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Risk of *Mycobacterium tuberculosis* Transmission in a Low-Incidence Country Due to Immigration from High-Incidence Areas

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Does immigration from a high-prevalence area contribute to an increased risk of tuberculosis in a low-incidence country? The tuberculosis incidence in Somalia is among the highest ever registered. Due to civil war and starvation, nearly half of all Somalis have been forced from their homes, causing significant migration to low-incidence countries. In Denmark, two-thirds of all tuberculosis patients are immigrants, half from Somalia. To determine the magnitude of *Mycobacterium tuberculosis* transmission between Somalis and Danes, we analyzed DNA fingerprint patterns of isolates collected in Denmark from 1992 to 1999, comprising >97% of all culture-positive patients ($n = 3,320$). Of these, 763 were Somalian immigrants, 55.2% of whom shared identical DNA fingerprint patterns; 74.9% of these were most likely infected before their arrival in Denmark, 23.3% were most likely infected in Denmark by other Somalis, and 1.8% were most likely infected by Danes. In the same period, only 0.9% of all Danish tuberculosis patients were most likely infected by Somalis. The Somalian immigrants in Denmark could be distributed into 35 different clusters with possible active transmission, of which 18 were retrieved among Somalis in the Netherlands. This indicated the existence of some internationally predominant Somalian strains causing clustering less likely to represent recent transmission. In conclusion, *M. tuberculosis* transmission among Somalis in Denmark is limited, and transmission between Somalis and Danes is nearly nonexistent. The higher transmission rates between nationalities found in the Netherlands do not apply to the situation in Denmark and not necessarily elsewhere, since many different factors may influence the magnitude of active transmission.

Denmark is an industrialized Scandinavian country with 5.3 million inhabitants. In 1999, the incidence of tuberculosis (TB) was as low as 3.3 and 10.1 per 100,000 for Danish-born patients and all patients, respectively. Somalia is an East African country with an estimated 10.1 million inhabitants and a TB incidence of 1,168 per 100,000, among the highest known in the world (14, 33). When civil war intensified in the late 1980s, nearly half of all Somalis were forced from their homes, and by 1992, starvation threatened about one-fourth of the population; both factors contributed to large-scale immigration (11).

In Denmark as well as in other industrialized countries, the numbers of TB cases and deaths due to TB have declined uniformly during most of the 20th century (19, 20). Since the mid-1980s, an increasing number of cases of TB among foreign-born residents has resulted in a change from this expected downward trend (20, 22). For instance, in Denmark in 1986, only 299 cases were reported, the lowest number ever registered (18, 23). In 1999, however, the number was 529 (1), an increase of 77%. This increase was mainly due to immigration from high-prevalence countries (17, 23), especially Somalia (18, 28) (Fig. 1), though a minor but specific increase in the capital, Copenhagen, was observed among socially marginalized Danish males aged 35 to 55 due to recent infection (5).

In other Western countries, the reasons for the resurgence of TB during the last 2 decades are not as clear (32), but socioeconomic factors, such as poverty and poor living conditions with overcrowding (7), as well as inconsistent treatment policies with poorly managed, underfunded, and incorrectly conceptualized TB control programs are important (20, 32, 34). Risk groups include people infected with the human immunodeficiency virus and chronic alcohol and/or intravenous-drug abusers (2, 10, 20, 23). What still remains to be clarified is to what extent the increased immigration from countries with a high incidence of TB contributes to an increased risk of TB among the resident populations of low-incidence countries (8).

This study was undertaken to analyze the importance of *Mycobacterium tuberculosis* transmission among Somalis in Denmark and between Somalis and Danes. We wanted to determine if *M. tuberculosis* was transmitted significantly to the resident population and if part of the high incidence of TB among Somalis in Denmark (18) could be explained by active *M. tuberculosis* transmission occurring after their arrival in the country. If so, both factors could indicate a poor-quality TB control program. We also wanted to determine if the DNA fingerprint patterns from clustered Somalian immigrants in Denmark could be retrieved among Somalian immigrants in

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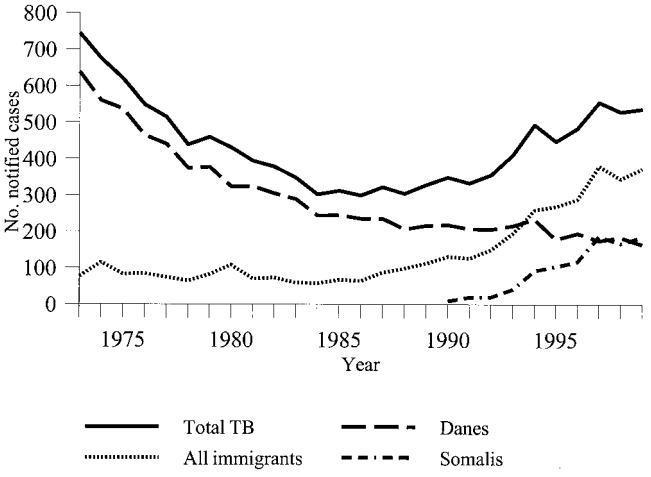

FIG. 1. Trends in tuberculosis cases in Denmark from 1973 through 1999.

The Netherlands, a finding that would indicate *M. tuberculosis* transmission at a stage prior to immigration or the existence of certain internationally predominant Somalian clusters.

MATERIALS AND METHODS

Study design. Retrospective cohort analysis of all *M. tuberculosis* culture-positive patients reported in Denmark from 1992 through 1999 combined conventional epidemiology and molecular subtyping (n = 3,320).

Data collection. In Denmark, reporting of TB has been mandatory since 1905, and since 1922, all microbiological analyses for mycobacteria have been carried out in the International Reference Laboratory for Mycobacteriology (IRLM) at Statens Serum Institut, Copenhagen (19). Information on the annual numbers, nationality, and date of entry into the country of reported immigrants with TB was obtained from the Statens Serum Institut Department of Epidemiology. Information concerning identification and bacteriology, such as identification codes, age, sex, site of disease, microscopy and culture results, and DNA fingerprint patterns, was collected at the IRLM. In Denmark, treatment of TB is centralized in respiratory, pediatric, and infectious-disease medical departments, which all provided clinical specimens from the TB patients. The study was approved by the local medical ethics committee (no. 11-087/99) and the Danish data protection agency (no. 1995-1200-371).

Specimen processing. The IRLM is the only laboratory performing TB diagnostics in Denmark, Greenland, and the Faroe Islands, processing 20,000 specimens on an annual basis (1999). In addition, the laboratory serves as an international reference laboratory for Iceland and Lithuania. Because all mycobacterial specimens from the whole country are processed in only one laboratory, and due to the mandatory and centralized TB reporting system, we believe that our data are nearly complete and highly representative for culture-positive TB. This is a factor of major importance in interpreting DNA fingerprint clustering (13). Denmark was the first country in the world to introduce nationwide DNA fingerprinting in 1992, and since then, isolates from more than 97% of the culture-positive patients have been analyzed by restriction fragment length polymorphism (RFLP), using the insertion element IS*6110* as a probe for strain differentiation. Since the discriminatory power of this probe is poor for strains harboring only one to five copies of the insertion element, and since these low-copy-number strains are more frequent among immigrants in Denmark, additional genotyping has been performed by the PCR-based method known as spoligotyping (4). Among all reported TB patients in 1992 through 1999, 82 to 91% have been *M. tuberculosis* culture positive every year. Altogether, 3,320 *M. tuberculosis* specimens were analyzed, representing one clinical specimen from each patient, excluding 79 patients with *Mycobacterium bovis* and *M. bovis* bacillus Calmette-Guérin (BCG) infections. Of the 3,320 isolates, 763 were from Somalian immigrants.

In general, as soon as growth was obtained, usually in the BACTEC culture system (Becton Dickinson and Company, Sparks, Maryland), and species identification by AccuProbe (Gene-Probe Inc., San Diego, Calif.) had revealed the presence of *M. tuberculosis* complex, the isolates were subcultured in Dubos medium containing Tween 80. After 3 to 4 weeks of growth in Dubos medium, the bacteria were harvested by centrifugation and heat killed (90°C for 30 min), and RFLP was performed by the standardized method (29). In brief, DNA is extracted and digested with the enzyme *Pvu*II. After electrophoresis on agarose gel, the digested DNA is transferred to nylon membranes (Hybond N+; Amersham, Little Chalfont, United Kingdom) and probed with a chemiluminescence-labeled 245-bp sequence of IS*6110*. For spoligotyping, DNA extraction was performed by mechanical disruption of the cells with glass beads by vortexing either a few colonies from solid media or 100 μl of the frozen culture stock (27). The membranes used (Isogen; Bioscience BV, Utrecht, The Netherlands) contained oligonucleotides derived from the spacer DNA sequences interspersed with the direct-repeat (DR) sequences in the DR region of the *M. tuberculosis* strain H37RV and *M. bovis* BCG. The presence or absence of these spacers in *M. tuberculosis* complex strains can be detected by hybridization following amplification of the spacer regions by primers complementary to the DR. PCR and hybridization were performed as previously described (16). The fingerprints obtained by RFLP and spoligotyping were scanned and analyzed by computer using GelCompar software version 4.0 (Applied Maths, Kortrijk, Belgium) as described previously (15).

Data analysis and definitions. The magnitude of *M. tuberculosis* transmission was analyzed by three different approaches, as follows.

(i) Calculation of theoretical expected transmission. The calculation of the theoretical expected *M. tuberculosis* transmission in Denmark was based on two "classical" epidemiological assertions: (i) the 5 to 10% lifelong risk of developing TB following primary infection (3, 12, 21, 24) and (ii) the assumption that one acid-fast bacillus (AFB) smear-positive patient on average infects 13 persons per year (25). This analysis was performed on a minor subgroup of all the reported Somalian TB cases in Denmark, including only the cases from 1997 (n = 187).

(ii) RFLP and spoligo cluster frequency analyses. In the RFLP and spoligo cluster frequency analyses, clusters were defined as groups of patients with *M. tuberculosis* isolates exhibiting 100% identical fingerprints, in contrast to patients with unique strains (13).

The following two steps were conducted: (i) an analysis of the cluster frequency every year in different age groups for all Somalian immigrants with *M. tuberculosis* culture-positive TB in 1992 through 1999, not going into any details of the probability of active transmission within each cluster (n = 763 [Table 1]); (ii) a detailed analysis of each clustered patient based on assumptions about the probability of transmission within each cluster for the subgroup of Somalian immigrants with *M. tuberculosis* culture-positive TB from 1996 through 1998 (n = 391 [Table 2]). The second group was chosen in order to allow a sufficient time for *M. tuberculosis* transmission to have occurred in Denmark before the patients of interest were diagnosed. As mentioned, nationwide DNA fingerprinting has been performed from 1992 on. Assuming that approximately 80% of all new TB cases develop within 2 years after the primary infection (26), the vast majority of active transmission in Denmark causing TB in the Somalian immigrants diagnosed from 1996 through 1998 would be discovered due to clustering fingerprints between the source from 1992 on and the case of interest. Furthermore, only very few Somalis arrived in Denmark before 1992 (Fig. 1), so that undiscovered transmission before the introduction of DNA fingerprinting would be extremely limited.

Having identified all Somalian TB patients who were part of a cluster, it was assumed that only patients with AFB smear-positive pulmonary TB could be the source of *M. tuberculosis* transmission in Denmark, although some transmission from other patients has been described (6, 30). Any cluster with possible active *M. tuberculosis* transmission in Denmark should thus include at least one patient with AFB smear-positive pulmonary TB. Therefore, any patients with unique DNA fingerprint patterns, or patients in a cluster without possible transmission in Denmark, were considered to have been infected outside the country. Patients in a cluster with possible transmission in Denmark were considered to have been infected by other Somalis if the cluster included only Somalian patients or, in the few cases of mixed Somalian-Danish clusters, if the vast majority of all patients in the same cluster were from Somalia (>75% was used as an arbitrary limit). Similarly, patients in a cluster with possible transmission in Denmark were considered to have been infected by Danes if the cluster included only Danish patients, except the Somalian patient analyzed, or if the vast majority (>75%) of all patients in the same cluster were from Denmark. Danish TB patients who were clustered with Somalian immigrants in a cluster with possible *M. tuberculosis* transmission in Denmark were considered to have been infected by the Somalian immigrants if the cluster included only Somalian patients, except for the Danish patient analyzed, or if the vast majority of all patients in the same cluster were from Somalia. Epidemiological linkage information from contact tracing was not used in the study, since it was not possible to obtain detailed

TABLE 1. All 763 cases of *M. tuberculosis*-positive TB[a] among Somalian immigrants in Denmark from 1992 through 1999 by sex, age group, and cluster frequency

Parameter	Value [no. (%)]										
	1992	1993	1994	1995	1996	1997	1998	1999	1992–1999		
									No. (%)	No. cluster[b] (%)	Total
Male	20 (95.2)	31 (70.5)	50 (65.8)	40 (42.6)	48 (50.5)	85 (55.9)	76 (52.8)	74 (54.0)	424 (55.6)	251 (32.9)	
Female	1 (4.8)	13 (29.5)	26 (34.2)	54 (57.4)	47 (49.5)	67 (44.1)	68 (47.2)	63 (45.7)	339 (44.4)	200 (26.2)	
≤15 yr old	0	10 (22.7)	13 (17.1)	10 (10.6)	13 (13.7)	20 (13.2)	13 (9.0)	20 (14.6)	99 (13.0)	63 (63.6)	
16–30 yr old	17 (81.0)	23 (52.3)	49 (64.5)	65 (69.1)	56 (58.9)	86 (56.6)	85 (59.0)	65 (47.4)	446 (58.5)	263 (59.0)	
31–45 yr old	4 (19.0)	7 (15.9)	10 (13.2)	14 (14.9)	21 (22.1)	38 (25.0)	42 (29.2)	47 (34.3)	183 (24.0)	105 (57.4)	
46–60 yr old	0	2 (4.5)	2 (2.6)	3 (3.2)	2 (2.1)	5 (3.3)	3 (2.1)	1 (0.7)	18 (2.4)	11 (61.1)	
61–75 yr old	0	2 (4.5)	2 (2.6)	2 (2.1)	2 (2.1)	3 (2.0)	1 (0.7)	4 (2.9)	16 (2.1)	9 (56.3)	
>75 yr old	0	0	0	0	1 (1.1)	0	0	0	1 (0.1)	0	
Total no.[c] (%)	21 (100.0)	44 (100.0)	76 (100.0)	94 (100.0)	95 (100.0)	152 (100.0)	144 (100.0)	137 (100.0)			763 (100.0)
No. cluster[b] (%)	7 (33.3)	29 (65.9)	50 (65.8)	44 (46.8)	55 (57.9)	95 (62.5)	90 (62.5)	81 (59.1)			451 (59.1)
No. LC cluster[b] (%)	2 (9.5)	12 (27.3)	17 (22.4)	17 (18.1)	24 (25.3)	29 (19.1)	30 (20.8)	29 (21.2)			160 (21.0)
No. LC unique spoligo[e] (%)	0	3 (25.0)	2 (11.8)	1 (5.9)	4 (16.7)	6 (20.7)	3 (10.0)	11 (37.9)			30 (3.9)

[a] More than 97% of all reported *M. tuberculosis* culture-positive Somalian patients in Denmark.
[b] Number of patients in any cluster.
[c] Total number of patients.
[d] Number of patients in a cluster with low-copy-number (LC) strains harboring only one to five copies of the insertion element IS*6110*.
[e] Number of patients in a cluster with low-copy-number strains with unique spoligo patterns.

information from all 391 Somalian immigrants with *M. tuberculosis* culture-positive TB from 1996 through 1998.

(iii) Comparison of 35 Somalian clusters with possible transmission in Denmark to the Dutch TB database. Finally, the IS*6110* RFLP patterns from all clusters with possible *M. tuberculosis* transmission among Somalian immigrants in Denmark were compared to the Dutch IS*6110* RFLP pattern database held in the *Mycobaterium* Reference Laboratory at the National Institute of Public Health and the Environment, Bilthoven, The Netherlands. This comparison was done to determine if these Somalian clusters were internationally frequent and therefore that this clustering would be less likely to represent recent transmission in Denmark (13). The Dutch database includes 9,387 DNA fingerprint patterns collected from all culture-positive TB patients in the Netherlands since 1993.

RESULTS

Cohort description. From 1992 through 1999, 3,814 patients with TB were reported in Denmark, including the bacteriologically unconfirmed cases. A total of 59.3% (2,260 of 3,814) of all patients were immigrants; 40.4% (913 of 2,260) were from Somalia. Males accounted for 64.4 and 55.1% among the Danes and immigrants, respectively. Every year, 82 to 91% of all patients were *M. tuberculosis* culture positive; three-fourths of these had pulmonary TB, and two-thirds of the pulmonary cases were AFB smear positive. Immigrants had a higher frequency of extrapulmonary TB than Danes through the whole period.

Theoretical transmission calculations. In 1997, 12,000 Somalian immigrants were living in Denmark; 187 of these developed TB, including 33 Somalis with TB not verified by culture. Two Somalian patients were excluded from this analysis due to a lack of clinical information. Of the remainder, 15.1% (28 of 185) were AFB smear positive and thus able to transmit *M. tuberculosis*, given our assumptions (see Materials and Methods). We also assumed that one AFB smear-positive patient on average infects 13 persons per year and that each person has a 5 to 10% lifelong risk of developing TB following the primary infection. If we further assume that *M. tuberculosis* transmission in Denmark occurs only among Somalian immigrants and that all infections are primary infections, we calculate the lifetime theoretical number of new TB cases caused by the Somalian immigrants with TB in 1997 as follows: 185 × 0.15 × 13 × (0.05 or 0.10) = 18 to 36 TB cases (i.e., number of TB patients reported in 1997 × fraction smear positive × number of persons infected by each AFB smear-positive patient × lifelong risk of developing TB following primary infection).

RFLP and spoligo cluster frequency analyses. Table 1 includes data from all 763 Somalis with *M. tuberculosis* culture-positive TB in Denmark from 1992 through 1999 analyzed by sex, age group, and cluster frequency. In total, 59.1% (451 of 763) of all patients were clustered, 21.0% (160 of 763) in low-copy-number clusters, and 3.9% (30 of 763) of the patients with clustered low-copy-number strains were harboring unique spoligo patterns, resulting in a total cluster frequency of 55.2% (59.1% − 3.9%). The cluster frequencies for males and females were equal at 59.2% (251 of 424) and 59.0% (200 of 339), respectively. In the first study year (1992), only one-third

TABLE 2. All 391 cases of *M. tuberculosis*-positive TB[a] among Somalian immigrants in Denmark from 1996 through 1998 by age group and cluster frequency using refined cluster analysis based on assumptions[b] about transmission

Age (yr)	No. cluster/total[c] (%)			
	1996	1997	1998	1996–1998
≤15	1/13 (7.7)	5/20 (25.0)	5/13 (38.5)	11/46 (23.9)
16–30	13/56 (23.2)	22/86 (25.6)	25/85 (29.4)	60/227 (26.4)
31–45	6/21 (28.6)	8/38 (21.1)	11/42 (26.2)	25/101 (24.8)
46–60	0	0/5 (0.0)	1/3 (33.3)	1/3 (33.3)
61–75	0	1/3 (33.3)	0/1 (0.0)	1/3 (33.3)
>75	0	0	0	0
No. cluster/total[c] (%)	20/95 (21.1)	36/152 (23.7)	42/144 (29.2)	98/391 (25.1)

[a] More than 97% of all reported *M. tuberculosis* culture-positive Somalian patients in Denmark.
[b] Assumptions are specified Materials and Methods; in brief, the cluster should include one or more AFB smear-positive source patients.
[c] Number of patients in a cluster divided by total number.

TABLE 3. Analysis of *M. tuberculosis* transmission among and between 391 Somalian immigrants with tuberculosis from 1996 through 1998[a] and the resident population

Most likely route of transmission:	No.[c](%)
Somalian immigrants infected outside DK[b]	293/391 (74.9)
Somalian immigrants infected in DK by other Somalis	91/391 (23.3)
Somalian immigrants infected in DK by Danes	7/391 (1.8)
Danes infected in DK by Somalian immigrants	4/470[d] (0.9)

[a] More than 97% of all reported *M. tuberculosis* culture-positive Somali patients.
[b] DK, Denmark.
[c] Number infected/total.
[d] *M. tuberculosis* culture-positive Danish patients from 1996 through 1998.

of the Somalian immigrants were clustered compared to one-half to two-thirds from 1993 through 1999.

A subgroup of 391 Somalis with *M. tuberculosis* culture-positive TB in the 3 years 1996 through 1998 was analyzed in order to obtain detailed information on *M. tuberculosis* transmission in Denmark (Tables 2 and 3). As seen in Table 2, 25.1% (98 of 391) of these patients belonged to clusters containing one or more AFB smear-positive "source" patients, increasing from 21.1% (20 of 95) in 1996 to 29.2% (42 of 144) in 1998, though the cluster frequency remained largely constant with increasing age. The clustered patients were grouped in 35 different clusters, and Fig. 2 shows the RFLP patterns, Danish cluster names, and numbers of patients included for the 16 most frequent of these clusters. These 16 RFLP patterns represent 89.8% (88 of 98) of the clustered patients. The figure also includes the five most frequent spoligo patterns for the patients in the low-copy-number clusters, representing 89.7% (26 of 29) of the clustered patients harboring low-copy-number strains. Out of the 35 different clusters, only 9 minor ones were "mixed," including both Somalian and Danish TB patients. In these nine clusters, the vast majority of all patients (>75%) had the same nationality, and actually, five out of the nine clusters included only one patient from the other nationality. The possible routes of *M. tuberculosis* transmission among and between the Somalian immigrants and Danes were analyzed. As indicated in Table 3, 74.9% (293 of 391) of all Somalian immigrants appeared to have been infected before their arrival in Denmark, either because they were harboring unique DNA fingerprints or because they belonged to clusters containing no

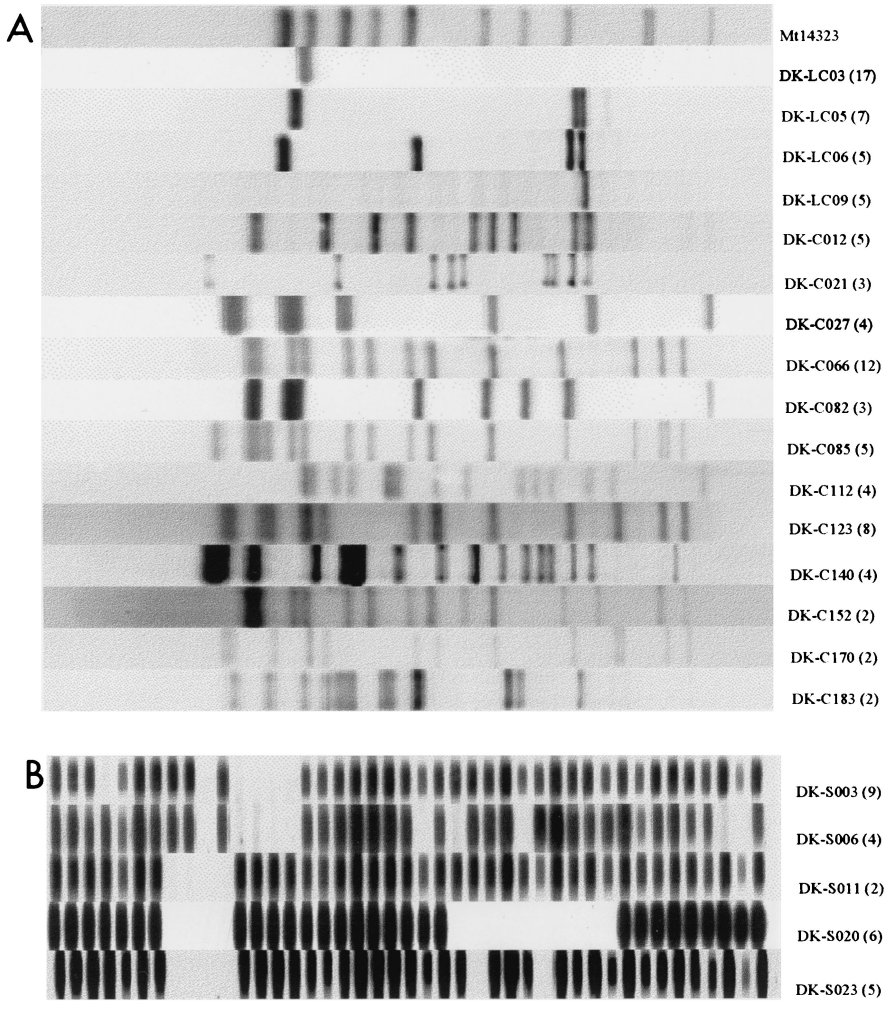

FIG. 2. RFLP (A) and Spoligo (B) patterns for frequent Somalian *M. tuberculosis* clusters with possible transmission in Denmark. Mt14323, international reference strain; DK, Denmark; C, cluster; LC, low-copy-number cluster. The figures in parentheses are the numbers of patients.

AFB smear-positive source patients. Furthermore, 23.3% were most likely infected by other Somalian immigrants, because they belonged to clusters where all, or the vast majority (>75%), of patients were Somalian immigrants. The last 1.8% were most likely infected by Danes, because they belonged to clusters where all, or the vast majority (>75%), of patients were Danes. In the same period, 0.9% of all Danish TB patients belonged to clusters where all, or the vast majority (>75%), of patients were Somalian immigrants.

Comparison to *Mycobacterium tuberculosis* isolates in the Dutch database. DNA fingerprint patterns from 18 out of the 35 Somalian clusters with possible *M. tuberculosis* transmission in Denmark were also found in the Dutch DNA fingerprint database, representing 60% (59 of 98) of the clustered Somalian TB patients in Denmark. Altogether, 578 Dutch matches were found distributed in 17 clusters and one unique strain. The 17 clusters included mainly Somalian patients, of which one cluster is the previously reported fourth-largest cluster in The Netherlands (31). Seven of the clusters included ≥10 patients. It was not possible to combine epidemiological linkage information between the clustered Somalian immigrants in Denmark and the Netherlands due to registry laws in the two countries and a lack of detailed information from all 578 Dutch and 59 Danish Somalian immigrants.

DISCUSSION

Since 1986, the number of TB patients reported in Denmark has increased by 91%, and while the incidence has remained fairly stable among Danes, the increase can be fully explained by immigration from countries with a high prevalence of TB, lately especially from Somalia (Fig. 1) (18, 28). The Somalis in Denmark have an incidence of 2,000 per 100,000, which is among the highest ever reported in the world (14, 18), and tuberculin skin tests among 300 Somalis have indicated that 90% of all adults (≥16 years old) and 25% of all children were infected at arrival (authors' unpublished data). Based on these high figures, we decided to investigate the magnitude of *M. tuberculosis* transmission among and between the Somalian immigrants, at high risk for TB, and the low-risk resident Danish population.

Our theoretical transmission calculations suggest that 18 to 36 new TB cases were caused by the 185 Somalian immigrants reported to have TB in Denmark in 1997. This would correspond to 10 to 19% (18 to 36 of 185) of all TB cases among the Somalian immigrants in 1997, although all new cases would not necessarily develop during the first year but could develop over a lifetime. In an earlier study from The Netherlands (26), approximately 80% of all new TB cases developed within 2 years after the primary infection. Our theoretical transmission calculation is influenced by many different factors tending to both increase and lower the number. The result should therefore be interpreted with care. We are aware that AFB smear-positive TB patients are the main but not the only source of *M. tuberculosis* transmission (6, 25, 30), and the average number of 13 persons infected annually by one AFB smear-positive patient (25) is influenced by social factors, such as different living conditions (e.g., crowding), and by differences in patients' and doctors' delays. Furthermore, the estimated 5 to 10% risk of developing TB is based on studies from Scandinavia (12, 21) and Canada (3), which may not be representative of Somalian immigrants in Denmark.

In the cluster frequency analyses of all culture-positive TB patients in Denmark from 1992 through 1999, 55.2% (421 of 763) of all Somalian immigrants had isolates that shared RFLP patterns, including 21.0% (160 of 763) with low-copy-number bands but excluding the 3.9% (30 of 763) of these with unique spoligo patterns (Table 1). It has previously been reported that the cluster frequency would be expected to decline with increasing age due to a higher frequency of cases caused by endogenous reactivation (5, 31, 37). However, among the Somalis, the cluster frequency tended to decline only slightly (Table 1), a finding previously described among non-Dutch compared to Dutch TB patients in The Netherlands (31). This may indicate that most DNA-fingerprinting studies have been performed in low-incidence settings and that the risk of recent *M. tuberculosis* transmission in relation to age may not be similarly pronounced in populations with a high incidence of TB. During the 8-year study period, the total frequency of clustered patients did not increase, as would be expected as the so-called "time window" increases (13) (Table 1). This observation supports the assumption that most *M. tuberculosis* transmission occurred among Somalian immigrants before their arrival in Denmark. Otherwise, we would expect the frequency of clustered cases to increase steadily due to more and more cases caused by recent transmission among the growing Somali population in Denmark during the 1990s.

It is generally assumed that the proportion of clustered patients in a population reflects the extent of recent *M. tuberculosis* transmission, but the inference of the proportion of disease due to recent transmission from a crude proportion of clustered patients is complicated and should be made with care (5, 8, 35; J. van Embden and D. van Soolingen, Editorial, Int. J. Tuberc. Lung Dis. **4:**285–286, (2000). Apparent clustering may be observed between individuals who have no recent direct link or common contacts but for whom there was a link at some time in the past and in whom reactivation of old infections happens to have occurred at about the same time (9, 13). This is especially true if a few strains predominate over a long period, like, for instance, the Danish clusters 1 and 2 (5). Until now, no predominant Somalian *M. tuberculosis* strain has been reported. However, in this study, nearly 90% of all the clustered Somalian immigrants could be included in only 16 different clusters, of which the 4 most frequent accounted for 45% of all clustered patients in Denmark (Fig. 2). Furthermore, 17 out of the 35 identified clusters with possible active transmission in Denmark were also identified as clusters in The Netherlands. These 17 clusters accounted for 60% of all the clustered patients. Based on these findings, we propose the existence of several predominant Somalian strains, making some clustering by chance likely, and we have reason to believe that the high numbers of clustered Somalis in Denmark, both the overall numbers (Table 1) and the detailed numbers (Table 2), are an overestimation not reflecting the actual extent of recent transmission in Denmark. This is also likely to be true because some of the Somalis clustered in Denmark could very well have acquired the infection in Somalia. We also recognize that some of the unique strains in the Somalian immigrants in Denmark may have arisen from recent transmission outside the country, for instance, in refugee camps, but this would not

influence the magnitude of recent transmission in Denmark. Furthermore, some of the unique strains could be clustered with some unknown source cases in Denmark, and such an event would tend to increase the "true" extent of recent transmission, but due to our nationwide fingerprinting, the centralized reporting and treatment system, and the significant number of Somalian immigrants in Denmark, we believe this is only true in a minority of cases.

Among the 391 Somalis with *M. tuberculosis* culture-positive TB from 1996 through 1998, 74.9% appeared to have been infected outside Denmark, 23.3% could have been infected in Denmark by other Somalis, and 1.8% could have been infected by Danes. In the same period, 0.9% of all Danish TB patients appeared to be infected by Somalis (Table 3). In conclusion, *M. tuberculosis* transmission among Somalis in Denmark is limited, and transmission between Somalis and Danes is nearly nonexistent. The majority of all Somalian immigrants were infected before their arrival in Denmark. In a recent Dutch study (8), 17% of the TB cases among the resident Dutch population were attributable to active transmission from non-Dutch source cases. In our study, 0.9% of all Danish TB patients appeared to have been infected by Somalian immigrants, although in total, 38.6% of all the immigrants in Denmark were from Somalia. We have not been able to explain the disparity between the Danish and the Dutch figures by differences in the methods used, and we anticipate that the disparity most likely is explained by major differences in the social integration of Somalian immigrants in the two countries, but it may also reflect social differences between the two groups of immigrants. In Denmark, the Somalian immigrants tend to live with only limited social contact with the Danish population. With time and integration, more *M. tuberculosis* transmission is likely to occur, as has been observed for Greenlanders and Danes (5, 36).

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