 times higher than in 52 suburban municipalities. The case rate increased significantly with the level of urbanization. In the multivariate analysis, cases in urban municipalities were more often male, younger than 65 years, immigrant, illicit drug user or homeless, and had more frequently a clustering *M. tuberculosis* strain. After stratification for country of birth, case rates were 1.7 times higher for immigrants and 2.8 times higher for

nonimmigrants in the urban municipalities than in suburban municipalities. At least 47% of urban immigrant TB cases had foreign-acquired disease against 62% of immigrant cases in the suburban areas. Immigrants in the urban municipalities had a 2.6 times higher recent transmission case rate than those in suburban municipalities; for nonimmigrants the case rate was 4.6 times higher.

In low-prevalence countries, TB case rates in urban and suburban areas show wide disparities and can even differ considerably between boroughs within large cities (5, 18). In our study, stratification for country of birth generated smaller differences of case rates in urban and suburban municipalities. Thus, the higher overall case rate in the urban municipalities was partly due to the larger proportion of immigrants in these areas, which is a characteristic of almost all metropolitan cities in low-prevalence countries (19, 20). DNA fingerprinting confirmed that TB among immigrants resulted most frequently from foreign-acquired infections. The highest case rate was among immigrants between 15 and 29 years, with similar rates in urban and suburban municipalities, also indicating that most of these infections were acquired before residence in the Netherlands.

Several studies demonstrated that the proportion of clustered cases in urban areas comprised 20 to more than 50 percent of RFLP-genotyped TB cases (19, 21-24). The clustering proportion of urban cases (59%) in our study was comparably high. Clustering, however, is not identical with recent transmission, and genotyping data should be analyzed together with conventional epidemiological information. Our finding that 40% of all urban cases were recently infected confirmed that recent transmission contributed substantially to the high TB caseload in the urban municipalities. Comparison of TB case rates of urban and suburban municipalities also showed that ongoing transmission was particularly an urban phenomenon. Recent transmission was more common among the urban immigrant population in our study, and is most likely related to the total high TB case rate among immigrants and possibly influenced by factors such as housing conditions, risk group behavior and delays in diagnosis. Ineffective contact investigation procedures may also have contributed to the high proportion of recent transmission among immigrants in urban municipalities. According to the Dutch TB guidelines, BCG-vaccinated contacts of smear-positive TB patients were not eligible for tuberculin skin testing (TST) during most of the study period and were examined only by radiograph. In our study population, 72% of urban cases were immigrants. Most of their close contacts were either BCG-vaccinated in their native country or vaccinated in the Netherlands as part of a targeted BCG-vaccination programme. Contact investigation with chest radiography failed to diagnose LTBI in these contacts.

Certain groups, such as illicit drug users and homeless persons, are at high risk for TB and are typically found in large cities (20, 25). In our study, 10.3% of urban TB cases were homeless or used illicit drugs. In a previous study, we calculated that the TB case rate among these risk groups varied between 250 and 500 per 100,000 and that 83% had a

clustering strain (26). Thus, ongoing transmission accounted for the high TB case rate among and illicit drug users and homeless persons in the urban municipalities and influenced the recent transmission case rate in the general urban population. We therefore calculated and compared recent transmission case rates of age groups and country of birth of urban and suburban inhabitants without these risk profiles and all significant rate ratios described in the results remained the same (data not shown). Thus, recent transmission was not only common among illicit drug users and homeless persons, but also among the other urban immigrant and nonimmigrant population.

One of the features of large cities is that many people travel together or gather for work, education and leisure, often in congested places (22). A substantial part of TB transmission occurs unrecognized and cannot be traced through contact investigation, as has been revealed by other genotyping studies (27). Pubs e.g. are well-known places for high TB transmission due to crowding and poor ventilation (28). Several of the molecular clusters in our study expanded due to pub-related outbreaks in the urban municipalities, which protracted over many years.

For nonimmigrants between 15 and 64 years, the case rate related to a unique fingerprint was several times higher in urban municipalities than in suburban areas, although the contribution to the overall case rate was low. Different risk and behavioral patterns such as visits of children of immigrants to their parents' native countries and international travel of young urban persons, such as students, may explain this unexpected finding and needs further study.

Our study showed that the high TB case rate in the urban municipalities was driven by ongoing transmission among certain identified risk groups, such as illicit drug users and homeless persons, but also among other immigrants and nonimmigrants in these cities. Furthermore, we showed that the high TB case rate was related to the high proportion of immigrants who frequently reactivate a foreign-acquired infections. Thus, metropolitan TB control should focus on a package of targeted interventions (18). These interventions should be aimed at hard-to-reach, marginalized urban risk groups, such as illicit drug users and homeless persons. For active case finding, radiographic screening can be very useful to identify asymptomatic cases in these populations (26, 29, 30). Case holding activities are key in TB control, and directly observed therapy is elementary to complete treatment in specific TB patients, to prevent defaulting and the possible development of resistant strains. TST examination of contacts of pulmonary TB patients is one of the cornerstones of TB control in low-incidence countries. The recent introduction of interferon-gamma release assays in TB control provides better opportunities to correctly identify latent infections among BCG-vaccinated contacts and to offer preventive treatment (31). Since the majority of cases are diagnosed once symptomatic, education should be directed at the health care providers who see these patients first, such as general practitioners in inner-cities, and social workers involved with the care of urban risk groups. Low-threshold services, such as public health

TB clinics, are needed for symptomatic persons without health insurance or otherwise marginalized in the society. In addition, screening immigrants for TB and LTBI are important interventions to reduce the burden of disease and prevent transmission in the host country (20, 32). Genotyping can identify groups of persons at high risk and offer opportunities to develop targeted interventions to actively screen for TB or LTBI, as has been described by others (26, 33). The lessons learned from TB control in metropolitan areas are almost universal and sharing of experience and interventions should be facilitated, e.g. in international working groups.

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5

From contact investigation to screening drug addicts and homeless persons

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Abstract

In early 2001 there were indications that tuberculosis (TB) was increasingly becoming a problem among drug addicts and homeless persons in Rotterdam, after a periodical screening was discontinued in 1997. A contact investigation around a homeless drug addicted man in Rotterdam with infectious pulmonary TB is described.
Contact investigation: A total of 507 drug addicts, homeless persons, and staff of facilities for these risk groups were examined with tuberculin skin testing (TST) and chest radiography. DNA fingerprinting of mycobacteriological cultures through Restricted Fragment Length Polymorphism methodology and molecular epidemiology investigation through cluster analysis were performed.
Outcome: TST showed an infection prevalence of 29%, especially among staff of services for drug addicts and homeless persons. Six persons with active intrathoracic TB were identified. Cluster analysis demonstrated no relation with the initial case but showed intense transmission of TB among drug addicts and homeless persons in Rotterdam by multiple sources. As a consequence of the findings, a proposal to the Council of the City of Rotterdam resulted in the re-introduction of a comprehensive TB screening programme among these risk groups with mobile digital X-ray units (MXUs).
Conclusion: This contact investigation gradually obtained the characteristics of a screening of drug addicts and homeless persons. Novel technologies, such as MXUs, facilitate appropriate and efficient outreach approaches to TB control among difficult-to-reach groups. This method and knowledge of individual fingerprints and clusters of TB patients are indispensable for underpinning proposals for change of local TB control strategies and convincing local authorities of the rationale.

Introduction

In the mid-1990s the number of hard drug addicts in Rotterdam (population 600 000) was estimated at ~3700 through the capture-recapture method (1). The hard core of homeless persons was estimated ~1100 persons, many of them also hard drug or alcohol addicted (2). On average 10 drug addicts or homeless persons were diagnosed with tuberculosis (TB) annually. Although a fraction of drug addicts and homeless persons were periodically screened for TB until 1996, still the majority of TB cases were found passively with symptoms. In 1997 screening was stopped because of limited financial and human resources and the Department of TB Control of the Rotterdam Municipal Health Service adopted a reactive strategy, i.e. an institution for drug addicts or homeless persons was examined only after an infectious case of TB was detected (contact investigation).

In early 2001 there were indications that TB was increasingly becoming a problem among drug addicts and homeless persons in Rotterdam. In 1999, TB was diagnosed in 13 drug addicts or homeless persons (8.8% of the total number of TB cases in Rotterdam), and in 2000 there were 17 cases (12.6%). DNA fingerprinting of the Mycobacterium tuberculosis isolates showed that a specific cluster had developed in Rotterdam with a substantial number of patients being drug addicted or homeless. According to the quarterly reports of the National Institute of Public Health and the Environment (RIVM), this was the fastest growing and largest TB cluster in the Netherlands.

We describe a contact investigation among drug addicts and homeless persons in Rotterdam turning into a screening exercise, and how DNA fingerprint cluster analysis contributed to the re-introduction of a comprehensive programme of periodic active case finding among these risk groups.

Initial case. A homeless, schizophrenic, drug addicted 50-year-old male came to the Department of TB Control in 2001 with a persistent cough that had worsened since 2 months. The chest X-ray (CXR) showed extensive infiltrative lesions with small cavities, compatible with infectious pulmonary TB (Figure 5.1). It appeared that the man had been referred by his general practitioner twice before but had never reported himself. This time he was accompanied by a social worker. After the CXR was made the man left immediately but the social worker managed to bring a sputum sample from the patient the next day. Microscopical examination of the sputum after Ziehl-Neelsen staining showed many alcohol and acid fast bacteria. The patient could be traced the same day and was admitted to a general hospital for isolation and treatment.

Considering the duration of his complaints and the extent of the abnormalities on the CXR, it was assumed that the patient had been infectious for a long time. Besides, the infectiousness was regarded high, considering the quantitative count of alcohol and acid fast bacteria in the sputum. Furthermore, the patient had a very social lifestyle, chatting with

many people every day, while his cough hygiene was minimal. In the months preceding the diagnosis, the man had stayed in three different shelters for homeless persons. He visited the regional institution for ambulatory mental health care, daily, for supervised intake of antipsychotic drugs.

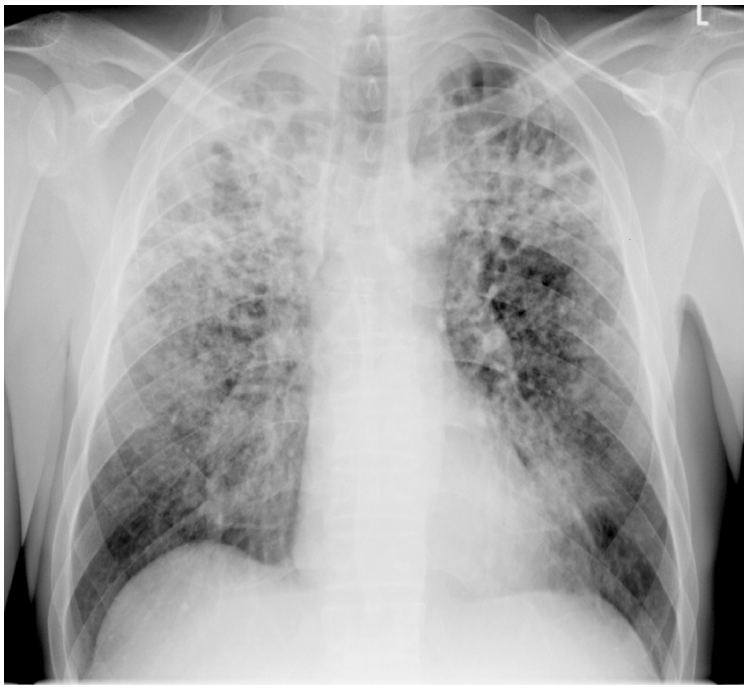

Figure 5.1. Chest X-ray of the initial patient.

Outline of the contact investigation. The Department of TB Control planned an extensive contact investigation that was initially aimed at the staff and visitors of the three homeless shelters, as well as the staff of the regional institution for ambulatory mental health care. According to the national guidelines for contact investigation, contacts with a previous history of TB, latent tuberculosis infection (LTBI) or BCG vaccination, and persons born before January 1, 1945 are excluded from tuberculin skin testing (TST) and are examined by CXR. Among persons examined with TST in the first round 20% had a LTBI. These results lead to an extension of the second round of the contact investigation to a larger group of residents of various shelters for homeless persons, as they often stay at different locations. The outlines of the contact investigation were, therefore, deliberately obscured and it obtained more the characteristics of a screening.

In the second round, contacts were checked at the Department of TB Control with TST or CXR. In addition, 312 residents of shelters for homeless persons were examined for TB on location. A mobile digital X-ray unit (MXU) was used, allowing one of the TB physicians to read the CXR immediately in the van (Figure 2). In this way evident abnormalities could be detected instantly and follow-up examination could be initiated at once. All CXRs were later reviewed at the Department of TB Control. In this group TST was not performed because of expected poor compliance with preventive treatment.

Figure 5.2. TB screening at a hostel for homeless persons in Rotterdam with a mobile digital X-ray unit.

Outcome of the contact investigation. In the contact investigation a total of 507 persons were examined. For 127 persons the examination consisted of one or more TSTs (Table 5.1). In 28 persons – 18 staff members and 10 residents – LTBI was found, resulting in an infection prevalence of 29% (only persons in whom the TST examination could be completed were counted in the denominator). The CXR of one shelter resident with a positive TST in the first round of the contact investigation was suspicious for pulmonary TB. The sputum culture for *M. tuberculosis* became positive after several months. In two larger shelters for homeless persons the infection prevalence among staff members was high with 26% (6 out of 23 persons) and 27% (4 out of 15 persons). Preventive treatment with isoniazid during 6 months was started for 17 persons while for the remaining 10 individuals it was decided to follow them up with CXRs every 6 months during 2 years.

Table 5.1. Results of tuberculin skin testing.

Conclusion	Number of persons tested
No LTBI[a]	67
Recent LTBI[b]	28 (23 persons had a positive TST in the first round (including one resident in whom active TB was diagnosed later); 1 person with a positive TST in the second round; 4 persons with a TST conversion)
Infection with mycobacterium other than TB	2
Examination not completed[c]	30
Total number of persons examined with TST	127

LTBI: latent tuberculosis infection; TB: tuberculosis; TST: tuberculin skin test
a: TST negative in first and second round, or TST negative when only the second round was performed
b: TST positive in the first round, or TST positive when only the second round was performed, or TST negative in the first
round but positive in the second round (conversion)
c: No TST performed in the second round, or TST in the second round not read

Table 5.2. Results of chest radiography examinations.

Conclusion	Number of persons examined
No indication of active intrathoracic TB	375
Active intrathoracic TB	5 Second round: 2 persons with smear-positive pulmonary TB; 2 persons with culture-positive pulmonary TB and 1 person with tuberculous pleurisy
Total number of persons examined with a CXR	380

TB: tuberculosis; CXR: chest X-ray

The other 380 persons were examined for active TB by means of a CXR (Table 5.2). Individuals examined with a CXR as a result of a positive TST are excluded from this number. As a result of the TB examination on location with the MXU, active intrathoracic TB was diagnosed in five persons: two persons with smearpositive pulmonary TB, two persons with culture-positive pulmonary TB, and one person with a tuberculous pleurisy. The results of this contact investigation translate into a prevalence rate of 1183 TB cases per 100 000 persons examined. All six patients with active TB completed 6 months of therapy under Directly

Observed Therapy. Remarkably, two of these patients, one of them smear-positive, stayed in a shelter of which the initial case had been a resident for 3 months, which until then was unknown. During follow-up, LTBI was found in several staff members of this shelter and in one staff member culture-positive TB was diagnosed. These results are not presented in the tables since it is considered a new contact investigation.

DNA fingerprint results. When *M. tuberculosis* is cultured from sputum or other patients' material in the Netherlands, the microbiology laboratory sends the isolate to the national mycobacteriological reference laboratory at the RIVM for DNA fingerprinting through Restricted Fragment Length Polymorphism (RFLP) methodology (3). When a RFLP pattern matches that of the mycobacterial DNA from at least one other patient, they belong to a so-called cluster. This often indicates transmission of TB between these persons or infection by a common source or sources.

The mycobacterial RFLP pattern of the initial case was unique, i.e. not previously found in the Netherlands. None of the six other TB cases found had the same DNA fingerprint as the initial patient. The DNA fingerprint of four out of these six TB cases – as well as the fingerprint of the staff member mentioned above-belonged to the largest TB cluster in the Netherlands, almost entirely consisting of patients from Rotterdam. One fingerprint belonged to another major cluster related to homelessness in Rotterdam and one fingerprint was unique.

Discussion

In this contact investigation, which gradually obtained the characteristics of a screening, many cases of LTBI and six patients with active TB were found. RFLP pattern analysis showed that none of the secondary TB cases were related to the initial patient but reflected intense transmission of TB in the group of drug addicts and homeless persons in Rotterdam by multiple sources.

Under Dutch public health laws the Local Authorities are responsible for infectious disease control, implemented by Municipal Health Services. Metropolitan areas harbour a disproportionate number of inhabitants belonging to various risk groups for TB, such as recent immigrants from TB high-endemic countries, hard drug users, or homeless persons. High rates of TB among homeless persons in large cities are well documented almost universally (4-9). Outbreaks of TB in homeless shelters have been reported (10, 11). Molecular epidemiology investigation through RFLP cluster analysis has contributed to the understanding of the significance and spread of TB in a community and demonstrated the importance of (recent) transmission of TB, e.g. among homeless persons (12-15). Transmission of TB among homeless persons can also affect shelter personnel and other staff of medical and social services contacted by homeless persons (16, 17). The many cases of LTBI we found among staff members of institutions that dealt with homeless persons were probably

the result of intense exposure to TB during several years. Active case finding has been reported to decrease TB transmission and overall case rate among urban homeless persons (17, 18). However, the yield of some TB screening programmes of homeless persons may be low due to poor health care system uptake and poor treatment completion rates (5, 19-23). Among risk groups such as homeless persons, conventional methods of contact investigation are not feasible for various reasons (8, 9). Outreach interventions, bringing active case finding and case holding services closer to certain risk groups, with incentives to maximize compliance, and in co-ordinated co-operation with hospitals, social services and the community, have been described (17, 22, 24). Among some groups of careless care-avoiders strong persuasion or even mandatory screening may be necessary and has resulted in a drastic reduction of TB incidence (17, 18). Novel technologies, such as MXUs with digital X-ray equipment facilitate appropriate and efficient approaches of targeted TB control among difficult-to-reach groups. A TB physician can read the CXR immediately and follow-up examination can be initiated instantly, such as a short medical history, spot sputum collection, or referral to a clinic accompanied by a TB nurse or social worker. In this way, the failure of many individuals in attending the medical services as a result of their care-avoiding behaviour can be prevented. Also, the bond created in the first encounter with health care providers will positively influence the trust of the patient and contribute to better compliance.

Many homeless persons are also alcohol or hard drug addicted. High rates of TB among hard drug users are also reported (25-27). Digital MXUs can also be employed in co-operation with needle exchange posts, methadone substitution programmes, or day-care facilities for hard drugs users. In the Netherlands MXUs have been used effectively for TB control among prisoners and in centres for asylum seekers (28). This has facilitated its use in case of an outbreak of TB or screening of other risk groups on location.

Considering the outcome of the DNA fingerprint analysis, this ‘contact investigation’ should be regarded as a screening, revealing several undetected TB cases among hard drug users and homeless persons in Rotterdam. As a consequence of these findings, a proposal to re-introduce TB screening for hard drug users, methadone users, and homeless persons in Rotterdam gained momentum from the Municipal Health Service and the institutions providing social services to these risk groups. It contributed to making TB screening among drug users, methadone users, and homeless persons one of the priority issues in the Council Programme 2003-6 of the Council of the City of Rotterdam. This resulted in the re-introduction of a comprehensive TB screening programme among these risk groups, aimed at semi-annual radiological examination, with a digital MXU already used for screening prisoners and asylum seekers. Also, arrangements were made with the institutions for social services for drug users, methadone users, and homeless persons for periodic examination of their staff members.

Conclusions

Contact investigation has not been effective among hard drug users and homeless persons in Rotterdam. The use of MXUs with digital X-ray equipment facilitates TB control among certain difficult-to-reach TB risk groups. Molecular technologies in TB control such as mycobacterial strain identification with DNA fingerprinting can recognize local epidemiological patterns previously undetectable. The knowledge of individual fingerprints and clusters of TB patients not only contributes to better risk group identification but is also indispensable for underpinning proposals for change of local TB control strategies and convincing local authorities of the rationale behind appeals for support, in this case for a screening programme.

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6

Impact of mobile radiographic screening on tuberculosis among drug users and homeless persons

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Abstract

Rationale: In 2002, a mobile radiographic screening programme was started in Rotterdam to respond to high rates of tuberculosis (TB) among illicit drug users and homeless persons.
Objectives: We studied trends and characteristics of TB among these risk groups and assessed the impact of the screening programme on transmission, using molecular typing.
Methods: Description of trends, and of demographic and disease-related characteristics of tuberculosis cases among these risk groups between 1993 and 2005. TB was considered to result from recent transmission if the mycobacterial DNA fingerprints of cases were identical to those of other cases in the risk groups in the previous 2 years.
Measurements and Main Results: During the study period, 206 individuals with TB among illicit drug users and homeless persons were notified, representing 11.4% of the total case load of 1,811 in Rotterdam. The annual number of tuberculosis cases declined from 24 at the start of the screening programme to 11 cases in 2005. The screening programme identified 28 cases (a prevalence rate of 327 per 100,000 radiographs), of which 12 were smear positive. In 1997–2002, more than 80% of the illicit drug users or homeless persons with TB were infected with one of the Mycobacterium tuberculosis strains prevalent among these risk groups. After nearly 4 years of systematic radiographic screening this proportion declined to 45% in 2005.
Conclusions: DNA fingerprinting can be a useful tool to evaluate the impact of a TB screening programme. We advocate that screening of illicit drug users and homeless persons should be continued to prevent a resurgence of TB.

Introduction

In low-prevalence countries, tuberculosis (TB) is becoming a problem especially affecting large cities, where high rates can be found among certain risk groups such as (recent) immigrants, asylum seekers, prisoners, illicit drug users, and homeless persons (1-3). These risk groups need targeted interventions for (early) case detection and adequate treatment supervision (4). In the Netherlands, immigrants and asylum seekers from TB high-endemic countries are screened twice per year by chest X-ray for 2 years, and new inmates are radiographically screened after entering a correctional facility (5). Mobile digital X-ray units (MDXUs) visit the asylum seeker centres and prisons on a weekly basis.

In the city of Rotterdam (about 600,000 inhabitants), a screening programme among clients of a limited number of facilities for illicit drug users and homeless persons was discontinued in 1996 (6). The number of TB cases among illicit drug users and homeless persons gradually doubled over an 8-year period to 23 in 2001, which translated into an annual notification rate of 511 per 100,000 persons, more than 50 times the TB notification rate of 9.0 per 100,000 persons in the Netherlands in that year (5). DNA fingerprinting of the *Mycobacterium tuberculosis* isolates revealed that a fast-growing cluster developed in the Netherlands with a substantial number of patients from Rotterdam being illicit drug users or homeless. A large contact investigation among these risk groups in 2001 identified multiple asymptomatic infectious TB cases, whereas cluster analysis of all six culture-positive secondary cases demonstrated that none of them was linked to the initial case (6). As a consequence of these findings, a comprehensive targeted TB screening programme with an MDXU was introduced in May 2002, aiming to detect cases early and to control the epidemic.

The objectives of this study were to describe the trends and characteristics of disease among illicit drug users and homeless persons with TB, to evaluate nearly 4 years of systematic screening, and to determine the effect of screening on transmission, using information from molecular typing.

Methods

Case Definition. An illicit drug user was defined as a person who, at the time of diagnosis, regularly used heroin or cocaine, or was receiving methadone replacement therapy. A homeless person was defined as a person who frequently used day-care, night-care, residential, or other facilities for homeless persons.

All notified TB cases among illicit drug users with a registered address in Rotterdam and homeless persons residing in Rotterdam who were diagnosed between January 1, 1993, and December 31, 2005, were included in the study. To compare demographic and disease-related characteristics, all TB cases not belonging to these risk groups, and

notified in the same period, were also studied. Information about the patients was extracted from the TB register of the Department of Tuberculosis Control and from individual patient records.

Screening. A targeted mobile TB screening programme among illicit drug users and homeless persons in Rotterdam was reintroduced in May 2002, visiting an incomplete number of services and facilities in that year. Since January 1, 2003, all known facilities – that is, four methadone-dispensing centres, six day- or night-care facilities (some of them also having safe drug consumption rooms), three residential homes, and the street prostitution zone – were visited twice per year with an MDXU. The screening of the 14 facilities was performed during 12 working days per half-year, with an average of 102 persons per day, ranging between 36 and 319. The screening results were obtained from the Client Information System of the Department of Tuberculosis Control. The yield of the screening was cross-checked with individual patient records.

Mycobacteria and DNA Fingerprints. Since 1993, all *M. tuberculosis* isolates in the Netherlands have been subject to standardized insertion sequence (IS)*6110*-based restriction fragment length polymorphism (RFLP) typing, so-called DNA fingerprinting (7). Clusters are defined as groups of patients having isolates with fully identical RFLP patterns or, if strains harbour fewer than five IS*6110* copies, with identical subtyping by use of the polymorphic GC-rich sequence probe (8). The mycobacterial DNA fingerprints of TB cases among illicit drug users and homeless persons were compared with those of other cases with the same risk profile in previous years. If the mycobacterial DNA clustered with a preceding case within a 2-year period, the disease was considered to be due to recent transmission related to illicit drug use or homelessness (9). Because of this 2-year time lag, only cases in the period 1995 to 2005 were included in the analysis of recent transmission.

Statistical Methods. We analyzed data with SPSS version 12 (SPSS, Inc., Chicago, IL). We calculated χ^2 trends for the proportion of TB cases among illicit drug users and homeless persons of the total caseload and the proportion of recently infected TB cases in these risk groups for three periods, that is, a first period from calendar years 1993 through 1996 and 1995 through 1996, respectively, with mobile screening, a second period from calendar years 1997 through 2001 without screening, and a third period from calendar years 2002 through 2005 with the MDXU screening programme. For comparison of the proportion of smear-positive TB cases and cases found through active case finding among illicit drug users and homeless persons, the third period started in May 2002. We calculated the odds ratio of demographic and disease-related characteristics of TB cases among illicit drug users and homeless persons. Multivariate logistic regression was performed, considering factors significant in univariate analysis.

Results

Trends of Disease. During the 13 years of study, 90 TB cases were notified as illicit drug users, 37 were homeless persons, and 79 were both illicit drugs user and homeless. These 206 cases represented 11.4% of the total TB caseload of 1,811 in Rotterdam (Table 6.1). The annual number of TB cases among illicit drug users and homeless persons doubled from an average of 12 in the years 1993 until 1998 to 23 in 2001. After the introduction of the mobile screening programme the annual number and the proportion fell between 2002 and 2005 from 24 to 11 and 16.4 to 8.5% ($p < 0.05$), respectively. The annual notification rate decreased from 533 per 100,000 persons in 2002 to 244 per 100,000 persons in 2005, based on an estimated number of 4,500 illicit drug users and homeless persons in Rotterdam.

Table 6.1. Tuberculosis (TB) cases among illicit drug users or homeless persons compared to the total number of cases of the city of Rotterdam 1993-2005.

Year	Number of TB cases	Illicit Drug Users or Homeless Persons with TB		$\chi 2$ Test for Trend for Proportions
		n	%	
1993	125	16	12.8	
1994	137	10	7.3	
1995	107	8	7.5	
1996	125	13	10.4	Years 1993-1996: p=0.58
1997	138	12	8.7	
1998	129	12	9.3	
1999	148	20	13.5	
2000	135	14	10.4	
2001	156	23	14.7	Years 1997-2001: p=0.11
2002	146	24	16.4	
2003	171	25	14.6	
2004	164	18	11.0	
2005	130	11	8.5	Years 2002-2005: p=0.03
Total	1,811	206	11.4	

The horizontal line demarcates the start of the screening programme in May 2002. In that year 7 cases were notified before and 17 cases after the screening programme was started.

Characteristics of Disease. Table 6.2 compares demographic and disease-related characteristics of illicit drug users and homeless persons with TB and cases without this risk profile in Rotterdam between 1993 and 2005.

Table 6.2. Demographic and disease-related factors for tuberculosis (TB) among illicit drug users and homeless persons and cases without this risk profile.

Characteristics	Illicit Drug User or Homeless at Time of Diagnosis *(n = 206)*		Not Using Illicit Drugs or Homeless at Time of Diagnosis *(n=1605)*		Unadjusted OR (95% CI)	p Value	Adjusted OR (95% CI)*	p Value
	n	%	n	%				
Male sex	152	73.8	958	59.7%	1.9 (1.4-2.6)	0.001	1.4 (1.0-2.1)	0.06
Age (Yrs)								
0-19	1	0.5	218	13.6	0.0 (0.0-0.2)	< 0.001	0.0 (0.0-0.2)	< 0.001
20-39	116	56.3	777	48.4	1			
40-59	86	41.7	389	24.2	1.5 (1.1-2.0)	0.01	1.3 (1.0-1.9)	0.09
≥ 60	3	1.5	221	13.8	0.1 (0.0-0.3)	< 0.001	0.1 (0.0-0.4)	0.001
Born in the Netherlands	78	37.9	412	25.7	1.8 (1.3-2.4)	< 0.001	2.2 (1.5-3.2)	< 0.001
Previous history of TB	13	6.3	103	6.4	1.0 (0.5-1.8)	0.95		
HIV infection	30	14.6	65	4.0	4.0 (2.6-6.4)	< 0.001	3.9 (2.3-6.6)	< 0.001
Pulmonary TB	186	90.3	960	59.8	6.3 (3.9-10.0)	< 0.001	2.6 (1.5-4.5)	0.001
Positive sputum or bronchoalveolar lavage fluid smears†	111	59.7	465	48.4	1.6 (1.2-2.2)	0.01	1.7 (1.2-2.5)	0.01
Culture positive	189	91.7	1244	77.5	3.2 (1.9-5.4)	< 0.001	0.6 (0.3-1.2)	0.16
Mycobacterium tuberculosis strains	189		1224‡					
Drug resistance against isoniazid or rifampicin (against both drugs, i.e. multidrug resistance)§	6 (1)	3.2 (0.5)	67 (7)	5.5 (0.6)	0.6 (0.2-1.3)	0.20		

Clustering mycobacteria (exclusive of first cases in a cluster)[||]	156	83.4	630	52.4	4.6 (2.9-7.2)	< 0.001	3.6 (2.3-5.5)	< 0.001
Active case finding (contact investigation and screening)	83	40.3	264	16.4	3.4 (2.5-4.7)	0.001	3.6 (2.4-5.2)	< 0.001

Definition of abbreviations: CI = confidence interval; HIV = human immunodeficiency virus; OR = odds ratio; TB = tuberculosis.
* Multivariate logistic regression was performed, considering all factors significant in the univariate analysis.
† One hundred and four pulmonary TB cases with positive smears for acid-fast bacilli and seven cases with positive bronchoalveolar lavage fluid smears for acid-fast
bacilli.
‡ In 20 cases, *Mycobacterium bovis* (17), *Mycobacterium bovis BCG* (1), and *Mycobacterium canetti* (2) strains were identified.
§ Drug sensitivity test results were not available for 10 cases. None of them was homeless or used illicit drugs.
|| DNA fingerprinting was not performed in 2 culture-confirmed cases among illicit drug users or homeless persons and in 21 cases without these risk factors.

In the multivariate analysis illicit drug users and homeless persons with TB were more often in the age group between 40 and 59 years, born in the Netherlands, and coinfected with human immunodeficiency virus (HIV). They more often had pulmonary disease with positive sputum or bronchoalveolar fluid smears for acid-fast bacilli. The proportion of smear-positive TB cases among illicit drug users and homeless persons was 55.3% (26 of 47), 58.0% (51 of 88), and 47.9% (34 of 71) during the three periods. The proportion of smear positivity during the MDXU screening programme decreased compared with the nonscreening years, although the decrease was not statistically significant ($p = 0.11$). The *M. tuberculosis* strains of illicit drug users and homeless persons clustered more frequently. TB cases among illicit drug users and homeless persons were more often identified through active case finding, such as contact investigation and screening.

Table 6.3. Number of persons screened, chest X-rays taken, tuberculosis cases and incidence rate of mobile radiographic screening programme among illicit drug users and homeless persons in Rotterdam.

	Number of Persons Screened	Number of Chest X-rays	Number of Tuberculosis cases	Smear-positive Tuberculosis Cases	Prevalence Rate (per 100,000 chest X-rays)
2002 (from May on)	1,229	1,484	11	4	741
2003	1,824	2,605	10	4	384
2004	1,712	2,431	1*	0	41
2005	1,507	2,039	6	4	294
Total	3,248	8,559	28	12	327

* Two persons had a suspected chest X-ray but could not be followed up. One person reported to a hospital with complaints one week after the chest X-ray was taken and the other person could not be traced, but reported two months later with symptoms to the Department of Tuberculosis Control of the Municipal Public Health Service. Both of them had TB.

Targeted Mobile Digital X-ray Screening and Other Active Case-finding Activities. During the 3 years and 8 months of the MDXU screening programme, 8,559 chest X-rays were taken of 3,248 individuals (Table 6.3). The total yield of the screening was 28 TB cases, of which 12 cases were smear positive and 27 cases were culture confirmed. This translated into a prevalence rate of 327 cases per 100,000 radiographs. In the same period, another 7 TB cases among illicit drug users or homeless persons were identified through contact investigation and 7 via the TB screening programme in prisons, resulting in 42 of a total of 71 TB cases (59.2%) among these risk groups found through active case finding during the time of the MDXU screening programme. In the first 4 years of the study period, 15 of a total of 47 TB cases (31.9%) were found through active

case finding, of which 9 cases were found via mobile screening of these risk groups, 2 cases through contact tracing, and 4 cases via the TB screening programme in prisons. During the nonscreening period, 26 of a total of 88 cases (29.5%) were actively traced, of which 17 cases were found through contact investigation and 9 via the TB screening programme in prisons. The difference in the proportion of cases identified through active case finding during the MDXU screening programme and during the nonscreening years was highly significant ($p < 0.001$).

Case-holding Activities. During the study period 1993–2005, initial hospitalization was required for two-thirds of the TB cases among illicit drug users and homeless persons, because of the high proportion of infectious cases. Fourteen patients (6.8%) were detained in a designated hospital for a mean period of 10 weeks (3–26 wk), because they did not comply with isolation procedures during the infectious period. Other aspects of case holding are addressed in the discussion. TB cases among illicit drug users and homeless persons had a significant lower treatment completion rate than did cases without the risk profile (79.1 vs. 86.8%, unadjusted odds ratio, 0.6 [95% confidence interval, 0.4–0.8]; $p <0.05$). However, 25 of the 28 cases (89.2%) identified through the mobile radiographic screening programme completed treatment.

Molecular Epidemiology. Table 6.4 gives cluster information of the mycobacterial strains of the culture-confirmed TB cases among illicit drug users or homeless persons in Rotterdam over the period 1993–2005. In 31 cases TB was caused by mycobacteria with an unique DNA pattern in the Netherlands, of which 9 were the first case of a cluster; 3 of these clusters (clusters 13, 19, and 30) also included other illicit drug users or homeless persons with TB in Rotterdam. In the remaining 156 TB cases (83.4%) the mycobacteria belonged to 27 different clusters. Four mycobacterial strains (clusters 1, 13, 17, and 19) were the causative microorganism in 62.8% (117 of 187) of the culture-confirmed cases with an RFLP.

In 1997–2002, more than 80% of the illicit drug users or homeless persons with TB were infected with one of the *M. tuberculosis* strains prevalent among these risk groups within 2 years before diagnosis (Figure 6.1). The proportion of prevalent strains declined from 82% in 2002 at the start of the screening programme to 45% in 2005 ($p <0.05$).

Table 6.4. Unique and clustered cases of culture-confirmed tuberculosis (TB) among illicit drug users and homeless persons in Rotterdam between 1993 and 2005 and the proportion of cases with prevalent strains between 1995 and 2005.

	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	Total
Total cases	16	10	8	13	12	12	20	14	23	24	25	18	11	206
No culture	1	3		3	1	2	1	1		1	1	3		19
Culture-confirmed	15	7	8	10	11	10	19	13	23	23	24	15	11	189
RFLP														
Unique fingerprint	4	1		1	2		1	2	3	1	4	1	2	22
Cluster 1	7		1	1	1	3	3	2	2	1	1	1	1	24
Cluster 2	1			1										2
Cluster 3	**1**													1
Cluster 4	1													1
Cluster 5	1													1
Cluster 6		1		1	2								1	5
Cluster 7		**1**												1
Cluster 8		**1**												1
Cluster 9		**1**												1
Cluster 10		**1**												1
Cluster 11		1												1
Cluster 12			1	3	1					1				6
Cluster 13			**1**	1	4		3	1	1	2	2	2		17
Cluster 14			2		1		1							4
Cluster 15			1											1
Cluster 16			1											1
Cluster 17				1		6	9	5	14	10	7	1		53
Cluster 18				1										1
Cluster 19						**1**		2	2	5	5	5	3	23

Cluster 20							1							1
Cluster 21							1							1
Cluster 22								1						1
Cluster 23									1					1
Cluster 24										1	3			4
Cluster 25										1				1
Cluster 26											2			2
Cluster 27												2		2
Cluster 28												1		1
Cluster 29												**1**		1
Cluster 30												**1**	1	2
Cluster 31													1	1
Cluster 32													1	1
Cluster 33													1	1
Total number of cases with an RFLP	15	7	7*	10	11	10	19	13	23	22*	24	15	11	187
Number of cases clustering with a case among the risk group within 2 yr of diagnosis			2	6	9	9	16	10	19	18	19	9	5	120
Proportion of culture-confirmed cases with clustering RFLP among risk group within 2 yr of diagnosis			29%	60%	82%	90%	84%	77%	83%	82%	79%	67%	45%	

Definition of abbreviation: RFLP = restriction fragment length polymorphism.
Cases in boldface (clusters 3, 7, 8, 9, 10, 13, 19, 29, and 30) represent the first cases in clusters and were initially unique. Clusters 15, 30, 31, and 32 have cases with isoniazid-resistant strains; cluster 23 had a multidrug-resistant strain.
* Excluding two culture-confirmed cases for which DNA fingerprinting was not performed. One of them, the patient reported in 2002, was previously diagnosed in 2000 with a strain belonging to cluster 17.

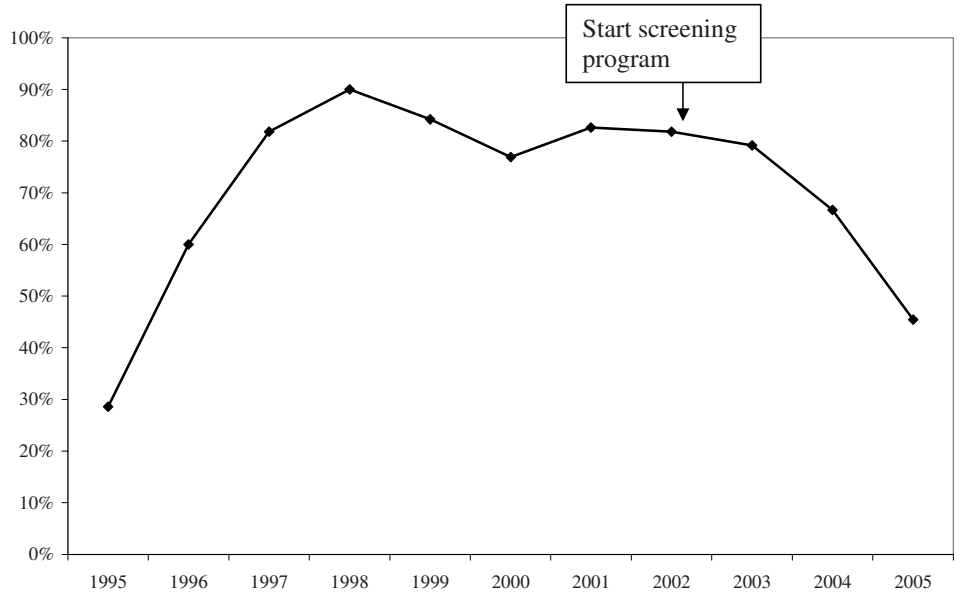

Figure 6.1. Annual proportion of tuberculosis cases among illicit drug users and homeless persons in Rotterdam with identical restriction fragment length polymorphism with cases in these risk groups 2 years before diagnosis. Fisher exact test for trend first (screening) period (1995–1996): $p = 0.22$; χ^2 test for trend second (nonscreening) period (1997–2001): $p = 0.77$; χ^2 test for trend third (MDXU screening) period (2002–2005): $p = 0.03$.

Figure 6.2 shows details of cluster 17, the largest TB cluster in the Netherlands with 150 cases in December 31, 2005. Initially it contained only TB cases with the same ethnic background and all residing in Rotterdam (13 cases in 1996–1997). One of them was an illicit drug user who was diagnosed at the end of 1996 with extensive disease. In the years 1998–2001 another 76 cases were added to this cluster, among them 34 cases from Rotterdam with a drug addiction or homeless status (46%) and also documented secondary cases among family members, and medical and social staff involved in the care for these persons. The number of cases among illicit drug users and homeless persons in this cluster declined during the screening years 2002–2005, with only one case during the last 2 years. The growth of cluster 17 continued in 2004 and 2005 due to pub-related transmission among migrants with the same ethnic background as the initial cases of the cluster.

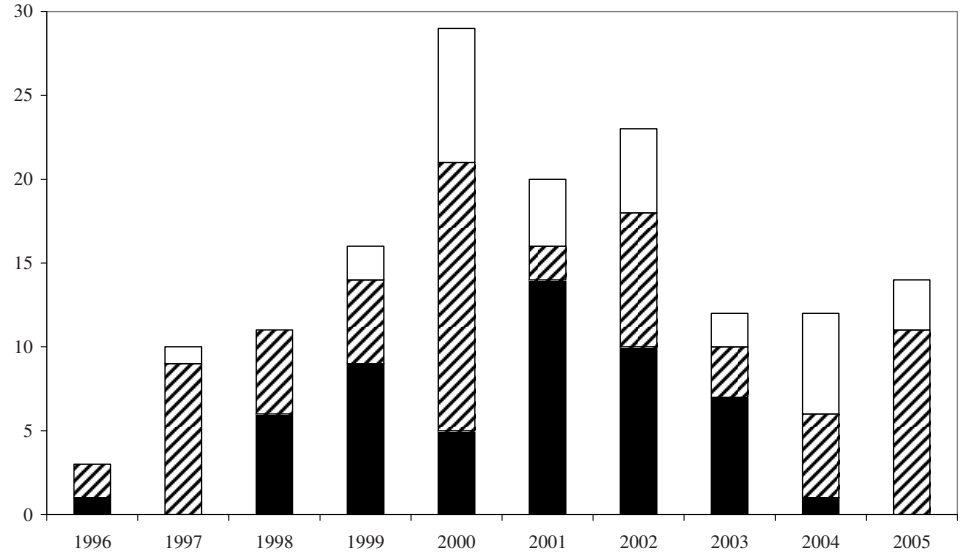

Figure 6.2. Epidemic curve of tuberculosis cases in cluster 17 in the Netherlands by year, residential area, and risk factors. Column patterns: solid, illicit drug user or homeless person in Rotterdam; hatched, resident of Rotterdam, not an illicit drug user or homeless; open, patient residing elsewhere in the Netherlands.

Discussion

As a response to a doubling of TB cases among illicit drug users and homeless persons a comprehensive targeted screening programme was introduced in May 2002 in Rotterdam.

Three years and 8 months after this introduction of periodic radiographic screening the annual number of TB cases among illicit drug users and homeless persons, and their proportion of the total number of TB cases, had declined by approximately 50% and reached preoutbreak incidence levels. Molecular genotyping showed a decrease of prevalent strains among these risk groups as well as a reduction in the number of incident TB cases among illicit drug users and homeless persons in relevant clusters, providing further evidence that transmission was reduced during the intervention.

TB among Homeless Persons and Illicit Drug Users. Outbreak reports of TB among residents of homeless shelters in large cities in Europe and the United States have cited high TB incidence rates ranging from 250 to more than 1,000 per 100,000 persons (9-14). In one review homeless TB cases represented between 6.1 and 6.7% of all cases between 1994 and 2003 in the United States (15). At the height of the outbreak in

Rotterdam, one of six TB cases (16.4%) used illicit drugs or was homeless at the time of diagnosis. We have combined these two risk factors because they were strongly interrelated, although illicit drug use was more dominant.

Homelessness and drug addiction are important risk factors for TB because transmission is facilitated by late presentation, common to marginalized care avoiders, crowding, and poor ventilation of shelters or safe drug consumption rooms. In addition, interrelated factors such as HIV-infection, malnutrition, and alcohol and drug abuse increase the risk of progression to active disease once infected (16-20). In our study cohort, HIV coinfection was three times more common among illicit drug users and homeless persons with TB than among cases without these risk factors.

Demographic and Disease Characteristics. Illicit drug users or homeless persons with TB in Rotterdam were more often born in the Netherlands than were cases without these risk factors, and DNA fingerprinting showed that the majority of cases among these risk groups clustered. This underpins the notion that the increase in the number of illicit drug users and homeless persons with TB was due to intensive and prolonged transmission in Rotterdam.

Illicit drug users or homeless persons with TB more often had disease located in the lungs, which more frequently had reached an infectious stage with positive sputum or bronchoalveolar lavage fluid smears after direct microscopy. They represented 19.3% (111 of 576) of all infectious cases in Rotterdam during the study period. DNA fingerprinting analysis, including all cases from the general population, showed that apart from other illicit drug users or homeless persons, also workers in close contact with the risk groups, such as shelter staff, social and health care workers and volunteers, as well as family members, had developed TB caused by *M. tuberculosis* strains prevalent among these risk groups.

Screening, Case-finding, and Case-holding Activities. Systematic chest radiographic screening for TB, especially with M(D)XUs, has been reported in a limited number of outbreaks of TB among homeless persons (21-23). In the Netherlands, MDXUs were already used for several other screening activities, so they could be easily used for the screening of illicit drug users and homeless persons. We considered screening with an MDXU the most appropriate intervention for active case finding because of the urgent public health situation and previous findings that asymptomatic infectious cases may persist undiagnosed for a long time in the targeted community (6). In addition, symptomatic cases may report late because of limited access to care and to patient delay (24). Alternative interventions to control TB among homeless persons, such as symptom screening, spot sputum screening, treatment of latent TB infections in HIV-infected populations, and environmental control activities have been

applied (9, 10, 25, 26), although the success of these approaches has been questioned (20).

The screening resulted in a prevalence rate of 741 per 100,000 radiographs in the first year, but declined after some years of systematic screening, suggesting that the epidemic was being controlled. The total yield was 327 TB cases per 100,000 radiographs, which is high compared with the outcomes of other TB screening programmes in the Netherlands, such as entry screening of immigrants (111 per 100,000 radiographs) or prisoners (72 per 100,000 radiographs) (5). Our findings were similar to the result of radiographic screening of opiate users in Amsterdam in the 1990s of the last century (400 per 100,000 radiographs) (11). Radiographic screening detects only active intrathoracic TB cases. Additional interventions, such as screening for latent TB infections, might be of use in a phase of a controlled epidemic to identify cases at risk and select candidates for preventive treatment, although low completion rates have been reported among homeless persons (27).

Apart from active case-finding activities, completion of treatment is essential to control TB and prevent new cases (28). High completion rates can be achieved by directly observed therapy, in which patients take their medication under supervision (28, 29). In Rotterdam, all illicit drug users and homeless persons with TB have a directly observed therapy indication, and as a result of the screening programme additional human and other resources were needed to maximize case holding. Incentives such as public transport tickets, priority accommodation in shelters, voluntary admission to specialized TB hospitals, or assistance in applying for temporary residence permits also contributed to the high treatment completion rate. The use of detention as a last resort to isolate noncompliant infectious patients and complete treatment has been successfully applied in other programmes to control tuberculosis (30).

DNA Fingerprint Analysis. DNA fingerprint studies, most of them based on RFLP, combined with conventional epidemiologic information, have increased our knowledge of TB transmission (10, 31-34). Community studies demonstrated unidentified outbreaks of TB and underlined the importance of ongoing transmission, also in low-prevalence countries (33, 34). Molecular studies have pointed out the limitations of standard contact-tracing procedures to identify or prevent new cases and have showed the contribution of DNA fingerprinting to surveillance and the evaluation of TB control programs (33-35). For adequate interpretation and comparison, molecular studies should ideally involve a high proportion of cases in a population, be conducted in conjunction with conventional epidemiologic investigations, provide information about patient characteristics, and report the time period of the study (36). In the Netherlands, a comprehensive database of DNA fingerprints of nearly all culture-confirmed TB cases over more than 10 years is available (37-39). Together with detailed epidemiologic information from the patient records the present study fulfils the above-cited criteria.

DNA fingerprinting has been used to document clonal relationships in shelter-associated outbreaks over long time periods (9, 40, 41). In our study, the four most prevalent mycobacterial strains continued to reoccur in the illicit drug–using or homeless population for 8 to 13 years. It is unlikely that these cases were due to a single point source, because 54% (data not shown) of the illicit drug–using or homeless cases in these clusters were infectious. Ongoing transmission from multiple sources among illicit drug users or homeless persons sustained the outbreak for many years. The growth of one of these clusters clearly illustrated the epidemic spread both inside and outside these risk groups. DNA fingerprint analysis also revealed that isoniazid-resistant strains were introduced in the homeless community in Rotterdam, leading to a secondary case and creating the potential for new and more complicated outbreaks, as documented elsewhere (42).

The proportions of TB cases among illicit drug users and homeless persons with prevalent strains varied between 80 and 90% at the height of the epidemic in Rotterdam and were higher than the reported proportions in Denver (49%), Los Angeles (53%), San Francisco (60%), New York (60%), or Budapest (70%) (9, 10, 13, 31, 43). One of these studies also measured the effect of an intervention on the proportion of prevalent strains, and showed a decrease from 49 to 14% after a mandatory screening programme using symptom screening and tuberculin skin testing was implemented (9). During the mobile radiographic screening programme in Rotterdam, which was not mandatory but used an opting-out strategy and strong persuasion of clients to comply with screening, the proportion of TB cases among illicit drug users and homeless persons with prevalent strains declined from 82% in 2002 to 45% in 2005.

Conclusions

After the introduction of systematic and targeted TB screening among illicit drug users and homeless persons with mobile digital X-ray units in Rotterdam, the annual number of notified TB cases among these risk groups decreased and transmission fell. This study demonstrates that DNA fingerprinting can be a useful tool to evaluate the impact of a TB screening programme. Although the yield declined after some years of systematic screening, we advocate that screening of illicit drug users and homeless persons in Rotterdam should be continued to prevent a resurgence of TB as was experienced after discontinuing a screening programme at the end of the last century.

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7

Health care workers with tuberculosis infected during work

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Abstract

The risk for healthcare workers (HCWs) of tuberculosis (TB) attributable to occupational exposure is difficult to determine, as are the conditions contributing to this risk. The objective of the present study was to determine which TB cases among HCWs in the Netherlands were infected during work and to analyse factors which contributed to infection and subsequent disease.

The total study population consisted of 101 cases over a 5-yr period. In 67 (66%) subjects the route of infection could be determined by epidemiological and microbiological information. Of these cases, 28 out of 67 (42%) were due to infection at work, 19 (28%) were community acquired, and 20 (30%) were infected abroad.

The 28 cases infected at work were subject to an in-depth analysis. Delayed diagnosis of the index case, especially in the elderly patient, was the main cause of patient-to-HCW transmission. In some circumstances, inadequate infection-control measures also contributed to transmission.

In conclusion, a high suspicion of tuberculosis by the clinician, adequate infection-control measures by hospital authorities, and early identification of latent tuberculosis infection by occupational and public-health specialists are necessary to prevent tuberculosis among healthcare workers.

Introduction

Healthcare workers (HCWs) are at risk of nosocomial infection with *Mycobacterium tuberculosis* (1). This risk was high in the pre-chemotherapy era, but declined rapidly after the introduction of effective treatment, which reduced the infectious period of tuberculosis (TB) patients, as well as the absolute number of TB patients in many countries (2). Along with the declining risk, the attention for infection-control practices in hospitals has lessened (2). Recognition of nosocomial transmission as a public-health issue was renewed when extensive HIV-related transmission of multidrug-resistant TB occurred in New York City hospitals (NY, USA), which also affected HCWs, some with fatal outcomes (3-5).

Several research methods, such as cohort studies and case–control studies, have been applied to study and estimate the extent of work-related TB among HCWs (2, 6-8). These studies have methodological limitations in determining the excess risk for HCWs and the importance of conditions of exposure leading to this risk (7, 8). Cohort studies and case–control studies are often unable to differentiate between occupational and nonoccupational risk, while outbreak reports may describe extraordinary situations with a high number of infecting inocula, particular virulent strains or HIV comorbidity, making it difficult to extrapolate such results to nonoutbreak conditions (4, 6, 7, 9).

In the Netherlands, all TB cases are reported to the Netherlands Tuberculosis Register (NTR), which also includes information on risk-group status (10). One of the variables within the register is ''working in the healthcare/social welfare sector''. Every year, on average 20–30 HCWs out of 1,500 cases are reported with TB. The objective of the present study was to determine which cases were really infected during healthcare work in the Netherlands and to analyse factors which contributed to infection and subsequent disease.

Methods

The current study cohort comprised of all consecutive TB patients registered in the Netherlands during the period of January 1, 1995 to December 31, 1999, who were classified as ''working in the healthcare/social-welfare sector''. After approval by the ethical committee of the NTR, all 37 Depts of Tuberculosis Control of the Municipal Health Services (MHSs) responsible for notification of the cases were identified and asked for their collaboration. This essentially meant that nurses who were involved in patient management and contact investigations related to these patients were interviewed to answer three basic questions as follows. 1) Was the patient really a HCW before or at the time of diagnosis? 2) What kind of work was the HCW involved in at the time of diagnosis or before diagnosis? 3) Does epidemiological or molecular information exist to prove or exclude infection during work in the

Netherlands? Cases were excluded if the patient was not a HCW or the diagnosis of TB was withdrawn after notification.

Since 1993, all *M. tuberculosis* isolates in the Netherlands are subject to standardised insertion sequence (IS) *6110*-based restriction fragment length polymorphism (RFLP) typing, socalled DNA fingerprinting (11). Clusters are defined as groups of patients having isolates with fully identical RFLP patterns or, if strains harbour less than five IS*6110* copies, with identical sub-typing by use of the polymorphic GC-rich sequence probe (12). The fingerprints of all culture-confirmed cases in the cohort were cross-checked with the National DNA fingerprint database.

With the information from the interviews, the NTR and DNA fingerprints, patients were classified into four categories as follows. Category 1: HCW infected during healthcare work in the Netherlands. Cases were included if an epidemiological nosocomial link was confirmed by matching DNA fingerprints, or TB was diagnosed during a contact investigation carried out at the workplace, or a well-documented epidemiological link was present without bacteriological confirmation of the diagnosis in the HCW. Category 2: HCW infected in the community. These cases had either matching DNA fingerprints with a close contact in the community or, if culture negative, were considered infected in the community based on convincing epidemiological evidence, such as contact investigation among household contacts and friends. Category 3: HCW infected abroad. This category includes Dutch HCWs who worked for a long period in a hospital within a TB endemic country, as well as foreign-born HCWs. Classification was also based on tuberculin skin test (TST) conversion after leaving the Netherlands and on DNA fingerprints (e.g. a unique fingerprint). Category 4: HCW place of infection unknown, including the remaining cases which could not be classified into one of the above categories.

Cases belonging to category one were investigated in more detail by contacting the TB departments of the MHSs and other health institutions involved. Patient and disease characteristics of both the HCW and the index case were obtained from patient records. In addition, information was collected about the circumstances under which transmission occurred. The results were discussed with involved professionals working in these settings. The study design did not allow for collection of standardised information about the airflow (ventilation) in relevant rooms.

Results

In the 5-yr study period, 123 patients were recorded as working in the healthcare/social welfare sector. Eight cases were misclassified as having TB, seven had a latent TB infection and one case had disease caused by *M. avium*. Another 21 cases were workers/volunteers involved in the social services in asylum seekers centres, penitentiary institutions and homeless centres that did not have a HCW status. In total, these 29 (24%) cases were excluded from the analysis, leaving a total of 94 eligible

HCWs with active TB. However, during the field research, seven additional cases were identified, which were not (yet) included in the present study. The box on the registration form was either not ticked or information was wrongly copied into the national TB register. These cases were included in the current study, giving a total study population of 101 HCWs.

The incidence of TB among HCWs was calculated for hospital workers involved in patient care, since they are at greatest risk for nosocomial infection and their denominator can be more accurately determined. In 1997, 126,500 persons were involved in patient care in Dutch hospitals (13). During the 5-yr study period, 50 cases were hospital-employed HCWs involved in patient care, resulting in a TB incidence of 7.9 per 100,000 per annum. The other 51 cases were employed in nursing homes, home-care organisations or were general practitioners, physiotherapists, student doctors or not employed in the healthcare setting at the time of diagnosis.

Table 7.1 shows the classification of the cases in the four categories. Of the 47 cases infected in the Netherlands, 28 were work-related (category one) and 19 community acquired (category two). The attributable risk (AR) of healthcare work in the Netherlands can be derived directly by dividing the number of cases in category one by all cases infected in the Netherlands (categories one and two) and is 0.6 (28 out of 47). The relative risk (RR) for healthcare work in the Netherlands can be calculated by AR=(1–1/RR) and is 2.47.

Of the 11 HCWs infected abroad (category three) and born in the Netherlands, all but one case was involved in patient care in developing countries, often for many years. One Dutch HCW had a negative TST when leaving the Netherlands to work in a refugee camp in Kenya and on returning the TST was 6 mm. No treatment for latent TB infection was prescribed in accordance with Dutch guidelines. The patient developed pleural TB within 1 yr of returning, with a *M. tuberculosis* strain identical to a strain prevalent among Somalian TB patients in the Netherlands.

In-depth investigation focused on the 28 cases infected during work in the Netherlands. Among them, 16 cases were classified based on both an epidemiological link and a matching DNA fingerprint, two cases had an epidemiological link and a unique fingerprint which could be explained (see below), eight cases had culture-negative TB diagnosed in a contact investigation at work, and two HCWs with culture-negative TB had a well-documented contact in the hospital, but were diagnosed after they reported with symptoms. However, five cases were excluded from analysis below since they had no identified patient contact: two HCWs were infected by a regular visitor of the hospital; two HCWs by another HCW; and one laboratory assistant developed cutaneous TB with a unique fingerprint after injuring herself with a needle in a laboratory.

Table 7.1. Classification of healthcare workers with tuberculosis (TB) in all four categories according to information from interviews, the Netherlands TB Register and DNA fingerprints 1995-1999.

	Category 1: HCW infected during health care work in the Netherlands		Category 2: HCW infected in the community		Category 3: HCW infected abroad		Category 4: HCW place of infection unknown		Total	
	n	%	n	%	n	%	n	%	n	%
Cases	28	28	19	19	20	20	34	34	101	
Born in the Netherlands	28	100	15	79	11	55	27	79	81	80
Average (yrs)	30		30		33		37		33	
Age range	21-65		21-56		24-87		17-84		17-87	
Pulmonary TB	13	46	13	68	16	80	28	82	70	69
Bacteriological confirmation	18	64	14	74	10	50	8	24	50	50
Clustered cases	16	89	13	93	1*	10	5	63	35	70

Characteristics of HCWs and index patients. The median (range) age of the HCWs was 28 (21–65) yrs with the majority being female (18 out of 23). The following professions were involved: nurses (n=14); doctors (n=4); ward assistants (n=2); bronchoscopy assistants (n=2); and an assistant of an outpatient department (n=1). In total, 21 were infected in the hospital and two outside the hospital. The hospital workers were deployed at the following departments: a pulmonology ward (n=9); an internal medicine (including AIDS) department (n=5); a bronchoscopy unit (n=2); an outpatient department (n=2); and at other wards (n=3). In total, 10 cases had pulmonary TB (among them two cases were smear positive), 12 had pleural TB and one had TB of the skin. None of the cases reported a previous history of TB. There were no HCWs with HIV co-infection. However, as the HCWs were not systematically tested for HIV, this information is incomplete. All isolates were drug susceptible and all 23 HCWs completed treatment.

In 21 out of the 23 patients, the infection could be attributed to a specific index case, while in two cases only incomplete information of the presumed index patient could be obtained. Of the known sources, 16 index patients each caused one secondary case among HCWs, one index case was the source of two secondary cases and one index case caused three secondary cases among HCWs. Almost all index patients had smear-positive pulmonary TB, except for two cases: one with disseminated TB with chest radiography abnormalities, a negative sputum culture and a positive stool culture for *M. tuberculosis*; and one case with a TB abscess of the knee joint that was surgically managed and drained. The median (range) age of the index patients was 45 (25–87) yrs, with 44% of the index patients (eight out of 18) aged >60 yrs. Two index cases were HIV infected.

Interval between infection and diagnosis of TB in HCW. The date of infection was determined as the date of admission of the index case to the hospital or, for nonhospitalised patients, the date of first contact with the HCW. The date of TB diagnosis of the HCW was the date of admission to the hospital, or for nonhospitalised HCWs, the date of specimen collection for TB examination or the first date of presenting with symptoms at a health post. For two HCWs, both infected by the same index patient, the interval could not be determined because the index patient was undiagnosed for a long time, probably >1 yr. One of these HCWs even developed pleural TB 4 months before the index patient was diagnosed.

The interval between infection and disease of TB cases among HCWs is presented in Figure 7.1. The median interval was 32 weeks for all 21 secondary cases and 34 weeks for the 13 culture-confirmed secondary cases. The factors that contributed to infection are summarised in Table 7.2. In 10 index cases, mostly elderly patients with comorbidity, TB was initially not suspected and, thus, adequate isolation was delayed. This diagnostic delay was the main cause of patient-to-HCW transmission, while in some circumstances inadequate isolation measures contributed to infection.

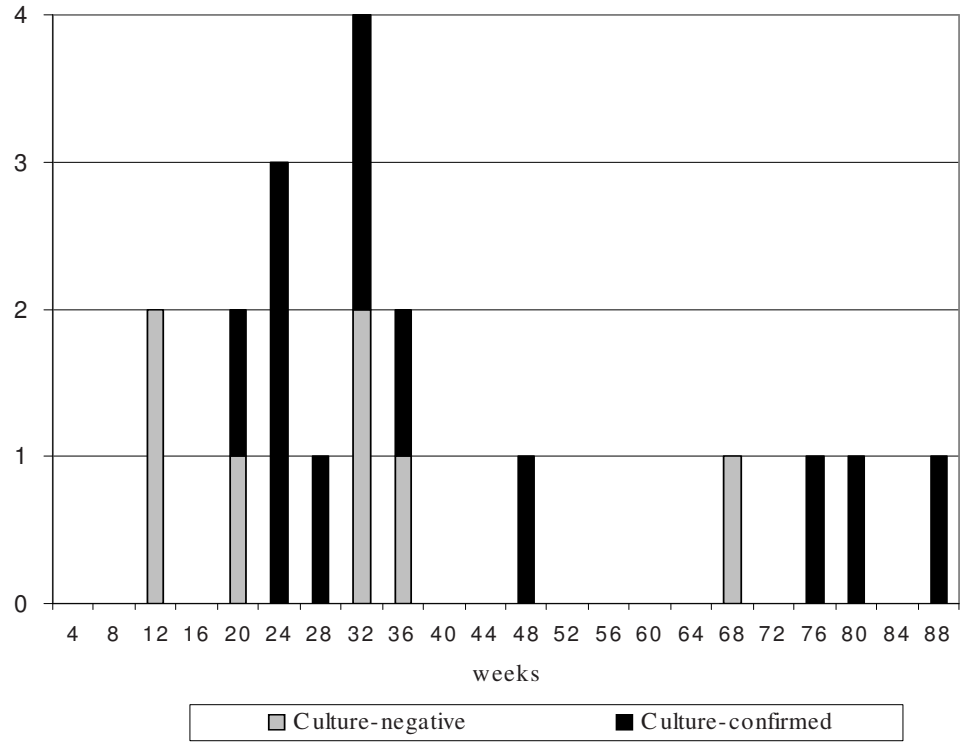

Figure 7.1. Interval between contact and diagnosis of 19 tuberculosis (TB) cases among healthcare workers. Pulmonary TB was diagnosed after 10, 12, 18, 23, 29, 31, 32, 75, 78 weeks and 4 yrs (last case not shown). In six cases, TB was confirmed by a positive culture. Pleural TB was diagnosed after 17, 23, 24, 26, 31, 32, 33, 45, 65, 85 weeks and 9 yrs (last case not shown). In seven of these cases, TB was confirmed by a positive culture. Two cases (pleural TB and TB of the skin) had an undetermined interval due to a long delay in diagnosis of the index patient (both infected by the same source case).

Table 7.2. Underlying factors for patient-to-healthcare worker (HCW) transmission of tuberculosis (TB).

	HCW with TB
Failure to identify and isolate index case	
For some days after admission	4
For 3 weeks	4[#]
During out-patient consultation	3
During irrigation of an abscess at home for several months	1
Total delay in diagnosis	**12**
Inadequate infection-control	
Surgical masks used during isolation period	3
Adequate protective masks used, but not all the time	2
Total Inadequate infection-control practices	**5**
High-risk procedures[¶]	2
No underlying factors identified	2
Incomplete information of the index patient[†]	2

Data are presented as n.
#: One 80-yr-old index patient, with chronic obstructive pulmonary disease, was diagnosed with smear-positive pulmonary TB 2 months after a previous hospital admission of 3 weeks and caused three secondary cases among HCWs: two among other elderly hospitalised patients (one died) and one in a visitor. The four bacteriologically confirmed cases had identical DNA fingerprints as the index case.
¶: i.e. bronchoscopies.
†: one HCW developed pleural TB 15 months after being diagnosed and treated for a latent TB infection (LTBI) after exposure in a hospital. The other HCW had pleural TB with a unique fingerprint. She was treated for LTBI 9 yrs previously after exposure to a highly infectious case, before DNA fingerprinting was carried out in the Netherlands. This case was included in the study population because no other risk factors were identified.

Screening for TB or latent TB infection. Eight HCWs were diagnosed with TB in a contact investigation or pre-employment screening. Three HCWs developed TB despite treatment for latent TB infection (LTBI) with 6 months isoniazid (one was diagnosed later in a pre-employment screening). In three HCWs, LTBI was missed due to a false-negative TST. Results are shown in Table 7.3.

Seven HCWs with TB were not enrolled in a contact investigation or periodical screening. Two of them should have been included in a periodical screening, both employed at a pulmonology ward. Another two should have been included in a contact investigation, but in one situation the index patient refused to mention contacts with health professionals. In the remaining three cases, contact investigation or periodical screening for TB was not indicated and the link with the index patient was only determined by matching DNA fingerprints retrospectively.

Table 7.3. Enrolment of healthcare workers (HCWs) with tuberculosis (TB) in active case finding activities.

In a contact investigation (n=10)
TB diagnosed six times
LTBI missed twice, TST false negative[#]
Two HCWs developed TB despite treatment with 6 months isoniazid for a latent TB infection.
In a pre-employment screening (n=2)
TB diagnosed twice[¶]
In periodical screening (n=4)
LTBI missed once, TST false negative[#]
Two HCWs were infected and developed TB within the screening interval
One BCG-vaccinated HCW was screened by periodical chest radiography, no TB found
Not enrolled in a contact investigation or periodical screening (n=7)

LTBI: latent TB infection; TST: tuberculin skin test; BCG: bacille Calmette–Guérin.
#: two HCWs had a negative TST in a contact investigation and one in a periodical screening, but later developed pleural TB with the same strain as the presumed index cases. Retrospectively, TSTs were false negative 7, 8 and 10 weeks after contact with the index patient, while they were 10 mm, 19 mm and ''strongly positive'' at the time of diagnosis, 31, 32 and 24 weeks after infection.
¶: One was diagnosed with LTBI before contact investigation at work. The HCW completed 6 months isoniazid preventive treatment, but had chest radiography abnormalities 4 yrs later at pre-employment screening. TB was confirmed by a positive culture of material obtained by video-assisted thoracoscopy and the restriction fragment length polymorphism pattern matched with the index patient.

Discussion

In the present study, a case series of HCWs with TB, information from a comprehensive national DNA fingerprinting database and detailed epidemiological information from TB departments was used to distinguish nosocomial and non-nosocomial routes of transmission. In 67 out of 101 (66%) HCWs with TB, the route of infection could be determined. Among them, 28 out of 67 (42%) were infected during work in the Netherlands, 19 (28%) were community acquired and 20 (30%) were infected abroad. In 34% of cases, the route of transmission could not be determined, mainly due to lack of bacteriological confirmation.

The TB incidence rate for hospital-employed HCWs with patient contacts (7.9 per 100,000) was approximately two times higher than the incidence rate for Dutch citizens during the study period (average 4.4 per 100,000), but still lower than the rates for all citizens in the Netherlands (average 9.8 per 100,000 with immigrants accounting for >50% of all cases) (10). The present authors also calculated the relative risk for

healthcare work in the Netherlands by classifying cases into categories. The RR of 2.47 compares with findings in England and Wales where TB rates in HCWs were two to three times higher than those in similar occupational groups (14). The fact that in one third of cases the route of transmission could not be determined (category four) probably does not influence the distribution of cases among the three categories and the RR in a significant manner.

Only 20% of all HCWs with TB were foreign born. This differs from studies in low-incidence countries with high percentages of foreign-born HCWs with TB (14-17). Almost all HCWs with work-related TB in the present study developed early manifestations of TB, such as primary pulmonary TB, pleural TB or TB of the skin. Although active-case finding activities such as pre-employment screening and contact investigation detect TB in an early stage, often without bacteriological confirmation, the current authors also found a relatively short interval between infection and disease for HCWs who presented with symptoms. In these cases, awareness of the HCW and a high suspicion of the physician might have limited diagnostic delay, as has been observed elsewhere (14), although in one other study the health-seeking behaviour was similar for HCWs and controls (17).

The reported TB cases among HCWs are the tip of the iceberg of nosocomial transmission of *M. tuberculosis* bacteria. After all, only 10% of TB infections will eventually lead to active TB (18), so many more infections have occurred. Furthermore, through pre-employment screening, contact investigation and periodical screening of HCWs, a number of latent TB infections are identified (250 annually in the Netherlands) and treated in the majority of cases (10). However, as the present data show, a significant proportion of these infections have been acquired outside the hospital, as has been observed in other studies (19-21). Furthermore, other patients (and visitors) might be at even greater risk if TB is not suspected and diagnosis is delayed. This is well illustrated by the transmission of *M. tuberculosis* from one index case to three HCWs, two other hospitalised patients and one visitor.

Early recognition of TB and adequate isolation of cases remain the most important interventions to prevent transmission (2, 8, 22, 23). In the current study, diagnostic delay was the main cause of patient-to-HCW transmission in 50% of the cases, often in an elderly Dutch patient with comorbidity. The association between initially missed diagnosis and older age has been described by others (23). Greater experience in TB management, with increased TB suspicion and compliance with diagnostic algorithms, has the potential to reduce diagnostic delay and nosocomial transmission (23). In some cases in the present study, failure to apply with infection-control procedures, such as the use of appropriate masks, contributed to infection. Hospital infection-control measures are relevant to prevent transmission of TB to healthcare workers (18, 23, 24). Although the study design did not allow for a standardised assessment of ventilation during the study period, the relevance of airflow is also well known (21). Four cases in the current study were infected during high-risk

procedures, i.e. two HCWs while assisting with bronchoscopies, one laboratory attendant due to a needle stick injury and one HCW during irrigation of a TB abscess (syringing). The occupational risk of these procedures, as well as autopsies, has previously been described in case reports and reviews (2, 8, 23, 25-27). Adequate personal protection measures should be taken during these procedures.

In spite of adequate infection-control measures, transmission of TB might still occur in healthcare institutions, and early diagnosis of latent TB infection or active TB in HCWs is needed in high-risk settings. In the present study, 70% of cases (16 out of 23) had been examined at least once because of high-risk activities or unprotected exposure to a patient at work. The reasons for not detecting and/or effectively treating LTBI were related to the inability of screening procedures to identify infections, the limitations of the TST and failure of (unsupervised) preventive treatment. However, the current study focused on a relatively limited number of HCWs in which infection-control measures failed. It needs to be emphasised that a much greater number of infected HCWs have benefited from screening and preventive treatment.

In conclusion, DNA fingerprint surveillance can be used to confirm expected and reveal unexpected cases of nosocomial transmission, thus providing the necessary evidence for policy makers and professionals to take appropriate action. High suspicion of tuberculosis by the clinician, adequate infection-control measures by the hospital authorities and early identification of latent tuberculosis infection in healthcare workers by occupational specialists form the essential components of a comprehensive package to prevent tuberculosis in healthcare workers.

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8

General discussion

This chapter provides answers to the three research questions posed in this thesis (section 8.1), discusses implications for future TB control in the Netherlands (section 8.2), and lists the main conclusions and recommendations (section 8.3).

8.1 Answer the research questions

8.1.1 Question 1: What has been the added value of DNA fingerprinting for TB control in a metropolitan area?

DNA fingerprinting confirmed outbreaks in the Rotterdam metropolitan area and was indispensable to develop and evaluate interventions for high-risk groups. Genotyping also supported decisions for extending contact investigations in circumstances with confirmed transmission, and contributed to surveillance of tuberculosis in the metropolitan area.

DNA fingerprinting and contact investigation. In our cluster analysis study (Ch.3), several transmission sites, such as a pub, a vocational training school and a children's ward of a hospital, were identified by genotyping. Matching DNA fingerprints of epidemiologically linked cases revealed or confirmed transmission at those places and as a consequence, contact investigations were extended to large numbers of contacts yielding secondary TB and LTBI cases. Thus, genotyping helped to decide upon and justify large scale contact investigations. The majority of the clustered TB cases in this study however were not identified through contact tracing. Other studies in low-incidence areas also reported that only 5-10% of clustered cases were identified by conventional contact investigation and called for improved strategies of active case finding targeted to specific populations (1-6). The difficulties to conduct contact tracing in pubs and treat latently infected contacts are discussed below.

In our study of a contact investigation around a homeless, drug-addicted man (Ch.5) all six secondary TB cases were not molecularly linked to the index case and thus genotyping confirmed that illicit drug users and homeless persons in the Rotterdam metropolitan area were at high risk for TB. Other molecular epidemiological studies also showed that sometimes cases identified by contact investigation have unrelated strains (7-10). In one large study 29% of TB cases discovered by contact investigation had non-matching fingerprints (11).

TB cases represent only the tip of the iceberg of transmission, since most *M. tuberculosis* infected cases never develop TB. The true amount of transmission can only be shown if culture negative TB and LTBI cases are included in research as we described in the cluster analysis study (Ch.3). Studies using only genotyping data exclude such cases and may thus underestimate the true extent of an outbreak and the value of active case finding. Thus, the effectiveness of contact tracing can only be established by combining conventional epidemiological investigation and molecular typing, including all TB and LTBI cases identified by contact investigation.

DNA fingerprinting and outbreak management. Outbreak management starts if a large number of cases are involved in recent chains of transmission or when disease is concentrated in certain risk groups. Typically, TB outbreaks have cases with identical *M. tuberculosis* isolates. Thus, cluster growth and cluster size are important indicators to monitor the occurrence and development of outbreaks. We described a cluster of 150 national cases (Ch.6) with more than 80% residing in our study area and with a high proportion of cases using illicit drugs or being homeless. Knowledge of transmission within these communities was indispensable for successfully applying for funds to reinstitute a TB screening programme among these risk group. Cluster investigations revealed intensive TB transmission in pubs in a number of our studies (Ch. 3 and Ch. 6), but interventions to control transmission and investigate contacts were very disappointing, as observed by others (12-14).

TB epidemics can also consist of different *M. tuberculosis* strains among risk groups as we described among illicit drug users and homeless persons (Ch.5). In a 13-year observation period, 55 different fingerprints were recovered from isolates of cases of these risk groups in Rotterdam, with four dominant strains (Ch.6). Thus, DNA fingerprinting highlighted the potential threat of new outbreaks, because new strains were introduced and circulated among these risk groups regularly and supported our conclusion that screening should be continued also in non-outbreak conditions.

DNA fingerprinting and surveillance. A standardised way of classifying TB into recently, remotely and foreign-acquired disease provides indicators for surveillance and programme performance that can be used to prioritise interventions and allocate resources (Ch.2). TB control programmes with predominately recent transmission may focus on a package of targeted active case finding activities, while those with a high proportion of imported strains should consider screening activities for TB and LTBI in immigrants, and those with mainly reactivated cases can monitor the gradual decline of TB or step-up interventions to eliminate TB.

DNA fingerprinting can help to estimate the number and timing of future cases in a cluster and also in a given area (1, 2, 15). In our cluster analysis study (Ch.3), we showed that TB developed within one year of infection in about 50% of cases and between one to five years in the other 50%, which agrees with previous estimates of disease development (16). Although more research is needed into the prediction of future cases, it is clear that the chance that related cases develop depends on the number and time interval of secondary cases with a pulmonary source case.

DNA fingerprinting and evaluation of programmes. In our health care worker (HCW) study (Ch.7), we looked at the role of active case finding to identify cases. We observed few missed opportunities to diagnose LTBI and TB in Dutch HCWs. Delayed diagnosis of the index cases, especially in the elderly patient, was the main cause of patient-to-HCW transmission. In some circumstances, inadequate infection-control measures contributed

to transmission and subsequently disease development. The majority of TB cases (70%) had been examined at least once because of high-risk activities or unprotected exposure to a patient and TB was actually diagnosed in half of these cases. Screening procedures and efficacy of preventive treatment limited case finding in some other cases.

We used molecular typing to assess the impact of a screening programme on TB transmission among illicit drug users and homeless persons (Ch.6). The study showed that screening with mobile radiographic X-ray units decreased the annual number of notified TB cases following an outbreak. Screening reduced transmission because the proportion of cases with prevalent strains declined significantly from 82% to 45% in four years. Few studies have used genotyping as an evaluation instrument, probably because it requires universal genotyping for a prolonged period of time.

8.1.2 Question 2: What are the causes of the high TB incidence in a metropolitan area, as revealed by DNA fingerprinting?

DNA fingerprinting showed that the high TB incidence in the Rotterdam metropolitan area was related to the high proportion of immigrants, and was caused by recent transmission in both the immigrant and nonimmigrant population. Illicit drug users and homeless persons were particularly at risk of recently acquired TB.

In the metropolitan study (Ch.4), the TB case rate in three highly urbanised municipalities (Rotterdam, Schiedam and Vlaardingen) was 3.8 times higher than in surrounding municipalities. The rate ratios were lower after stratification for country of birth, i.e. 1.7 for immigrants and 2.8 for nonimmigrants. The reason for these lower incidences is the higher proportion of immigrants in highly urbanised areas; immigrants formed 23% of the population in the three urban municipalities and 8% in the surrounding areas. These immigrants are mainly from high-endemic countries and more frequently latently infected, that may reactivate many years after entering the Netherlands (17).

Recent transmission caused 40% of the case rate in the urban and 28% in surrounding municipalities, translating into a 5.5 times higher recent transmission case rate in the urban population. Illicit drug users and homeless persons were particularly affected by recent transmission, and in fact a number of strains caused the outbreak among these risk groups in Rotterdam, as described in Ch.5 and 6. Recent transmission was also more common among the urban immigrant and nonimmigrant population not using illicit drugs or being homeless. Thus, ongoing transmission contributed substantially to the high TB incidence in the urban municipalities.

In the metropolitan study, we also noticed a higher TB rate of foreign-acquired disease among the nonimmigrant urban population. Different risk and behavioural patterns such as frequent visits of second

generation immigrants to their parents' native countries and international travel of young urban persons may explain this unexpected finding. The contribution of foreign-acquired disease among nonimmigrants to the overall caseload in urban municipalities was however small.

In our studies, we identified a number of relevant locations for TB transmission in urban areas. First of all, transmission in homeless shelters and safe drug consumption rooms probably caused the high TB incidence among illicit drug users and homeless persons. Secondly, a number of our studies pointed at pubs as sites of TB transmission; several of the molecular clusters expanded due to pub-related outbreaks (18). Furthermore, the identification, investigation and treatment of infected contacts were limited. The social and behavioural problems in large cities in well-developed countries are very similar, as is the picture of these cities' epidemiological features of TB as shown by DNA fingerprinting.

8.1.3 Question 3: What do our studies add to the knowledge of TB transmission?

A standardised use of both molecular and conventional epidemiological information improves place and time determination of transmission and the identification of persons and people at risk for tuberculosis.

Place and time. We developed and applied a transmission classification model to determine whether TB cases were infected in a foreign country, or recently (≤ 2 years) or remotely (> 2 years) infected in the Netherlands. Molecular typing studies rarely distinguished foreign-acquired disease from TB resulting from transmission within the country of residence (19, 20). In our transmission classification study (Ch.2), one-third (38%) of all cases acquired infection in a foreign country. For immigrant TB cases in urban and suburban municipalities (Ch.4), these proportions were respectively 47% and 62%. Thus, transmission in a foreign country accounted for a substantial proportion of TB in our study area, which is beyond direct control of a local TB programme.

Our studies highlighted that homeless shelters, safe drug consumption rooms and bars were places for TB transmission in the Netherlands, as has been reported by others (13, 21-25). The poor ventilation of these settings, the crowding of people and delayed diagnosis of infectious cases facilitate the spread of *M. tuberculosis* bacteria in these institutions.

Genotyping more accurately ascertained time of infection for epidemiologically linked TB cases. In fact, for some of the HCWs (Ch.7) the actual date of nosocomial transmission could be determined because they had contact with their source case only once, e.g. during bronchoscopy. In the HCWs study, 71% of cases developed disease within one year. Another estimate of timing of infection was obtained in our transmission classification study (Ch.2), in which 76% of epidemiologically linked clustered cases developed disease within two years of infection. In

both studies however, cases with a remote infection probably have less chance to be epidemiologically linked due to patient recall bias. In the HCWs study earlier identification of disease may also limit generalisation of results. The best estimate of disease development was obtained in our cluster analysis study (Ch.3) which allowed us to draw a curve of secondary cases. In this study, TB developed within one year of infection in 52%, within two years in 71%, and between two to five years in 29% of patients, which more or less agreed with a previous estimate that 60% of infected cases developed disease within one year, 85% within two years, and 15% within two to five years (16).

Person (risk factors and risk groups). In the metropolitan study (Ch.4), immigrants were identified at high risk for TB both due to reactivation of foreign-acquired infection and recent transmission in the Netherlands. Urban immigrants had a 2.6 times higher recent transmission case rate than suburban immigrants, and 6.1 times higher than urban nonimmigrants. Possible explanations are the higher risk of exposure to infectious TB in urban areas, limitations of contact tracing procedures, poor housing conditions and delays in diagnosis. DNA fingerprinting and epidemiological investigation of an illustrative cluster (Ch.3) proved that these foreign-born clustered cases were infected in the Netherlands and not in their native countries, as might have been assumed. Cluster analysis showed that one single infectious TB case, a foreigner visiting the Netherlands, caused an extensive outbreak. Patient delay seeking medical attention and delay by the health system to diagnose infectious TB facilitated transmission and resulted in several secondary cases. The effective spread of *M. tuberculosis* by a single infectious case, so-called supertransmitters or superspreaders, was reported elsewhere (3, 8, 26-29).

Some of our studies drew attention to other well-known risk factors for TB, such as homeless persons, illicit drug users and alcoholics (3, 13, 21, 30, 31). DNA fingerprinting showed that several *M. tuberculosis* strains were circulating among illicit drug users and homeless persons and were responsible for the high TB caseload among persons of these risk groups. Extensive pub-related transmission contributed to the growth of nearly all large clusters in our study area (Ch.3) (18). Recently, we described another TB outbreak in urban pubs that caused unprecedented high case rates among a specific ethnic population (26).

Molecular typing has been used to determine whether recurrent TB was caused by a new strain (reinfection) or by the same strain (relapse) (32-36). In one of our studies we analysed a subset of 18 recurrent cases with two culture-confirmed episodes (Ch.2). Five cases (28%) had two different fingerprints and eleven (72%) identical fingerprints. Six recurrent cases with identical fingerprint shared epidemiological characteristics with one or more pulmonary TB cases that were added to the cluster since the first episode. This study suggested that reinfection with the same strain (ping-pong transmission) should be considered in recurrent cases if they are at high-risk for TB.

HIV-infected persons are at increased risk for TB. In Rotterdam, illicit drug users and homeless persons with TB were more often coinfected with HIV (15%) than cases without these risk factors (4%) (Ch.4). Genotyping results of the *M. tuberculosis* isolates were not assessed in this study, but clustering rates were higher in HIV-infected cases with the risk profile (62%; 18 of 29) than for HIV-infected cases without the risk profile (38%; 25 of 65). These differences were statistically significant (p=0.03) and further support the finding that ongoing transmission contributed to the high TB incidence among illicit drug users and homeless persons.

Drug resistance is an emerging public health threat in the world. In our studies, transmission of drug resistant strains was not a subject of research, although an outbreak of MDR cases occurred during the study period (37). The clustering rate of resistant strains in the transmission classification study (50%; 66 of 131 cases) was lower than that of fully sensitive strains (55%; 1042 of 1895). Although the difference did not reach statistical significance (p=0.3), it is in concordance with the observation of Van Soolingen *et al.* that drug resistant strains generate less secondary cases than fully sensitive strains (31).

In infectious disease outbreak management, it is relevant to know which secondary cases are caused by an index patient and present this graphically in a time line to detect epidemics, plan interventions and evaluate these efforts. Universal genotyping offers opportunities to visualise cases in a cluster diagram, which helps to find transmission locations and similarities and links between patients. It also assists monitoring an outbreak and estimating the number and timing of future cases.

8.2 Implications for future TB control in the Netherlands

DNA fingerprinting has tremendously improved our understanding of TB transmission (38), but as for any new technical achievement translation into real improvements remains a major challenge (39). Campbell *et al.* described the process and elements of using data for decision-making in health (40, 41). Some of the critical components are a sense of ownership and tools for self-assessment that help to identify achievements, constraints and possible actions. The tools and capacity to optimise the use of DNA fingerprinting for TB control in the Netherlands are not yet fully developed, especially not at local level, and need attention. Based on our experience and research, and in accordance with the guide on the application of genotyping issued by the the Center of Disease Control and Prevention in the United States (42), I suggest to prioritise cluster investigations, i.e. an investigation to identify epidemiological links between TB patients with matching fingerprints, as outlined in Table 8.1 (18).

Table 8.1. Prioritising cluster investigation (11, 18, 42).

High priority: • clusters with high-risk patients with a possible epidemiological link, e.g. institutionalised patients (prisons, asylum seekers centres, homeless shelters, hospitals), immunocompromised patients (HIV-coinfection), or patients with (multi)drugresistant strains. • Fast-growing clusters, e.g. with three or more patients in a year. • Large clusters, e.g. with more than 10 patients, that continue to expand. Low priority: • last preceding pulmonary TB occurred more than two years previously. • patient did not reside in the Netherlands with another pulmonary TB case in the cluster. • fingerprint confirmed an expected epidemiological link.

In the Netherlands, results from DNA fingerprinting and cluster investigation are best discussed in the recently developed TB control regions. TB specialists in these areas are knowledgeable of cases, risk groups and the epidemiological situation. They also know the local political situation and managerial possibilities. The studies in this thesis demonstrate such use of data for planning local TB control activities. Areas with high numbers and a high proportion of recent transmission benefit particularly from genotyping, and these are usually found in urban areas. We concur with Diel *et al.* that DNA fingerprinting should become an integral component of surveillance strategies aimed at TB control in metropolitan areas (1).

Metropolitan TB control should focus on a package of interventions addressing the different causes of the high TB incidence in large cities (43). In our metropolitan study we distinguished two main pathways, i.e. reactivation of foreign-acquired infections, predominantly among immigrants, and ongoing transmission among risk groups and the general urban population. Entry screening of immigrants for TB and LTBI are important interventions to identify disease early and prevent transmission in a country. In principal these interventions do not differ in urban and non-urban areas, although the number of immigrants varies. Interventions aimed at reducing ongoing transmission are more complex and should be tailored e.g. to the metropolitan setting. Radiographic screening can be very useful to identify asymptomatic cases in hard-to-reach, marginalised urban risk groups, such as illicit drug users and homeless persons. Case holding activities are key in TB control, and directly observed therapy is elementary to complete treatment in groups of TB patients that are more prevalent in urban areas. Treatment supervision is also necessary to prevent development of resistant strains. Contact tracing efforts in urban areas will benefit from interferon-gamma

release assays to correctly identify latent infections among BCG-vaccinated contacts and offer opportunities for preventive treatment. Education targeted at health care providers in cities, and social workers involved with urban risk groups may reduce diagnostic delay and diminish transmission. Low-threshold services, such as public health TB clinics, are needed for marginalized and often uninsured persons. Lastly, genotyping can contribute to identify high-risk areas within metropolitan areas and offer opportunities for location-based TB screening (44).

The Netherlands has no system for the systematic exchange of data and sharing experience between TB control departments. Some developing countries have excellent examples of the use of health management information systems (40, 41) that can be beneficial for TB control. Data of DNA fingerprinting and its use for action planning provide a good opportunity to conduct for instance routine peer performance meetings of the seven regions in the Netherlands or among Western European cities.

Another challenge is to use DNA fingerprinting for predicting future cases in an area (15). As a rule of thumb I applied the principle that – without ongoing transmission – a cluster will grow in the second year by 50%, in the third year by 25%, in the fourth year by 12.5% and in the fifth year by 6.3% of cases diagnosed in the first year (18). This is an area for further research that can help programme development in the elimination phase of TB.

Our metropolitan study showed that even among the urban population the majority of TB cases were infected in a foreign, often high-endemic country. Although screening can identify TB and LTBI cases early, the long term perspective should emphasise controlling TB in these high-endemic countries. This has become even more urgent with the event of MDR-TB and XDR-TB.

8.3 Conclusions and recommendations

Conclusions

- The added value of DNA fingerprinting for TB control in the Rotterdam metropolitan area has been its use for identifying and confirming outbreaks, and developing and evaluating interventions for high-risk groups.
- DNA fingerprinting also supported decisions for extending contact investigations in circumstances with confirmed transmission, and contributed to surveillance of tuberculosis in the metropolitan area.
- The high TB incidence in the Rotterdam metropolitan area was related to the high proportion of immigrants, and was caused by recent transmission. Illicit drug users and homeless persons were particularly at risk.
- DNA fingerprinting confirmed reduced TB transmission among illicit drug users and homeless persons after four years of screening.
- A standardised use of both molecular and conventional epidemiological information improves place and time determination of transmission and the identification of groups of persons at risk for tuberculosis.
- DNA fingerprinting enables the classification of cases as recently or remotely infected within a country, or infected in a foreign country. The number and distribution of these classified cases is relevant for programme performance and planning action.
- DNA fingerprinting assists to monitor an outbreak and estimate the number and timing of future cases, in particular in high-transmission settings such as metropolitan areas.

Recommendations

- Metropolitan TB control should include a package of targeted interventions of active case finding and case holding activities, focused on immigrants and hard-to-reach, marginalised urban risk groups, such as illicit drug users and homeless persons.
- Universal genotyping in the Netherlands should be continued to support TB outbreak investigation, surveillance and programme evaluation.
- Cluster investigation should be prioritised to cases with unknown epidemiological links and to possible recently infected cases.
- The tools and capacity to optimise the use of DNA fingerprinting for local and regional TB control in the Netherlands needs to be further developed and strengthened.
- Regional performance meetings should be conducted to share data and exchange experience to further enhance the use of DNA fingerprinting for TB control in the Netherlands.
- A European network should be established to facilitate the exchange of experience between metropolitan TB control programmes.

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Summary

This thesis addresses the question what has been the use of DNA fingerprinting for tuberculosis control in a metropolitan area.

Chapter 1 contains an outline of tuberculosis (TB), DNA fingerprinting methods and the molecular epidemiology of TB. Although TB is still highly endemic in many less-developed countries, incidence declined in the Netherlands to the lowest ever recorded in 2006 with 6.2 cases per 100,000 population. Urban case rates however are several times higher than the national average, not only in the Netherlands but also in other low-endemic countries. DNA fingerprinting has been very useful for a better understanding of TB transmission. Molecular studies have confirmed outbreaks, identified risk factors for recent transmission, revealed the importance of reinfection in recurrent TB and pointed at the occurrence of laboratory cross contamination. In the Netherlands, DNA fingerprinting methods have been used since 1993 to distinguish *Mycobacterium tuberculosis* strains. This chapter ends with the three research questions posed in this thesis: 1) What has been the added value of DNA fingerprinting for TB control in a metropolitan area; 2) What are the causes of the high TB incidence as shown by DNA fingerprinting in a metropolitan area, and 3) What do our studies add to the knowledge of TB transmission?

Chapter 2 describes a transmission classification model to determine place and time of infection of all culture-positive and culture-negative TB cases in the Rotterdam metropolitan area. Cases were divided into those with a unique mycobacterial DNA fingerprint, a clustering fingerprint and with an unknown fingerprint. Classification trees for each category determined whether cases were infected in a foreign country, or recently (≤ 2 years) or remotely (> 2 years) infected in the Netherlands. During the 12-year study period, 38% of all TB cases were infected in a foreign country, 36% resulted from recent transmission in the Netherlands and 18% from remote infections in the Netherlands, while for the remaining cases (9%) either time or place of infection could not be determined. Conventional epidemiological data suggested that at least 29% of clustered cases were not part of recent chains of transmission. Our findings highlighted that genotyping should be combined with conventional epidemiological investigation to establish place and time of infection of TB cases as accurately as possible.

Chapter 3 presents an illustrative cluster demonstrating the use of genotyping in metropolitan TB control. Of 21 secondary cases related to the index case, 52% developed TB within one year, 19% within one to two years and 29% within two to five years. Cluster analysis underscored relevant issues for TB control in a metropolitan setting, such as the early recognition of an outbreak, the importance of reinfections, the consequences of delayed diagnosis, the contribution of pub-related trans-

mission and the value of DNA fingerprinting for decisions upon extension of contact investigations. Visualising cases in a cluster diagram was particularly useful in finding transmission locations and the similarities and links between patients.

Chapter 4 addresses the high TB case rate in the Rotterdam metropolitan area. Characteristics of patients and mycobacteria in three highly urbanised municipalities were compared with those of surrounding less-urbanised municipalities. The TB case rate in the highly urbanised municipalities was 3.8 times higher than in the surrounding municipalities. After stratification for country of birth, case rates were 1.7 times higher for immigrants and 2.8 times higher for nonimmigrants. Recent transmission caused 40% of the total urban TB case rate and was 5.5 times higher than the rate in surrounding municipalities. Illicit drug users and homeless persons accounted for 10.3% of all urban TB cases. We concluded that the high urban TB case rate was related to the high proportion of immigrants in these cities, as well as recent transmission among urban immigrants and nonimmigrants. Illicit drug users and homeless persons in urban municipalities were particularly at risk for TB due to recent infection.

Chapter 5 describes an extensive contact investigation among illicit drug users and homeless persons in Rotterdam. None of the six secondary TB cases in this investigation were molecularly linked to the index case. Genotyping revealed intense transmission by multiple sources. As a consequence, a mobile radiographic TB screening programme among illicit drug users and homeless persons was reintroduced. **Chapter 6** evaluates the impact of the screening programme using DNA fingerprinting. In four years, the annual number of TB cases with these risk profiles decreased from 24 to 11 cases and the proportion of disease due to prevalent *M. tuberculosis* strains fell from more than 80% to 45%. We concluded that radiographic screening reduced transmission among illicit drug users and homeless persons.

Chapter 7 describes the use of DNA fingerprinting to determine whether health care workers (HCWs) with TB were infected during their work in the Netherlands. In 67 (66%) out of 101 HCWs with TB, the place of infection could be determined. Among them, 42% was infected during work in the Netherlands, 28% had a community-acquired infection and 30% was infected abroad. Delayed diagnosis, especially in the elderly patient, was the main cause of patient-to-HCW transmission. Inadequate control measures contributed in a number of circumstances to TB transmission. We concluded that a high suspicion of TB by the clinician, adequate infection-control measures by hospital authorities and early identification of latent TB infection by occupational health specialists are essential to prevent TB among HCWs.

The general discussion in **Chapter 8** answers the research questions and describes the findings of the studies in this thesis. This chapter also describes the implications for future TB control in the Netherlands. Finally, the conclusions and recommendations that follow from the research in this thesis are formulated, and are described below.

Conclusions

- The added value of DNA fingerprinting for TB control in the Rotterdam metropolitan area has been its use for identifying and confirming outbreaks, and developing and evaluating interventions for high-risk groups.
- DNA fingerprinting also supported decisions for extending contact investigations in circumstances with confirmed transmission, and contributed to surveillance of tuberculosis in the metropolitan area.
- The high TB incidence in the Rotterdam metropolitan area was related to the high proportion of immigrants, and was caused by recent transmission. Illicit drug users and homeless persons were particularly at risk.
- DNA fingerprinting confirmed reduced TB transmission among illicit drug users and homeless persons after four years of screening.
- A standardised use of both molecular and conventional epidemiological information improves place and time determination of transmission and the identification of groups of persons at risk for tuberculosis.
- DNA fingerprinting enables the classification of cases as recently or remotely infected within a country, or infected in a foreign country. The number and distribution of these classified cases is relevant for programme performance and planning action.
- DNA fingerprinting assists to monitor an outbreak and estimate the number and timing of future cases, in particular in high-transmission settings such as metropolitan areas.

Recommendations

- Metropolitan TB control should include a package of targeted interventions of active case finding and case holding activities, focused on immigrants and hard-to-reach, marginalised urban risk groups, such as illicit drug users and homeless persons.
- Universal genotyping in the Netherlands should be continued to support TB outbreak investigation, surveillance and programme evaluation.
- Cluster investigation should be prioritised to cases with unknown epidemiological links and to possible recently infected cases.
- The tools and capacity to optimise the use of DNA fingerprinting for local and regional TB control in the Netherlands needs to be further developed and strengthened.
- Regional performance meetings should be conducted to share data and exchange experience to further enhance the use of DNA fingerprinting for TB control in the Netherlands.
- A European network should be established to facilitate the exchange of experience between metropolitan TB control programmes.

Samenvatting

Dit proefschrift richt zich op de vraag wat de rol van DNA fingerprinting is geweest voor de tuberculosebestrijding in een grootstedelijk gebied.

Hoofdstuk 1 bevat een overzicht van de ziekte tuberculose, DNA fingerprinting methodes en de moleculaire epidemiologie van tuberculose. Hoewel tuberculose nog steeds hoogendemisch is in veel ontwikkelingslanden, is de incidentie in Nederland in 2006 gedaald tot de laagste ooit geregistreerd met 6,2 gevallen per 100.000 inwoners. De stedelijke tuberculose-incidentie is echter vele malen hoger dan het landelijk gemiddelde, niet alleen in Nederland maar ook in andere laagendemische landen. DNA fingerprinting is zeer bruikbaar geweest om de transmissie van tuberculose beter te begrijpen. Moleculaire studies hebben tuberculose-uitbraken bevestigd, risicofactoren voor recente transmissie geïdentificeerd, het belang van re-infectie bij recidief tuberculose aangetoond en gewezen op het voorkomen van laboratorium kruiscontaminatie. Sinds 1993 wordt in Nederland DNA fingerprinting toegepast om *Mycobacterium tuberculosis* stammen te onderscheiden. Dit hoofdstuk eindigt met de drie onderzoeksvragen die onderwerp zijn van het proefschrift: 1) Wat is de toegevoegde waarde geweest van DNA fingerprinting voor de tuberculosebestrijding in een grootstedelijk gebied; 2) Wat laat DNA fingerprinting zien als de oorzaken van de hoge tuberculose-incidentie in een grootstedelijk gebied, en 3) Wat voegen onze onderzoeken toe aan de kennis van de transmissie van tuberculose.

Hoofdstuk 2 beschrijft een transmissie-classificatie model voor de bepaling van plaats en tijdstip van infectie van alle kweekpositieve en kweeknegatieve tuberculosepatiënten in het grootstedelijk gebied rondom Rotterdam. Patiënten werden onderverdeeld in diegenen met een unieke mycobacteriële DNA fingerprint, een clusterende fingerprint en met een onbekende fingerprint. Voor elke categorie bepaalde een beslisboom of patiënten in het buitenland geïnfecteerd waren of recent (≤2 jaar) of lang geleden (>2 jaar) in Nederland waren geïnfecteerd. Tijdens een studieperiode van 12 jaar was 38% van alle tuberculosepatiënten in het buitenland geïnfecteerd, 36% het gevolg van recente transmissie in Nederland en 18% lang geleden in Nederland geïnfecteerd, terwijl van de overige patiënten (9%) plaats of tijdstip van infectie niet bepaald kon worden. Traditionele epidemiologische gegevens suggereerde dat 29% van de clusterende patiënten niet recent geïnfecteerd was. Onze bevindingen benadrukken dat genotypering gecombineerd moet worden met conventioneel epidemiologisch onderzoek om plaats en tijdstip van infectie van tuberculosepatiënten zo nauwkeurig mogelijk te bepalen.

Hoofdstuk 3 beschrijft een cluster die het gebruik van genotypering in een grootstedelijk gebied illustreert. Van 21 secundaire tuberculosepatiënten gerelateerd aan een index patiënt, ontwikkelde 52% de ziekte binnen een jaar, 19% tussen een en twee jaar en 29% tussen twee en vijf

jaar. Clusteranalyse onderstreepte relevante aandachtspunten voor de tuberculosebestrijding in een grootstedelijk gebied, zoals het tijdig onderkennen van een uitbraak, het belang van re-infecties, de gevolgen van vertraagde diagnostiek, de bijdrage van transmissie in cafés en de waarde van DNA fingerprinting voor de besluitvorming om contact-onderzoeken uit te breiden. Visualisering met behulp van een cluster diagram was vooral nuttig voor het vinden van transmissielocaties en de overeenkomsten en verbanden tussen patiënten.

Hoofdstuk 4 bespreekt de hoge tuberculose-incidentie in de Rotterdamse regio. Kenmerken van tuberculosepatiënten en mycobacteriën in drie grootstedelijke gemeenten werden vergeleken met die van omliggende minder-verstedelijkte gemeenten. De tuberculose-incidentie in de grootstedelijke gemeenten was 3,8 keer hoger dan in de omliggende gemeenten. Na stratificatie voor geboorteland was de tuberculose-incidentie in de grootstedelijke gemeenten 1,7 keer hoger voor immigranten en 2,8 keer hoger voor niet-immigranten. Recente transmissie veroorzaakte 40% van de totale tuberculose-incidentie in de grootstedelijke gemeenten en was 5,5 keer hoger dan in de omliggende gemeenten. Harddruggebruikers en daklozen vertegenwoordigden 10,3% van alle grootstedelijke tuberculosepatiënten. Wij concludeerden dat de hoge tuberculose-incidentie in de grootstedelijke gemeenten gerelateerd was aan het hoge percentage immigranten in deze steden, alsmede aan recente transmissie zowel onder immigranten als niet-immigranten. In het bijzonder hadden harddruggebruikers en daklozen in de grootstedelijke gemeenten een verhoogd risico voor tuberculose als gevolg van recente transmissie.

Hoofdstuk 5 beschrijft een uitgebreid contactonderzoek onder harddruggebruikers en daklozen in Rotterdam. Geen van de zes secundaire gevallen in dit contactonderzoek had een moleculaire link met de bronpatiënt. Genotypering liet intense transmissie door meerdere bronnen zien. Deze bevinding was onder andere aanleiding om een mobiel röntgenologisch tuberculose-screeningsprogramma voor harddruggebruikers en daklozen opnieuw te introduceren. **Hoofdstuk 6** evalueert dit screeningsprogramma met behulp van DNA fingerprinting. In 4 jaar daalde het aantal tuberculosepatiënten met dit risicoprofiel van 24 tot 11 gevallen per jaar en verminderde het percentage patiënten met tuberculose veroorzaakt door een prevalente *M. tuberculosis* bacterie van 80% naar 45%. We concludeerden dat röntgenologische screening de transmissie onder harddruggebruikers en daklozen verminderde.

Hoofdstuk 7 beschrijft het gebruik van DNA fingerprinting om te bepalen of gezondheidswerkers met tuberculose tijdens hun werk in Nederland geïnfecteerd waren. Bij 67 van 101 (66%) gezondheidswerkers met tuberculose kon de plaats van infectie worden vastgesteld. Van deze was 42% geïnfecteerd tijdens werk in Nederland, 28% buiten het werk om geïnfecteerd en 30% in het buitenland. Vertraagde diagnostiek, vooral bij de oudere tuberculosepatiënt, was de belangrijkste oorzaak van transmissie van patiënt naar gezondheidswerker. Inadequate infectie-preventiemaatregelen droeg in een aantal omstandigheden bij aan de

tuberculose transmissie. Wij concludeerden dat een sterke verdenking op tuberculose door de clinicus, adequate infectiepreventiemaatregelen door ziekenhuisautoriteiten en vroege opsporing van latente tuberculose-infectie door ARBO-diensten nodig zijn om tuberculose bij gezondheidswerkers te voorkomen.

De algemene discussie in **Hoofdstuk 8** beantwoordt de onderzoeksvragen en beschrijft de bevindingen van de onderzoeken. Dit hoofdstuk beschrijft tevens mogelijkheden van gebruik van DNA fingerprinting voor de toekomstige tuberculosebestrijding in Nederland. Tot slot worden de conclusies en aanbevelingen voortkomend uit het onderzoek voor dit proefschrift geformuleerd, zoals hieronder nogmaals beschreven.

Conclusies

- DNA fingerprinting voor de tuberculosebestrijding in het grootstedelijk gebied van Rotterdam is van waarde geweest voor het herkennen en bevestigen van uitbraken en het ontwikkelen en evalueren van interventies voor risicogroepen.
- DNA fingerprinting ondersteunde de besluitvorming om contactonderzoeken uit te breiden doordat het transmissie bevestigde, en droeg verder bij aan de surveillance van tuberculose in het grootstedelijk gebied.
- De hoge tuberculose-incidentie in Rotterdam was gerelateerd aan het hoge percentage immigranten, en werd tevens veroorzaakt door recente transmissie. Harddruggebruikers en daklozen hadden in het bijzonder een verhoogd risico.
- DNA fingerprinting bevestigde een verminderde tuberculose transmissie onder harddruggebruikers en daklozen na vier jaar screening.
- Gestandaardiseerd gebruik van zowel moleculaire en conventionele epidemiologisch informatie helpt bij de vaststelling van plaats en tijdstip van transmissie en bij de bepaling van groepen van personen die risico lopen op tuberculose.
- DNA fingerprinting helpt bij de classificatie van patiënten die recent of lang geleden in Nederland of in het buitenland geïnfecteerd zijn. Het aantal en distributie van deze patiënten is van belang om bestrijdingsprogramma's te evalueren en beleid te bepalen.
- DNA fingerprinting helpt bij het monitoren van een uitbraak en kan bijdragen aan de voorspelling van aantal en tijdstip van toekomstige gevallen, in het bijzonder in settings met veel transmissie zoals in grootstedelijke gebieden.

Aanbevelingen

- De tuberculosebestrijding in grote steden dient een pakket van interventies te omvatten die bedoeld zijn om patiënten actief op te sporen en eenmaal vastgestelde patiënten adequaat uit te behandelen, en moet gericht zijn op immigranten en moeilijk-bereikbare,

gemarginaliseerde stedelijke risicogroepen, zoals harddruggebruikers en daklozen.
- Universele genotypering in Nederland dient voortgezet te worden ter ondersteuning van tuberculose-uitbraakonderzoek, surveillance en programma-evaluatie.
- Clusteronderzoek moet zich vooral richten op patiënten met nog onbekende epidemiologische verbanden en patiënten bij wie recente transmissie een mogelijkheid is.
- De instrumenten en capaciteit om DNA fingerprinting optimaal te gebruiken voor de lokale en regionale tuberculosebestrijding in Nederland moet verder ontwikkeld en versterkt worden.
- Regionale bijeenkomsten dienen georganiseerd te worden om data te vergelijken en ervaringen uit te wisselen waarbij de toepassing van DNA fingerprinting voor de tuberculosebestrijding in Nederland toeneemt.
- Een Europees netwerk moet worden opgericht om de uitwisseling van ervaringen van grootstedelijke tuberculosebestrijdingprogramma's te faciliteren.

Dankwoord

Mijn interesse voor tuberculose dateert al uit mijn studietijd. Hoewel ik me niet kan herinneren een tuberculosepatiënt tegengekomen te zijn tijdens mijn coschappen, zag ik bestrijding van deze ziekte in de Nederlandse setting wel als een logisch vervolg op een tropencarrière. In de tropen groeide mijn overtuiging dat sommige ziekten, vaak met fatale gevolgen, simpel te voorkomen zijn waardoor mijn aandacht naar de preventieve aspecten van de gezondheidszorg verschoof. Daarbij had ik ook geleerd dat organisatie van een ziekenhuis, district of een programma van cruciaal belang is voor goede zorg. Tijdens het werk in de Rotterdamse GGD kwam daar geleidelijk het belang van onderzoek bij. In juni 2004, tijdens een werkbezoek aan de tuberculosebestrijders in St. Petersburg spoorde collega Rob van Hest mij aan om te gaan promoveren. Tijdens een vakantie aan de Corsicaanse kust heb ik daarover zitten brainstormen en ben de uitdaging met mezelf aangegaan. Het resultaat heeft zijn weerslag in dit proefschrift. In dit nawoord wil ik graag een aantal mensen bedanken die een belangrijke rol hebben gespeeld in mijn loopbaan en in het bijzonder tijdens mijn promotie.

Dik Habbema, mijn promotor, bedank ik voor de mogelijkheid om onder zijn supervisie dit promotieonderzoek te doen. Dik, ik herinner me ons eerste contact toen je me kritisch vroeg of een promotieonderzoek wel zou passen naast een fulltime baan als tuberculosearts en hoofd van een afdeling. Nou, dat leek mij niet zo'n probleem, want ik had natuurlijk ook nog vrije tijd en barstte van de energie. Er lag bovendien genoeg om uit te zoeken en op te schrijven. Dik, ik wil je graag bedanken voor de plezierige gesprekken en je waardevolle commentaar, de aanvullingen op de onderzoeksvragen en de structurering van het proefschrift. In het bijzonder mijn dank voor de laatste maanden toen je me keer op keer verzekerde dat de deadline van juli 2008 gehaald zou worden. Je had gelijk.

Jan Hendrik Richardus, mijn copromotor, bedank ik voor de intensieve en zeer prettige begeleiding. Jan Hendrik, jij hebt me bij mijn nekvel gepakt toen ik met de gedachte rondliep om te promoveren. Je vertelde me dat er maar één instituut was waar ik dat het beste kon doen en dat was bij de afdeling Maatschappelijke Gezondheidszorg van het Erasmus MC. En jij werd mijn copromotor. Tijdens onze constructieve gesprekken legde jij de lat elke keer wat hoger wat soms tot gepuf van mijn kant leidde, maar uiteindelijk steeds weer een beter resultaat opleverde. Het is volgens mij waar het om een promotiebegeleiding om gaat. Ik wil je ook graag bedanken voor de aandacht die jij gaf aan allerlei zaken die niet direct met mijn promotieonderzoek te maken hadden. Soms waren de gesprekken van coachende aard, waarbij ik je heb leren kennen als een zeer integer persoon. Je hebt me ook geïnspireerd om mijn oude foto's van een lepra survey in Ghana weer eens uit de kast te

halen, waarbij onze gemeenschappelijke passie voor de bestrijding van mycobacteriële ziekten naar voren kwam.

Er zijn verschillende personen die mij op het tuberculose- en public health pad hebben gezet of daarop hebben gehouden. Ger Steenbergen, destijds verantwoordelijk voor het nationale tuberculose- en leprabestrijdingsprogramma in Zambia, inspireerde me tijdens de jaarlijkse 'witte ronde' op de tuberculoseafdeling van het missieziekenhuis waar ik toen werkzaam was. Dr. E.K. Sory, regionaal medisch directeur in Cape Coast, Ghana en Rien Gotink, medisch adviseur van UNICEF in Accra, Ghana waren van grote waarde voor mijn definitieve keuze voor de public health. Daniel Chandramohan, mijn mentor tijdens de MSc opleiding in Londen bestendigde deze keus voor de volksgezondheid. Toen ik eenmaal gekozen had voor een carrière in de Nederlandse tuberculosebestrijding was Sytze Keizer, tuberculosearts van de GGD Amsterdam mijn opleider. Sytze, ik heb je persoonlijke betrokkenheid bij mijn opleiding en loopbaan altijd heel erg gewaardeerd. Jaap Klein, longarts Medisch Spectrum Twente, Enschede was mijn opleider tijdens de klinische stage. Jaap, van jou mocht ik leren hoe je ook met patiënten en collega's communiceert.

I like to thank the TB patients in Zambia, Ghana and the Netherlands for accepting me as their doctor and getting the opportunity to learn so many clinical and social aspects of the disease. Furthermore, I thank the staff of the two mission hospitals for their support during the years I worked in Africa.

Tuberculosis physicians in the world form a pleasant international family. During my training, I had the opportunity to travel to Denver, USA and to be trained by Prof. Michael Iseman and his team. Mike, I still have the signed copy of your book, which is really the guide for tuberculosis doctors. I also had the opportunity to visit Departments of Tuberculosis Control in St. Petersburg, San Francisco, Shanghai and Cape town. It is great to share knowledge and experience and learn from each other. Masae Kawamura thanks for the opportunity to visit you during my holidays and for the genotyping guide which you took from your cupboard and gave it to me. Our discussions on urban tuberculosis control were very inspiring and one of your quotes is included in my thesis.

Dick van Soolingen en de medewerkers van het Nationale Mycobacteriën Referentie Laboratorium van het Rijksinstituut voor Volksgezondheid en Milieu bedank ik de jarenlange prettige samenwerking, voor het controleren en corrigeren van de moleculaire data van de Rotterdamse patiënten en de mogelijkheden om fingerprinting in de praktijk te zien. Martien Borgdorff en medewerkers van KNCV Tuberculosefonds bedank ik voor de samenwerking op verschillende terreinen. In het bijzonder is mijn dank voor de financiële ondersteuning van het onderzoek naar transmissiepatronen in onze regio. De GGD'en Hollands Midden, Zuidhollandse Eilanden en Zuid-Holland Zuid bedank ik voor de steeds intensievere samenwerking en het beschikbaar stellen van de data voor een aantal onderzoeken die in dit proefschrift beschreven zijn.

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de GGD Rotterdam-Rijnmond, heb ik afgelopen jaren een bijzondere band ontwikkeld. Richard, jij was altijd online als ik 's avonds aan mijn onderzoek werkte, hoewel het me vaak niet duidelijk was of je in de kliniek, thuis of in Spanje of elders in de wereld was. Ik ben je erg dankbaar voor je toewijding en betrokkenheid bij onze patiënten. Marleen, je komst naar Rotterdam was een versterking voor de Rotterdamse tuberculosebestrijding. Je consulentschap bij de GGD en de reizen naar Zuid-Afrika versterkten de samenwerking tussen public health en kliniek; je enthousiasme voor de ziekte stimuleerde me in brede zin. Conny, jij hebt vele jaren meegeholpen en me gesteund in het management van de afdeling. Jouw vragen hebben mij bovendien geprikkeld om meer aandacht te besteden aan andere zaken in het leven. Richard, Marleen en Conny, dank voor jullie steun en vriendschap.

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Curriculum vitae

Gerard de Vries is geboren op 1 juni 1959 te Grootegast (Gr.). Hij volgt zijn middelbare schoolopleiding aan het College Groevenbeek te Ermelo en behaalt in 1977 het Atheneumdiploma. In datzelfde jaar start hij met de studie Geneeskunde aan de Vrije Universiteit te Amsterdam en behaalt in 1986 het artsdiploma. Hij werkt tussen 1986 en 1988 als arts-assistent Chirurgie en Verloskunde & Gynaecologie in het ziekenhuis Zonnegloren te Soest, een voormalig tuberculosesanatorium. In 1988 volgt hij de Nationale Tropencursus voor Artsen aan het Koninklijk Instituut voor de Tropen in Amsterdam. Van 1989 tot 1992 werkt hij via Memisa als tropenarts en als medical superintendent in het Our Lady's Hospital Chilonga in Mpika, Zambia. In Nederland werkt hij vervolgens als basisarts in twee asielzoekerscentra en volgt de International Course on District Health Care aan het Koninklijk Instituut voor de Tropen in Amsterdam. Van 1993 tot 1996 gaat hij via Memisa naar Ghana om als tropenarts in het St. Francis Xavier Hospital in Assin Foso en als District Director of Health Services van het Assin District te werken. Van 1996 tot 1997 studeert hij aan de London School of Hygiene and Tropical Medicine in London, Engeland en behaalt de Master of Science graad in Public Health in Developing Countries. Hij werkt voor de Wereldgezondheidsorganisatie als consulent van het Polio Eradicatie Programma in Zambia en als cursuscoördinator van de Nederlandse Tropencursus aan de Netherlands School of Public Health in Utrecht, alvorens hij in september 1999 bij zijn huidige werkgever, de Gemeentelijke Gezondheidsdienst (GGD) Rotterdam-Rijnmond, in dienst treedt. Hij volgt de opleiding Sociale Geneeskunde (bijzondere tak tuberculosebestrijding) aan de Netherlands School of Public Health in Utrecht en verkrijgt in 2001 zijn registratie als sociaal-geneeskundige. Tussen 2001 en 2008 is hij hoofd van de afdeling tuberculosebestrijding van de GGD Rotterdam-Rijnmond en werkt tevens als tuberculosearts voor de GGD Hollands Midden in Gouda. In 2004 start hij zijn proefschrift in samenwerking met de afdeling Maatschappelijke Gezondheidszorg Erasmus MC van het Universitair Medisch Centrum in Rotterdam. Onlangs is hij benoemd als hoofd van de Unit Nationaal van het KNCV Tuberculosefonds te Den Haag en blijft parttime werkzaam bij de afdeling tuberculosebestrijding van de GGD Rotterdam-Rijnmond. Hij is geregistreerd als epidemioloog-A en als praktijkopleider voor artsen maatschappij en gezondheid, en heeft deelgenomen aan verschillende samenwerkingsactiviteiten met andere tuberculoseafdelingen in de wereld. Naast dit proefschrift publiceerde hij diverse artikelen over verschillende aspecten van tuberculose en tuberculosebestrijding in internationale en Nederlandse tijdschriften. Gerard is getrouwd met Florence van Wijngaarden en zij hebben twee zoons: Douwe en Koen.

Curriculum vitae

Gerard de Vries was born on June 1, 1959 in Grootegast (Gr.), the Netherlands. He completed his secondary school at College Groevenbeek in Ermelo in 1977. He started his medical studies in Amsterdam at the Free University and obtained his medical degree in 1986. His medical career started with a housemanship in Surgery and Obstetrics & Gynaecology at the Zonnegloren Hospital in Soest, a former tuberculosis sanatorium in the Netherlands. In 1988, he participated in the Dutch Course for Tropical Medicine for Doctors at the Royal Tropical Institute in Amsterdam. From 1989 to 1992 he worked via Memisa as medical officer and as medical superintendent at Our Lady's Hospital Chilonga in Mpika, Zambia. In the Netherlands, subsequently he worked as medical doctor in two asylum seeker centres and participated in the International Course on District Health Care at the Royal Tropical Institute in Amsterdam. From 1993 to 1996 he worked again via Memisa as medical officer at St. Francis Xavier Hospital in Assin Foso and as District Director of Health Services of the Assin District, Ghana. From 1996 to 1997 he studied at the London School of Hygiene and Tropical Medicine, London, United Kingdom and obtained a Master of Science degree in Public Health in Developing Countries. He worked for the World Health Organisation as a consultant for the Polio Eradication Programme in Zambia and as a course coordinator for the Netherlands Tropical Course at the Netherlands School of Public Health in Utrecht, the Netherlands, before he joined his present employer, the Municipal Public Health Service Rotterdam-Rijnmond, in September 1999. He started the training for public health physician (tuberculosis control) at the Netherlands School of Public Health in Utrecht and obtained his registration as public health physician in 2001. Between 2001 and 2008 he was head of the Department of Tuberculosis Control of the Municipal Public Health Service Rotterdam-Rijnmond and at the same time worked as tuberculosis public health physician at the Municipal Health Service Hollands Midden in Gouda. In 2004 he started his PhD in collaboration with the Department of Public Health of the Erasmus MC, University Medical Center Rotterdam. Recently, he was appointed as head of the National Unit of KNCV Tuberculosis Foundation in The Hague and will continue to work part-time with the Department of Tuberculosis Control of the Municipal Public Health Service in Rotterdam. He is registered as epidemiologist and as trainer for public health physicians, and has participated in various collaborative activities with other Departments of Tuberculosis Control in the world. Apart from this thesis, he has published several articles on different aspects of tuberculosis control in international and Dutch medical journals. Gerard is married with Florence van Wijngaarden and they have two sons: Douwe and Koen.

Publications

This thesis

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Non-indexed publications

15. Bakker M, Later-Nijland, HMJ, van Hest NAH, van Altena R, de Vries G. Gedwongen isolatie voor infectieuze tuberculose in het kader van de Infectieziektenwet. Tijdschr Infect (Geaccepteerd)
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18. Kidgell-Koppelaar D, Koster S, de Vries G. Poster: The yield of tuberculosis contact investigations in the metropolitan area of Rotterdam. 38th Union World Conference on Lung Health, 2007. Cape Town, South Africa.
19. de Vries G, Arend SM, Burdo CCA, Franken WP. 2007. Poster: Application of interferon-gamma assays in tuberculosis control in the Netherlands. Rethinking the Epidemiology of Tuberculosis Infection: The First Global Symposium on Interferon-gamma Assays, 2007. Vancouver, Canada.
20. de Vries G, Arend SM, Burdo CCA, Franken WPJ. Praktijkervaringen met interferon-gammatesten. Tegen de Tuberculose 2006;102(1):3-5
21. de Vries G, van Hest NAH. Een impressie van het IUATLD-congres in Moskou en de tuberculosebestrijding in Sint-Petersburg. Tegen de Tuberculose 2005;101(1):17-20.
22. de Vries G, van Hest NAG, Sebek MMGG. Poster: Active tuberculosis screening with mobile digital X-ray units among drug addicts and homeless people in Rotterdam. International Union against Tuberculosis and Lung Disease (IUATLD). 3rd Congress of European Region. IUATLD, 2004. Moscow, Russia.
23. de Vries G. Evaluatie tuberculosescreening van immigranten: ook een zaak van de GGD. Tegen de Tuberculose 2004;100(2):34-8.
24. de Vries G, van Hest NAH, Sebek MMGG. Tuberculose bij drugsverslaafden en dak- en thuislozen in Rotterdam. Tijdschr Soc Geneesk 2004;88:33.
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28. de Vries G. The clinical management and control of tuberculosis course: een impressie uit Denver. Tegen de Tuberculose 2002;98(3):79-81.
29. de Vries G. Tuberculose bij gezondheidswerkers in Nederland, 1995-1999. Beschrijving van de tuberculosegevallen bij gezondheidswerkers die tijdens hun werk besmet werden in de Nederlandse gezondheidszorg. Thesis. Netherlands School of Public Health, 2001.
30. de Vries G, van der Valk PDLPM, Bok H, Severin WPJ. Preventie van ziekenhuisinfectie met *Mycobacterium tuberculosis*: van richtlijn tot praktische invulling. Tegen de Tuberculose 2000;96(4):93-6.
31. de Vries G, van der Valk PDLPM, Bok H, Severin WPJ. Het ziekenhuis als infectiebron. Tuberculosepreventie in het ziekenhuis. Medisch Contact 2000;55(29/30):1062-5.
32. de Vries G, van Hest R, van Bergen J. Partnership in International Health. Bundel ter gelegenheid van het 90-jarig jubileum van de Nederlandse Vereniging voor Tropische Geneeskunde. Amsterdam: Het Spinhuis, 1998.
33. de Vries G. What's New? The Internet and Public Health in Developing Countries: a critical appraisal of applications. Thesis. London School of Hygiene & Tropical Medicine, University of London, 1997.
34. de Vries G, Voorhoeve HWA. Subcutaneous emphysema in measles. Ped Clinics Amsterdam 1997;8:3-4.
35. van den Dool M, de Vries G, Voorhoeve HWA. Kinderen met ingeslikte vreemde voorwerpen. Memisa Medisch 1993;5:117-20.
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Erasmus MC
Universitair Medisch Centrum Rotterdam

PhD Portfolio Summary

Summary of PhD training and teaching activities

Name PhD student: Gerard de Vries Erasmus MC Department: Dept of Public Health Research School:	PhD period: 2004-2008 Promotor(s): Prof.dr. J.D.F. Habbema Supervisor: Dr. J.H. Richardus

1. PhD training

	Year	Workload (Hours/ECTS)
General academic skills -		
Research skills -		
In-depth courses (e.g. Research school, Medical Training) -		
Presentations		
- De toepassing van DNA fingerprinting voor de lokale tuberculosebestrijding. Laboratorium voor Infectieziekten en Screening, Rijksinstituut voor Volksgezondheid en Milieu, Bilthoven	2005	20 hours
- Specific strategies in metropoles: the experience of Rotterdam. Colloque européen sur la tuberculose. La tuberculose dans les mégapoles européennes, Paris, France.	2005	20 hours
- Molecular epidemiology: a tool for public health. Using DNA fingerprinting for outbreak management in tuberculosis control. European Congress of Clinical Microbiology and Infectious Diseases. Nice, France.	2006	20 hours
- Using DNA fingerprinting for outbreak management in tuberculosis control, Wetenschappelijke congres van de Nederlandse Vereniging voor Medische Microbiologie, Papendal	2006	10 hours
- Tuberculosis: ancient disease, modern tools and targeted interventions. Experience from Rotterdam. Health Protection Agency national congress, Warwick, United Kingdom.	2006	20 hours
- Molecular epidemiology: a tool for public health. Experience from Rotterdam. The Vilnius Tuberculosis Meetings, EuroTB / Wolfheze Workshop, Vilnius, Lithuania.	2006	10 hours
- The epidemiology of tuberculosis in the Netherlands, Clinical Tuberculosis Course, Cape Town, South Africa.	2008	20 hours

PhD Portfolio Summary (continued)

International conferences		
- 3rd Congress of International Union against Tuberculosis and Lung Disease (IUATLD), European Region. Moscow, Russia (including poster presentation).	2004	40 hours
- Rethinking the Epidemiology of Tuberculosis Infection: The First Global Symposium on Interferon-gamma Assays. Vancouver, Canada (including poster presentation).	2007	40 hours
- Powering Up Political Will for TB Control. 11th Annual Conference of the International Union against Tuberculosis and Lung Disease (IUATLD), North American Region. Vancouver, Canada.	2007	12 hours
Seminars and workshops		
- Clinical Tuberculosis Course, Cape town, South Africa	2006	20 hours
Didactic skills		
-		
Other		
-		
2. Teaching activities		
	Year	**Workload (Hours/ECTS)**
Lecturing		
- Module 'Infection disease hygiene' for pulmonologists and TB physicians, Dutch Thoracic Society, Utrecht.	2005-2008	24 hours
- Module 'Tuberculosis Control' for medical students Erasmus MC (Social Medicine), Rotterdam	2005	6 hours
Supervising practicals and excursions		
- Registered trainer for public health medicine, Netherlands School of Public and Occupational Health, Amsterdam	2006-2008	100 hours
Supervising Master's theses		
-		
Other		
-		