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Transmission Classification Model To Determine Place and Time of Infection of Tuberculosis Cases in an Urban Area▽†

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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 program performance that can be used to decide on interventions and allocation of resources.

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 (16). 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 (5, 22).

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 (1, 14, 15). The proportion of recent transmission is also an important indicator for surveillance and TB control program performance (25, 27).

DNA fingerprinting of *Mycobacterium tuberculosis* isolates provides a tool to disentangle the different transmission pathways (12, 15, 26). 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 (4, 18, 30). Furthermore, fingerprinting studies alone ignore culture-negative 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 (17, 25, 27). A standardized way of classifying transmission will help to monitor and compare TB control programs.

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

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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 center 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. (34). *Mycobacterium 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 (33). Clusters are defined as groups of patients having isolates with fully identical RFLP patterns or, if mycobacterial strains harbor fewer than 5 IS*6110* copies, isolates with identical subtyping in assays using the polymorphic GC-rich sequence (PGRS) probe (35). 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 (11).

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 1 and the supplemental material) 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 behavioral patterns (such as homelessness, illicit drug use, and pub visiting) with other patients in the cluster (23, 27). 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.

TABLE 1. Selected demographic and disease-related factors of 2,636 TB cases in the Rotterdam region, 1995 to 2006

Patient characteristic	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

RESULTS

Table 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.

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). Travel history suggested in 32 of 219 (15%) cases in nonimmigrants with an

TABLE 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 TB 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					
Cases in nonimmigrants 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
Total (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, presented in boldface in this table, is explained in Fig. 1.

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). Figure 1 shows the classi-

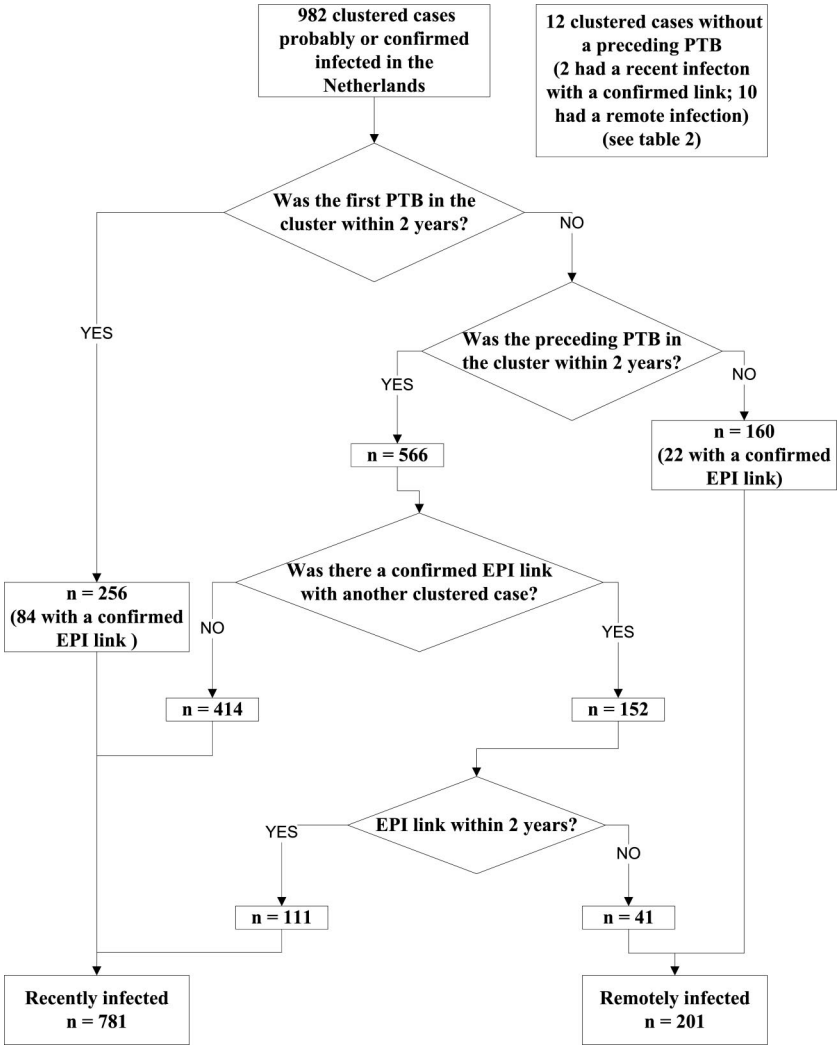

FIG. 1. Classification of time of infection for clustered cases from the Rotterdam area that were probably or confirmed infected in The Netherlands, 1995 to 2006 (n = 994). PTB, pulmonary tuberculosis; EPI link, epidemiological link.

fication of time of infection for clustered cases infected in The Netherlands, indicated in Table 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 behavioral characteristics. The other cases did not fulfill 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 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.

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

TABLE 3. Contribution 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 patients 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)

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 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.

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 (18, 29). 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 program (17, 18). 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 (1, 8, 9, 31), although some studies have limited the latency period to 1 year (6, 15, 21) or extended it to 5 years after infection (36). There is a need to decide on the latency period of recent disease development so that outcomes, such as program 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 (25). The half-life of RFLP genotypes is unclear but has been estimated to vary between 3 and 10 years in certain situations (10, 37). 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 programs 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 (24).

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 (4, 12, 20). 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 (19, 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 (2, 28).

In most developed countries, the diagnosis of TB is confirmed by culture in 80% of all cases at the most (7, 13). 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 con-

firmed 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 program performance that can be used to decide on interventions and allocate resources. Programs 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.

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