Mycobacterium tuberculosis Transmission among Elderly Persons, Yamagata Prefecture, Japan, 2009–2015

Junji Seto, Takayuki Wada, Yu Suzuki, Tatsuya Ikeda, Katsumi Mizuta, Taro Yamamoto, Tadayuki Ahiko

In many countries with low to moderate tuberculosis (TB) incidence, cases have shifted to elderly persons. It is unclear, however, whether these cases are associated with recent *Mycobacterium tuberculosis* transmission or represent reactivation of past disease. During 2009–2015, we performed a population-based TB investigation in Yamagata Prefecture, Japan, using in-depth contact tracing and 24-loci variable-number tandem-repeat typing optimized for Beijing family *M. tuberculosis* strains. We analyzed 494 strains, of which 387 (78.3%) were derived from elderly patients. Recent transmission with an epidemiologic link was confirmed in 22 clusters (70 cases). In 17 (77.3%) clusters, the source patient was elderly; 11 (64.7%) of the 17 clusters occurred in a hospital or nursing home. In this setting, the increase in TB cases was associated with *M. tuberculosis* transmissions from elderly persons. Prevention of transmission in places where elderly persons gather will be an effective strategy for decreasing TB incidence among predominantly elderly populations.

The World Health Organization End TB strategy (*1*) calls for every country, depending on their tuberculosis (TB) situation, to accelerate efforts designed to end TB. In Japan, 14.4 TB cases/100,000 population were reported in 2015. A small percentage of those cases occurred in foreign-born (6.4%) and HIV-positive (<0.1%) persons, who thus are not currently considered to pose a transmission threat; however, 71.8% of the reported cases were in elderly persons (≥60 years of age) (*1*,*2*). Given that the incidence of TB was high in Japan until the 1970s (*3*), the current elderly population is regarded as vulnerable to TB onset from reactivation of remotely acquired latent infection (*4*,*5*). However, TB transmission among elderly populations has not been determined worldwide (*6*).

Author affiliations: Yamagata Prefectural Institute of Public Health, Yamagata, Japan (J. Seto, Y. Suzuki, T. Ikeda, K. Mizuta, T. Ahiko); Nagasaki University, Nagasaki, Japan (T. Wada, T. Yamamoto)

DOI: http://dx.doi.org/10.3201/eid2303.161571

Molecular epidemiology can help identify recent TB transmission and, thereby, contribute to the creation of specific intervention programs for advanced TB control. Variable-number tandem-repeat (VNTR) typing is a useful method for rapidly detecting TB infections caused by the same strain of *Mycobacterium tuberculosis* (*7*); such cases might occur from recent transmission. A combination of VNTR typing and in-depth contact tracing has detected previously unrecognized recent TB transmission in various settings (*8*–*11*). However, data are scarce from areas such as Japan that might include large numbers of patients with reactivated TB (i.e., reactivation of past latent TB infection because of a weakened immune system).

The Beijing family, within lineage 2 (East Asian) of *M. tuberculosis*, has shown cumulative microevolution while spreading globally from its origin in eastern Asia (*12*–*16*). Worldwide, the modern Beijing subfamily predominates (*16*), but in Japan, the ancient Beijing subfamily, which is subdivided into 4 sublineages (ST11/26, STK, ST3, and ST25/19), accounts for >75% of Beijing family strains (*17*–*19*). Thus, appropriate VNTR subsets were proposed to discriminate Beijing *M. tuberculosis* lineages (*20*,*21*); a 24-loci subset, named 24_{Beijing}, is used to achieve high discriminatory power for *M. tuberculosis* Beijing clinical isolates in Japan (*19*,*21*). The subset comprises 15-loci mycobacterial interspersed repetitive units (MIRUs) (*7*) and 9 additional loci, including 3 hypervariable loci (*21*).

We performed a population-based TB investigation in Yamagata Prefecture, Japan, combining 24_{Beijing}-VNTR typing and in-depth contact tracing. We aimed to clarify the overall picture of recent *M. tuberculosis* transmission and thereby contribute to preventing the spread of TB in Yamagata Prefecture, where the annual TB incidence during the past decade was 7–13 cases/100,000 persons (*3*) and where 80.5% of reported patients were ≥60 years of age in 2015 (*2*). The investigation was designed to provide insight about TB among predominantly elderly populations as an aid to countries experiencing an increase in TB cases among the elderly (*1*,*22*,*23*). This work was approved by

the Ethics Committees of Yamagata Prefectural Institute of Public Health (approval no. YPIPHEC H24–04 and YPIPHEC 16–08) and of the Institute of Tropical Medicine, Nagasaki University (approval no. 130606112).

Materials and Methods

Study Setting

Yamagata Prefecture, located in the northern part of Japan's main island of Honshu, is subdivided into 4 topographically separated areas, each of which has a public health center (online Technical Appendix 1 Figure 1, https://wwwnc.cdc.gov/EID/article/23/3/16-1571-Techapp1.pdf). In 2014, Yamagata Prefecture had 1.1 million inhabitants, of whom 38.2% were elderly (≥60 years of age) and 0.5% were noncitizen residents. Statistical data used in the study was provided by the Statistics Planning Division, Yamagata Prefecture.

Study Population

The study included most patients in Yamagata Prefecture with culture-confirmed TB reported during January 1, 2009–December 31, 2015. On the basis of Japan's Law Regarding Infectious Disease Prevention and Medical Care for the Patients, which was implemented on April 1, 1999, public health centers collect *M. tuberculosis* strains isolated from patients in order to conduct molecular investigations. During 2009–2015, public health centers routinely delivered collected strains to the Yamagata Prefectural Institute of Public Health within 2 months after notification of TB cases. These collected strains corresponded in part to those described in our earlier works, which mainly analyzed genetic features of *M. tuberculosis* (e.g., phylogenetic classification and the genome sequence) (*19*,*24*,*25*).

Genotyping

We usually finished $24_{Beijing}$-VNTR typing within 3 days after arrival of the strains. We confirmed the amplified PCR fragment sizes by using a microchip electrophoresis system (MCE–202; Shimadzu Corp., Kyoto, Japan) and agarose gel electrophoresis. We calculated the number of repeats for each locus from the sizes of PCR products, in agreement with published allelic tables (*26*). For this study, we defined a preliminary TB cluster when the $24_{Beijing}$-VNTR profile of a strain was a single-locus variant (SLV) or was indistinguishable from that of other strains.

We estimated *M. tuberculosis* lineages of the clinical isolates by using maximum a posteriori estimation with the $24_{Beijing}$-VNTR profile, as described previously (*19*). We divided the strains into 6 lineages: the group of non-Beijing *M. tuberculosis* lineages, 4 sublineages (ST11/26, STK, ST3, and ST25/19) of ancient Beijing subfamily, and the modern Beijing subfamily.

Data Collection

Public health centers routinely collect demographic (age, sex, country of birth, and address), clinical (site of disease, acid-fast bacilli sputum smear status, and treatment history), epidemiologic (family members, occupation, and contacts during onset), and microbiologic (culture and drug-susceptibility status of *M. tuberculosis* strain) characteristics for all reported TB patients. Public health centers use these data to determine whether interferon-γ release assays and chest radiography should be used to determine whether contacts of patients have latent TB infection. When a preliminary TB cluster is confirmed, public health nurses investigate the behavior history of patients within clusters to determine recent *M. tuberculosis* transmission. If patients in a cluster live in dispersed areas, the investigations are performed in cooperation with the responsible public health centers. In addition, after November 2013, public health centers asked all patients with culture-confirmed TB to complete a long or short version of a self-administered questionnaire that specifically elicits responses associated with residence, travel history, transportation, and places of social aggregation (online Technical Appendix 2, https://wwwnc.cdc.gov/EID/article/23/3/16-1571-Techapp2.pdf). Public health centers decide which version of the questionnaire to use, depending on patient willingness and ability to fill out the form. For example, in this study, the short version was used for elderly TB patients (especially those ≥80 years of age) who exhibited forgetfulness, tremulousness of hands, or an unwillingness to complete the questionnaire. Using social interaction data gathered from the case investigations and questionnaires, public health centers graded patients within clusters as epidemiologically linked (i.e., patients had shared space at the same time); possibly linked (i.e., patients had shared space but not at the same time); or not linked (i.e., no shared space was found for patients), according to the classification method of Walker et al. (*27*).

Cluster Analysis

We defined cases as a cluster when their $24_{Beijing}$-VNTR profiles were indistinguishable from each other or when a social interaction was graded as epidemiologically linked or possibly linked in the SLV group. We excluded nonlinked cases in the SLV group from the cluster, based on the assertion by Allix-Béguec et al. that "even for hypervariable loci, at least in the absence of further specific epidemiological or contact tracing evidence, the definition of molecular clustering should remain restricted to full identity of the markers" (*28*). By comparing 24-loci MIRU-VNTR profiles proposed by Supply et al. (*7*) and results of whole-genome sequencing, Walker et al. (*27*) showed that *M. tuberculosis* strains in the SLV group contained many more single-nucleotide polymorphisms than those in the indistinguishable group.

We calculated the proportion of clustered cases resulting from recent *M. tuberculosis* transmission by using the $n - 1$ method, defined as $(N_c - n_c)/N_o$, where N_c stands for the total number of clustered cases, n_c signifies the number of clusters (i.e., equal to the number of source cases), and N_o denotes the total number of cases in this study (*9*,*29*). In addition, we calculated the percentage of cases resulting from epidemiologic links, including only cases and clusters with confirmed links in the calculation [(no. of clustered cases with epidemiologic links − no. of clusters with epidemiologic links)/N_o] (*9*). We visualized cluster diagrams displaying epidemiologic links by using Cytoscape 3.3.0, an open-source bioinformatics software platform (*30*).

Statistical Analysis

We calculated odds ratios and 95% CIs by using logistic regression analysis. To determine the association between cluster formation and epidemiologic features (especially age groups), we applied multivariate logistic regression analysis using, for example, age group, sex, and *M. tuberculosis* lineage as explanatory variables. We used backward, stepwise variable selection to select the multivariate model with a probability entry of <0.2. Residual analysis was used when logistic regression analysis was not applicable. We considered $p<0.05$ as statistically significant. All statistical analyses were conducted using R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

Results

During 2009–2015, a total of 854 TB cases were reported in Yamagata Prefecture; 676 of the cases were diagnosed as pulmonary TB, of which 513 were culture-confirmed. We studied 494 (57.8%) of the 854 cases (469 [91.4%] of the 513 pulmonary cases and 25 extrapulmonary cases). We collected *M. tuberculosis* strains from the patients and determined the $24_{Beijing}$-VNTR profiles. Patients had a mean (±SD) age of 72.3 ± 19.9 (range 18–100) years. Most patients were ≥60 years of age (387 patients, 78.3%), Japanese (478 patients, 96.8%), and undergoing initial TB treatment (466 patients, 94.3%), and most had pulmonary symptoms (469 patients, 94.9%) and non–multidrug-resistant TB (491 patients, 99.4%). No patients were HIV-positive, illicit drug users, or homeless.

Results of $24_{Beijing}$-VNTR typing showed that 173 strains formed 52 preliminary clusters, which were aggregations of indistinguishable and SLV profiles (online Technical Appendix 1 Table 1). Of note, the proportion of nonlinked cases in the SLV group was remarkably high (45/57, 78.9%) when we separated cases belonging to the preliminary cluster into indistinguishable and SLV groups (Table 1). Considering these findings and the assertion of Allix-Béguec et al. (*28*), we excluded 45 cases for which $24_{Beijing}$-VNTR profiles formed SLV clusters without epidemiologic links. The remaining 128 cases formed 42 clusters (online Technical Appendix 1 Table 1), each of which contained 2–17 cases; 45.3% (58/128) of cases were in small clusters (2 cases), 25.0% (32/128) were in medium clusters (3 or 4 cases), and 29.7% (38/128) were in 3 large clusters (7, 14, and 17 cases, respectively). Cases attributable to recent transmission accounted for 17.4% (128 clustered cases – 42 clusters/494 total cases).

In both univariate and multivariate analyses, odds ratios for cluster formation were lower for patients ≥60 years of age than those ≤39 of age (Table 2). However, among the *M. tuberculosis* lineages, only ancient Beijing lineage ST11/26, which was represented by cluster 12, the largest cluster (n = 17; online Technical Appendix 1 Table 1), showed a markedly high odds ratio against the modern Beijing subfamily in both univariate and multivariate analyses. The odds ratios of ancient Beijing lineages STK and ST3 against the modern Beijing lineage were significant only with univariate analysis. We also determined the risk for infection with the different *M. tuberculosis* lineages by age group (Table 3). The proportion of infections with STK, ST3, and ST25/19 was remarkably high among patients ≥60 years of age, whereas the proportion of infections with the modern Beijing subfamily was significantly higher ($p<0.01$) among patients ≤59 years of age.

We confirmed epidemiologic links in 22 (52.4%) of the 42 TB clusters that occurred during 2009–2015; the linked cases consisted of 20 source cases (i.e., the source of *M. tuberculosis* transmission) and 50 secondary TB cases (Figure) (online Technical Appendix 1 Figure 2). For each cluster, we identified a source case by information about the patients (e.g., the time of diagnosis, degree of infectiousness, the start of the infectious period based on sputum smear status, severity and duration of respiratory symptoms, findings on chest radiographs, and sociability of the patient). Source cases in clusters 12 and 34 were not included in this study because they occurred outside the study period or lacked a VNTR profile. The most common transmission setting was hospitals that had cared for TB

Table 1. Crude odds ratio for single-locus variant among 173 *Mycobacterium tuberculosis* strains forming preliminary clusters, by epidemiologic links of tuberculosis cases, Yamagata Prefecture, Japan, 2009–2015

Epidemiologic link	$24_{Beijing}$-VNTR profile, no. (%)		Crude odds ratio (95% CI)*
	Indistinguishable, n = 116	Single-locus variant, n = 57	
Linked	42 (36.2)	11 (19.3)	1.0
Possibly linked	16 (13.8)	1 (1.8)	0.2 (0.03–2.0)
Not linked	58 (50.0)	45 (78.9)	**3.0 (1.4–6.4)**

*CIs that do not overlap the null value of odds ratio = 1 are shown in bold.

Table 2. Odds ratio for cluster formation among 494 persons with tuberculosis, Yamagata Prefecture, Japan, 2009–2015*

Patient characteristic	$24_{Beijing}$-VNTR profile, no. (%)		Odds ratio (95% CI)†	
	Not clustered, n = 366	Clustered, n = 128	Univariate	Multivariate‡
Age group				
≤39	31 (8.5)	30 (23.4)	1.0	1.0
40–59	25 (6.8)	21 (16.4)	0.9 (0.4–1.9)	0.8 (0.3–1.8)
60–79	96 (26.2)	35 (27.3)	**0.4 (0.2–0.7)**	**0.4 (0.2–0.8)**
≥80	214 (58.5)	42 (32.8)	**0.2 (0.1–0.4)**	**0.2 (0.1–0.4)**
Sex				
F	143 (39.1)	59 (46.1)	1.3 (0.9–2.0)	1.6 (0.99–2.4)
M	223 (60.9)	69 (53.9)	1.0	1.0
Birthplace				
Japan	351 (95.9)	127 (99.2)	5.4 (0.7–41.5)	**16.7 (2.0–137.0)**
Other	15 (4.1)	1 (0.8)	1.0	1.0
Site of disease				
Pulmonary, sputum smear–positive	251 (68.6)	78 (60.9)	1.0	–
Pulmonary, sputum smear–negative	97 (26.5)	44 (34.4)	1.5 (0.9–2.3)	–
Extrapulmonary	18 (4.9)	6 (4.7)	1.1 (0.4–2.8)	–
Treatment history				
Initial	343 (93.7)	123 (96.1)	1.6 (0.6–4.4)	–
Retreatment	23 (6.3)	5 (3.9)	1.0	–
M. tuberculosis lineage				
Non-Beijing	102 (27.9)	38 (29.7)	0.7 (0.4–1.4)	1.2 (0.6–2.3)
ST11/26	14 (3.8)	19 (14.8)	**2.7 (1.2–6.3)**	**2.5 (1.02–6.1)**
STK	72 (19.7)	10 (7.8)	**0.3 (0.1–0.6)**	0.4 (0.2–1.1)
ST3	68 (18.6)	15 (11.7)	**0.4 (0.2–0.9)**	0.8 (0.3–1.7)
ST25/19	60 (16.4)	21 (16.4)	0.7 (0.4–1.4)	1.0 (0.5–2.2)
Modern Beijing	50 (13.7)	25 (19.5)	1.0	1.0

*–, no variables.
†CIs that do not overlap the null value of odds ratio = 1 are shown in bold.
‡Adjusted for the other factors used in the multivariate model.

patients (15 [30.0%] of 50 secondary cases), followed by households (14 [28.0%] of 50 secondary cases). We found unsuspected links for 23 (46.0%) of 50 secondary cases by conducting in-depth contact tracings after VNTR typing. Among the 23 cases, 22 (95.7%) aggregated with other TB cases in settings outside the household: hospitals (13 cases), pachinko parlors (7 cases), a nursing home (1 case), and a sporting event (1 case). The proportion of cases attributable to recent transmission after adjustment for epidemiologic links was 9.7% (70 clustered cases with epidemiologic links – 22 clusters with epidemiologic links/494 total cases). Furthermore, because unsuspected transmission settings were found within clusters 03, 12, and 34 after VNTR typing, we performed interferon-γ release assays on samples from close contacts of TB case-patients in those settings; none of the results were positive (data not shown).

For the large clusters (clusters 12, 26, and 34), we confirmed that there had been a delay between symptom onset and diagnosis for the source patients or that the source patients were highly socially active (online Technical Appendix 1 Table 2). In cluster 12, we studied 17 cases reported during 2009–2015; another 18 cases were excluded from the study because they occurred outside the study period (online Technical Appendix 1 Figure 2). In cluster 34, a diagnosis of TB in the probable source case-patient was missed because the patient had been diagnosed with lung cancer. However, a typical tuberculous cavity was found by retrospective viewing of a chest radiograph taken before the patient's death.

Seventeen clusters originating from elderly patients were of small or medium size; the exception was cluster 34, for which the source case had not been diagnosed as TB (online Technical Appendix 1 Figure 2). Source cases of the clusters aggregated with secondary cases in hospitals (9 clusters), households (7 clusters), and nursing homes (2 clusters). Almost all of the secondary case-patients aggregating at households and nursing homes were elderly persons, whereas most secondary case-patients within the

Table 3. Lineages of 494 *Mycobacterium tuberculosis* strains by patient age group in Yamagata Prefecture, Japan, 2009–2015

Patient age group, y	No. (%) patients	*M. tuberculosis* lineage, no. (%)					
		Non-Beijing	Ancient Beijing				Modern Beijing
			ST11/26	STK	ST3	ST25/19	
≤39	61 (100)	16 (26.2)	13 (21.3)*	4 (6.6)	2 (3.3)	3 (4.9)	23 (37.7)*
40–59	46 (100)	12 (26.1)	4 (8.7)	2 (4.3)	1 (2.2)	10 (21.7)	17 (37.0)*
60–79	131 (100)	37 (28.2)	6 (4.6)	19 (14.5)	24 (18.3)	30 (22.9)†	15 (11.5)
≥80	256 (100)	75 (29.3)	10 (3.9)	57 (22.3)*	56 (21.9)*	38 (14.8)	20 (7.8)

*Significantly higher proportion by residual analysis (p<0.01).
†Significantly higher proportion by residual analysis (p<0.05).

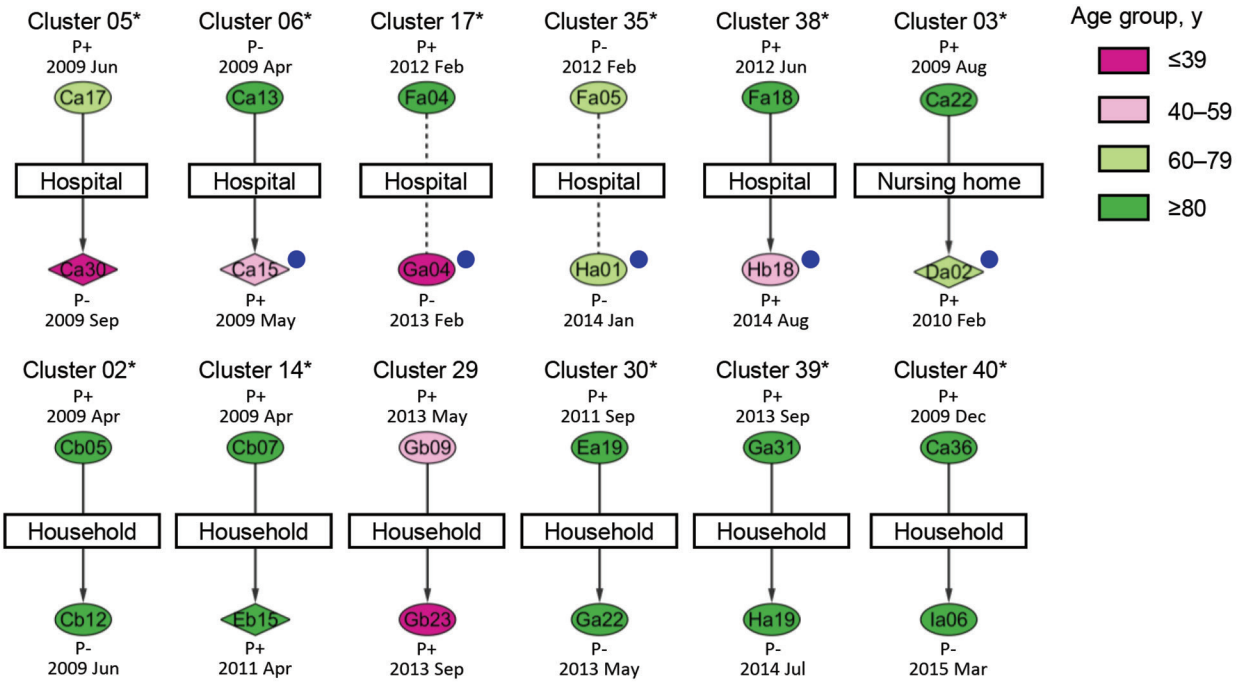


Figure. Twelve small tuberculosis (TB) clusters (2 cases each) among a total of 22 clusters with epidemiologic links between patients, Yamagata Prefecture, Japan, 2009–2015. Ovals and diamonds represent individual cases, by patient age group, in each cluster; numbers inside symbols are patient identification codes. Ovals indicate cases with an indistinguishable 24-loci variable-number tandem-repeat typing profile optimized for Beijing family *M. tuberculosis* strains ($24_{Beijing}$-VNTR profile); diamonds indicate cases with a single-locus variant profile. Vertical arrows and dotted lines between cases within a cluster indicate linked and possibly linked cases, respectively. Transmission settings for linked cases are shown within rectangles. Case notification dates and patient disease sites are shown above/below the case symbol; P+ and P– indicate pulmonary smear–positive and –negative cases, respectively. Black dots indicate confirmation of the epidemiologic link by in-depth contact tracings after $24_{Beijing}$-VNTR typing. Asterisks indicate clusters that began with a TB source patient who was ≥60 years of age. An expanded version of this figure is available as online Technical Appendix 1 Figure 2 (https://wwwnc.cdc.gov/EID/article/23/3/16-1571-Techapp1.pdf).

hospital clusters were younger persons. Twelve (80.0%) of 15 secondary cases in hospital settings were in women ≤59 years of age, including 11 patients who were working as nurses or nurse's aides.

To investigate risk factors for nonlinked results of contact tracings, we analyzed characteristics of 128 clustered cases (Table 4). Multivariate analysis showed that elderly persons and *M. tuberculosis* lineages (non-Beijing and ancient Beijing) were independent risk factors for nonlinked results of contact tracings.

Discussion

We conducted molecular and epidemiologic TB investigations among a population in which 78.3% of the patients were ≥60 years of age. Among 22 TB clusters with epidemiologic links, 17 (77.3%) were traced to an elderly source patient (online Technical Appendix 1 Figure 2). Of those 17 clusters, 11 occurred in hospitals and nursing homes. Transmission in these settings involved secondary cases mostly among younger persons. Our findings indicate that elderly persons must be included in TB prevention and control measures in countries with low to moderate incidence that have experienced a shifting of TB cases toward the elderly (*1*,*22*,*23*).

Our results suggest that elderly patients with TB are a source of TB spread involving younger persons. Borgdorff et al. showed that the number of secondary cases generated per source case decreased concomitantly with increasing age of the source patient; source cases involving elderly persons tend to form clusters of TB cases among elderly persons (*6*). Our findings show that 16 of 17 clusters that began with an elderly source patient were small or medium size; however, cluster 34, caused by an elderly person who was not diagnosed with TB, formed a large cluster. Eleven clusters that had an elderly source patient included transmissions in hospitals and nursing homes, and 80% of secondary cases in hospital settings were in persons ≤59 years of age. Elderly patients with TB often lack typical symptoms, such as cough and fever (*31*,*32*). In addition, diagnosis of TB in elderly persons might be delayed because they sometimes have multiple underlying diseases such as aspiration pneumonia and chronic obstructive pulmonary disease. Given that various factors may delay TB diagnosis in elderly persons, efforts should be taken to decrease

Table 4. Risk factors for nonlinked results of contact tracings among 128 TB cases clustered in Yamagata Prefecture, Japan, 2009–2015

Characteristic	No. (%) cases with epidemiologic link		Odds ratio (95% CI)*	
	Linked or possibly linked	Not linked	Univariate	Multivariate†
Patient age group				
≤59	37 (52.9)	14 (24.1)	1.0	1.0
≥60	33 (47.1)	44 (75.9)	**3.5 (1.6–7.6)**	**2.9 (1.3–6.6)**
Patient sex				
F	39 (55.7)	20 (34.5)	1.0	1.0
M	31 (44.3)	38 (65.5)	**2.4 (1.2–4.9)**	2.2 (0.994–4.9)
Mycobacterium tuberculosis lineage				
Non-Beijing	16 (22.9)	22 (37.9)	**15.8 (3.3–76.9)**	**12.5 (2.5–63.3)**
Ancient Beijing	31 (44.3)	34 (58.6)	**12.6 (2.7–57.9)**	**10.1 (2.1–48.2)**
Modern Beijing	23 (32.9)	2 (3.4)	1.0	1.0

*CIs that do not overlap the null value of odds ratio = 1 are shown in bold.
†Adjusted for the other factors used in the multivariate model.

such delays in order to stop or control the spread of TB. Furthermore, information regarding TB prevention should be provided to workers who have close and routine contact with elderly persons (e.g., hospital and nursing home staff).

Contact investigations are necessary to acquire the greatest public health benefit for most infectious diseases. Our findings confirmed unsuspected links in 46.0% of secondary TB cases through in-depth contact tracing after VNTR typing (online Technical Appendix 1 Figure 2). In addition, we sought undiscovered latent TB infection cases by using interferon-γ release assays to find patient contacts in unsuspected settings. Given that earlier studies performed similar investigations and found latent and active TB cases (*8*,*9*,*11*), a combination of population-based molecular typing and further contact investigation is expected to be an effective strategy for discovering unknown latent TB infections or active TB cases. Moreover, we examined links in detail for 57 patients infected with *M. tuberculosis* strains in the SLV group; our findings confirmed epidemiologic links in 21.1% (12/57) of the cases (Table 1). These results indicate that in-depth contact tracings should be performed for TB cases caused by *M. tuberculosis* strains with SLV profiles.

The results of our study suggest that clusters that include elderly persons without epidemiologic links might be caused by the past endemic *M. tuberculosis* strains. We estimated, on the basis of molecular typing (*9*), that 17.4% (128 clustered cases – 42 clusters/494 total cases) of TB cases in this study were attributable to recent transmission; however, after we made adjustment for epidemiologic links, only 9.7% (70 clustered cases with epidemiologic links – 22 clusters with epidemiologic links/494 total cases) of the cases were attributable to recent transmission. The main cause for this difference in case numbers is that epidemiologic links were difficult to confirm for clustered cases among elderly persons (Table 4). In Japan, cases of TB among the elderly are attributable mainly to the endogenous relapse of *M. tuberculosis* infections that occurred near the time of World War II, when TB was highly prevalent (*4*,*5*). This fact suggests that past endemic strains, having indistinguishable VNTR profiles, have been isolated from elderly persons who have onset of TB in modern times caused by reactivation of latent TB infection. The confirmation of settings with recent *M. tuberculosis* transmission can guide interventions to control the spread of TB; however, the lack of such confirmation, despite in-depth contact tracings, may suggest infection with the past endemic *M. tuberculosis* strain, and the transmission settings for such infections can be difficult to detect.

Epidemiologic data together with phylogenetic information for *M. tuberculosis* strains causing infections might be useful in determining the time of TB infection. In this study, more than half of the strains were of the ancient Beijing subfamily, and the proportion of several sublineages from this family were markedly high in persons ≥60 years of age, whereas modern Beijing subfamily strains were prevalent in younger age groups (Table 3). Given that an earlier Japanese study found a similar tendency (*17*), predominant lineages will have shifted over time, at least in Japan. Such historical dynamics might provide valuable clues for estimating the background of isolated strains. For example, if nonclustered strains with STK or ST3 were isolated from elderly patients in Japan, then it might be reasonable that such cases were regarded as sporadic because of reactivation.

Our study had several potential limitations. First, contact tracings might not clarify all epidemiologic links among clustered TB cases. In particular, findings of the true location of transmission for young and active persons with TB may be missed because those persons tended to frequent many locations. By contrast, elderly persons with TB frequented fewer locations, but their disabilities (e.g., forgetfulness and impaired hearing) or death immediately after TB diagnosis compromised our data gathering. To overcome this limitation, the use of whole-genome sequencing, which has higher discriminatory power than VNTR typing, might indicate whether clustered strains are derived from recent transmission (*27*,*33*). Second, *M. tuberculosis*

genotyping studies cannot investigate culture-negative TB cases. This unavoidable limitation caused a decrease of overall coverage in our study (i.e., >40% of TB cases were beyond the scope of investigation). However, our comprehensive investigation of culture-confirmed TB cases, which are more infectious than culture-negative cases (*34*) and form the basis of TB transmission, may have been sufficient for determining the representative transmission settings in our study area. Our findings from Yamagata Prefecture provide empiric evidence that nonhousehold settings populated or frequented by elderly persons (e.g., hospitals and social gathering settings) are hotspots for *M. tuberculosis* transmission among this population. The last limitation is that our study was restricted to a local setting. However, Theron et al. (*35*) recently proposed that an important factor for ending TB epidemics is to emphasize strategies at the local level, where TB transmission occurs. The accumulation of empiric evidence for various other local settings in Japan is expected to become a higher priority for decision-making for nationwide policies regarding TB.

In summary, molecular genotyping methods make it possible to perform evidence-based TB control (*8–11*,*27*). We confirmed the effectiveness of these methods in a mostly elderly population by using VNTR typing with in-depth contact tracing. Our results suggest that prevention of *M. tuberculosis* transmissions in places where elderly persons gather can be an effective strategy for decreasing TB incidence. A combination of molecular and epidemiologic data can assist public health officials in obtaining an overview of recent transmission and in detecting unsuspected transmission settings, thereby enabling further informational activities and interventions to prevent the spread of TB.

Acknowledgments

We thank all parties involved in public health centers and hospitals for their cooperation with our study. In particular, we thank public health nurses for their exhaustive work in completing contact tracings.

This work was supported by the Ministry of Health, Labour and Welfare (Research on Emerging and Re–emerging Infectious Diseases, Health Sciences Research Grants: grants H21-Shinko-Ippan-024, H22-Shinko-Ippan-042, H23-Shinko-Ippan-032, H24-Shinko-Ippan-019, H25-Shinko-Ippan-001, and H26-ShinkoJitsuyoka- Ippan-001) and by the Research Program on Emerging and Re-emerging Infectious Diseases (grants 15fk0108017h0002 and 15fk0108004h001) from the Japan Agency for Medical Research and Development, AMED. This work was also partly supported by a Grant-in-Aid for Young Scientists (A) (grant JP24689034) from the Japan Society for the Promotion of Science.

Mr. Seto is a senior research specialist at the Yamagata Prefectural Institute of Public Health, Yamagata, Japan. His research interests include molecular and field epidemiology of infectious diseases, especially tuberculosis.

References

1. World Health Organization. Tuberculosis (TB). Global tuberculosis report 2016 [cited 2016 Dec 22]. http://www.who.int/tb/publications/global_report/en/
2. Research Institute of Tuberculosis, Japan Anti-tuberculosis Association. Statistics of TB 2016 [in Japanese]. Tokyo: The Institute; 2016.
3. The Tuberculosis Surveillance Center. The Research Institute of Tuberculosis/JATA. Statistics of TB 2015 [cited 2016 Dec 22]. http://www.jata.or.jp/rit/ekigaku/en/statistics-of-tb/
4. Mori T. Recent trends in tuberculosis, Japan. Emerg Infect Dis. 2000;6:566–8. http://dx.doi.org/10.3201/eid0606.000602
5. Ohmori M, Ishikawa N, Yoshiyama T, Uchimura K, Aoki M, Mori T. Current epidemiological trend of tuberculosis in Japan. Int J Tuberc Lung Dis. 2002;6:415–23.
6. Borgdorff MW, Nagelkerke NJ, de Haas PE, van Soolingen D. Transmission of *Mycobacterium tuberculosis* depending on the age and sex of source cases. Am J Epidemiol. 2001;154:934–43. http://dx.doi.org/10.1093/aje/154.10.934
7. Supply P, Allix C, Lesjean S, Cardoso-Oelemann M, Rüsch-Gerdes S, Willery E, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis.* J Clin Microbiol. 2006;44:4498–510. http://dx.doi.org/10.1128/JCM.01392-06
8. Munang ML, Browne C, Khanom S, Evans JT, Smith EG, Hawkey PM, et al. Tuberculosis microepidemics among dispersed migrants, Birmingham, UK, 2004-2013. Emerg Infect Dis. 2015;21:524–7. http://dx.doi.org/10.3201/eid2103.140209
9. Anderson LF, Tamne S, Brown T, Watson JP, Mullarkey C, Zenner D, et al. Transmission of multidrug-resistant tuberculosis in the UK: a cross-sectional molecular and epidemiological study of clustering and contact tracing. Lancet Infect Dis. 2014;14:406–15. http://dx.doi.org/10.1016/S1473-3099(14)70022-2
10. Wang W, Mathema B, Hu Y, Zhao Q, Jiang W, Xu B. Role of casual contacts in the recent transmission of tuberculosis in settings with high disease burden. Clin Microbiol Infect. 2014;20:1140–5. http://dx.doi.org/10.1111/1469-0691.12726
11. Malakmadze N, González IM, Oemig T, Isiadinso I, Rembert D, McCauley MM, et al. Unsuspected recent transmission of tuberculosis among high-risk groups: implications of universal tuberculosis genotyping in its detection. Clin Infect Dis. 2005;40:366–73. http://dx.doi.org/10.1086/427112
12. Mokrousov I. Insights into the origin, emergence, and current spread of a successful Russian clone of *Mycobacterium tuberculosis.* Clin Microbiol Rev. 2013;26:342–60. http://dx.doi.org/10.1128/CMR.00087-12
13. Hill V, Zozio T, Sadikalay S, Viegas S, Streit E, Kallenius G, et al. MLVA based classification of *Mycobacterium tuberculosis* complex lineages for a robust phylogeographic snapshot of its worldwide molecular diversity. PLoS One. 2012;7:e41991. http://dx.doi.org/10.1371/journal.pone.0041991
14. Cowley D, Govender D, February B, Wolfe M, Steyn L, Evans J, et al. Recent and rapid emergence of W-Beijing strains of *Mycobacterium tuberculosis* in Cape Town, South Africa. Clin Infect Dis. 2008;47:1252–9. http://dx.doi.org/10.1086/592575
15. Hanekom M, Gey van Pittius NC, McEvoy C, Victor TC, Van Helden PD, Warren RM. *Mycobacterium tuberculosis* Beijing genotype: a template for success. Tuberculosis (Edinb). 2011;91:510–23. http://dx.doi.org/10.1016/j.tube.2011.07.005
16. Merker M, Blin C, Mona S, Duforet-Frebourg N, Lecher S, Willery E, et al. Evolutionary history and global spread of the

Mycobacterium tuberculosis Beijing lineage. Nat Genet. 2015;47:242–9. http://dx.doi.org/10.1038/ng.3195
17. Iwamoto T, Fujiyama R, Yoshida S, Wada T, Shirai C, Kawakami Y. Population structure dynamics of *Mycobacterium tuberculosis* Beijing strains during past decades in Japan. J Clin Microbiol. 2009;47:3340–3. http://dx.doi.org/10.1128/JCM.01061-09
18. Wada T, Iwamoto T, Maeda S. Genetic diversity of the *Mycobacterium tuberculosis* Beijing family in East Asia revealed through refined population structure analysis. FEMS Microbiol Lett. 2009;291:35–43. http://dx.doi.org/10.1111/j.1574-6968.2008. 01431.x
19. Seto J, Wada T, Iwamoto T, Tamaru A, Maeda S, Yamamoto K, et al. Phylogenetic assignment of *Mycobacterium tuberculosis* Beijing clinical isolates in Japan by maximum *a posteriori* estimation. Infect Genet Evol. 2015;35:82–8. http://dx.doi.org/10.1016/j.meegid.2015.07.029
20. Murase Y, Mitarai S, Sugawara I, Kato S, Maeda S. Promising loci of variable numbers of tandem repeats for typing Beijing family *Mycobacterium tuberculosis.* J Med Microbiol. 2008;57:873–80. http://dx.doi.org/10.1099/jmm.0.47564-0
21. Iwamoto T, Grandjean L, Arikawa K, Nakanishi N, Caviedes L, Coronel J, et al. Genetic diversity and transmission characteristics of Beijing family strains of *Mycobacterium tuberculosis* in Peru. PLoS One. 2012;7:e49651. http://dx.doi.org/10.1371/journal.pone.0049651
22. Lönnroth K, Migliori GB, Abubakar I, D'Ambrosio L, de Vries G, Diel R, et al. Towards tuberculosis elimination: an action framework for low-incidence countries. Eur Respir J. 2015;45:928–52.
23. Borgdorff MW, van der Werf MJ, de Haas PE, Kremer K, van Soolingen D. Tuberculosis elimination in the Netherlands. Emerg Infect Dis. 2005;11:597–602. http://dx.doi.org/10.3201/eid1104.041103
24. Seto J, Ahiko T, Wada T, Hase A, Yamada K. Effectiveness of comprehensive variable number of tandem repeat (VNTR) analysis in areas with a low incidence of tuberculosis [in Japanese]. Kekkaku. 2013;88:535–42.
25. Wada T, Iwamoto T, Tamaru A, Seto J, Ahiko T, Yamamoto K, et al. Clonality and micro-diversity of a nationwide spreading genotype of *Mycobacterium tuberculosis* in Japan. PLoS One. 2015;10:e0118495. http://dx.doi.org/10.1371/journal.pone.0118495
26. Iwamoto T, Yoshida S, Suzuki K, Tomita M, Fujiyama R, Tanaka N, et al. Hypervariable loci that enhance the discriminatory ability of newly proposed 15-loci and 24-loci variable-number tandem repeat typing method on *Mycobacterium tuberculosis* strains predominated by the Beijing family. FEMS Microbiol Lett. 2007;270:67–74. http://dx.doi.org/10.1111/j.1574-6968.2007.00658.x
27. Walker TM, Ip CL, Harrell RH, Evans JT, Kapatai G, Dedicoat MJ, et al. Whole-genome sequencing to delineate *Mycobacterium tuberculosis* outbreaks: a retrospective observational study. Lancet Infect Dis. 2013;13:137–46. http://dx.doi.org/10.1016/S1473-3099(12)70277-3
28. Allix-Béguec C, Wahl C, Hanekom M, Nikolayevskyy V, Drobniewski F, Maeda S, et al. Proposal of a consensus set of hypervariable mycobacterial interspersed repetitive-unit-variable-number tandem-repeat loci for subtyping of *Mycobacterium tuberculosis* Beijing isolates. J Clin Microbiol. 2014;52:164–72. http://dx.doi.org/10.1128/JCM.02519-13
29. Glynn JR, Vyonycky E, Fine PE. Influence of sampling on estimates of clustering and recent transmission of *Mycobacterium tuberculosis* derived from DNA fingerprinting techniques. Am J Epidemiol. 1999;149:366–71. http://dx.doi.org/10.1093/oxfordjournals.aje.a009822
30. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003;13:2498–504. http://dx.doi.org/10.1101/gr.1239303
31. Van den Brande P. Revised guidelines for the diagnosis and control of tuberculosis: impact on management in the elderly. Drugs Aging. 2005;22:663–86. http://dx.doi.org/10.2165/00002512-200522080-00004
32. Korzeniewska-Kosela M, Krysl J, Müller N, Black W, Allen E, FitzGerald JM. Tuberculosis in young adults and the elderly. A prospective comparison study. Chest. 1994;106:28–32. http://dx.doi.org/10.1378/chest.106.1.28
33. Gardy JL, Johnston JC, Ho Sui SJ, Cook VJ, Shah L, Brodkin E, et al. Whole-genome sequencing and social-network analysis of a tuberculosis outbreak. N Engl J Med. 2011;364:730–9. http://dx.doi.org/10.1056/NEJMoa1003176
34. Grzybowski S, Barnett GD, Styblo K. Contacts of cases of active pulmonary tuberculosis. Bull Int Union Tuberc. 1975;50:90–106.
35. Theron G, Jenkins HE, Cobelens F, Abubakar I, Khan AJ, Cohen T, et al. Data for action: collection and use of local data to end tuberculosis. Lancet. 2015;386:2324–33. http://dx.doi.org/10.1016/S0140-6736(15)00321-9

Address for correspondence: Junji Seto, Yamagata Prefectural Institute of Public Health, Tokamachi 1-6-6, Yamagata, 990-0031, Japan; email: setoj@pref.yamagata.jp

Article DOI: http://dx.doi.org/10.3201/eid2303.161571

Mycobacterium tuberculosis Transmission among Elderly Persons, Yamagata Prefecture, Japan, 2009–2015

Technical Appendix 1

Technical Appendix 1 **Table 1.** Genotyping results of 173 *Mycobacterium tuberculosis* strains forming preliminary clusters and epidemiologic data of clustered TB cases in Yamagata Prefecture, Japan, 2009–2015*

Preliminary cluster, strain no.†	Cluster	Isolate	Age, y	Sex	Residential area	Registration month/year	Epidemiologic link			VNTR profile of $24_{Beijing}$-VNTR‡	SLV	*M. tuberculosis* lineage
							Linked	Possibly linked	Not linked			
01												
1	–	Fa02	92	F	A	02/2012			x	3 4 2 3 3 4 3 5 4 4 7 5 3 2 5 4 3 5 7 7 8 12 12 11	x	ST25/19
2	–	Gb03	81	F	B	01/2013			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 7 6 8 12 12 11	x	ST25/19
3	01	Ha36	70	M	A	10/2014			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 7 7 8 12 12 11		ST25/19
4	01	Ha37	80	M	A	10/2014			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 7 7 8 12 12 11		ST25/19
02												
5	02	Cb05	82	F	B	04/2009	x			4 4 2 3 3 3 3 3 4 2 7 3 3 9 4 3 4 3 5 13 8 10 13 12		STK
6	02	Cb12	86	M	B	06/2009	x			4 4 2 3 3 3 3 3 4 2 7 3 3 9 4 3 4 3 5 13 8 10 13 12		STK
03												
7	03	Ca22	82	F	A	08/2009	x			4 7 2 3 3 3 3 3 4 2 7 5 4 8 4 3 3 4 7 7 8 3/13 16 6	x	STK
8	03	Da02	76	F	A	02/2010	x			4 7 2 3 3 3 3 3 4 2 7 5 4 8 4 3 3 4 7 7 8 13 16 6	x	STK
04												
9	04	Ca02	72	M	A	01/2009			x	4 4 2 3 3 3 4 4 4 4 7 5 3 8 3 3 3 4 7 8 5 14 14 11		Modern Beijing
10	04	Ca31	81	M	A	10/2009			x	4 4 2 3 3 3 4 4 4 4 7 5 3 8 3 3 3 4 7 8 5 14 14 11		Modern Beijing
11	04	Da17	83	F	A	08/2010		x		4 4 2 3 3 3 4 4 4 4 7 5 3 8 3 3 3 4 7 8 5 14 14 11		Modern Beijing
12	04	Ea28	50	F	A	11/2011		x		4 4 2 3 3 3 4 4 4 4 7 5 3 8 3 3 3 4 7 8 5 14 14 11		Modern Beijing
05												
13	05	Ca17	78	M	A	06/2009	x			3 4 2 4 3 4 3 6 4 4 7 5 3 2 5 4 3 5 8 10 8 16 12 8	x	ST25/19
14	05	Ca30	25	F	A	09/2009	x			3 4 2 4 3 4 3 6 4 4 7 5 3 2 5 4 3 5 8 10 13 16 12 8	x	ST25/19
06												

Preliminary cluster, strain no.†	Cluster	Isolate	Age, y	Sex	Residential area	Registration month/year	Epidemiologic link			VNTR profile of $24_{Beijing}$-VNTR‡	SLV	*M. tuberculosis* lineage
							Linked	Possibly linked	Not linked			
15	06	Ca13	84	M	A	04/2009	x			4 4 2 3 3 3 3 7 4 4 7 5 3 10 5 2 3 4 7 10 5 15 12 8	x	ST25/19
16	06	Ca15	41	F	A	05/2009	x			4 4 2 3 3 3 3 7 4 4 7 5 3 10 5 2 3 4 7 10 5 15 13 8	x	ST25/19
17	–	Ha22	82	M	A	09/2014			x	4 4 2 3 3 3 3 7 4 4 7 5 3 10 5 2 3 4 7 10 5 15 12 9	x	ST25/19
07												
18	07	Ca38	50	M	A	12/2009			x	4 4 2 3 3 3 3 7 2 4 7 5 3 10 5 2 3 4 7 10 >20 15 12 8		ST25/19
19	07	Da04	80	M	A	03/2010			x	4 4 2 3 3 3 3 7 2 4 7 5 3 10 5 2 3 4 7 10 >20 15 12 8		ST25/19
08												
20	08	Ca28	63	M	A	09/2009			x	2 3 2 1 3 3 1 3 3 2 5 3 8 3 3 3 2 4 12 5 2 5 5 2		Non-Beijing
21	08	Dc05	93	F	C	05/2010			x	2 3 2 1 3 3 1 3 3 2 5 3 8 3 3 3 2 4 12 5 2 5 5 2		Non-Beijing
09												
22	–	Dc08	77	M	C	08/2010			x	4 4 2 3 3 3 3 7 4 4 7 4 3 8 5 3 3 4 7 10 8 14 12 5	x	ST25/19
23	09	Da03	77	M	A	02/2010			x	4 4 2 3 3 3 3 7 4 4 7 5 3 8 5 3 3 4 7 10 8 14 12 5		ST25/19
24	–	Da34	85	M	A	01/2010			x	4 4 2 3 3 3 3 7 4 4 7 5 3 8 5 3 3 4 7 10 8 15 12 5	x	ST25/19
25	09	Ha11	77	M	A	05/2014			x	4 4 2 3 3 3 3 7 4 4 7 5 3 8 5 3 3 4 7 10 8 14 12 5		ST25/19
10												
26	10	Ca03	84	F	A	01/2009		x		4 4 2 3 3 3 4 5 3 4 7 5 3 7 3 2 3 4 7 8 5 14 20 8		Modern Beijing
27	10	Ea04	45	F	A	01/2011		x		4 4 2 3 3 3 4 5 3 4 7 5 3 7 3 2 3 4 7 8 5 14 20 8		Modern Beijing
28	10	Ea13	42	M	A	04/2011	x			4 4 2 3 3 3 4 5 3 4 7 5 3 7 3 2 3 4 7 8 5 14 20 8		Modern Beijing
29	10	Ea17	49	F	A	08/2011	x			4 4 2 3 3 3 4 5 3 4 7 5 3 7 3 2 3 4 7 8 5 14 20 8		Modern Beijing
11												
30	–	Ca19	79	M	A	08/2009			x	2 3 2 1 3 3 1 3 3 2 5 4 5 4 3 3 2 4 12 5 2 5 5 2	x	Non-Beijing
31	11	Ca34	82	M	A	01/2009			x	2 3 2 1 3 3 1 4 3 2 5 4 5 4 3 3 2 4 12 5 2 5 5 2		Non-Beijing
32	11	Ea16	83	M	A	07/2011			x	2 3 2 1 3 3 1 4 3 2 5 4 5 4 3 3 2 4 12 5 2 5 5 2		Non-Beijing
33	11	Ia15	79	M	A	08/2015			x	2 3 2 1 3 3 1 4 3 2 5 4 5 4 3 3 2 4 12 5 2 5 5 2		Non-Beijing
12												
34	12	Ca14	72	M	A	05/2009			x	3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 7		ST11/26
35	12	Ca18	29	M	A	07/2009		x		3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 7		ST11/26
36	12	Ca21	88	F	A	08/2009	x			3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 7		ST11/26
37	12	Ca23	43	M	A	08/2009		x		3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 7		ST11/26
38	12	Ca25	35	M	A	02/2009	x			3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 8	x	ST11/26
39	12	Ca32	31	M	A	10/2009		x		3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 7		ST11/26
40	12	Da07	26	F	A	03/2010		x		3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 9 8 12 9 7	x	ST11/26
41	12	Da14	24	M	A	07/2010			x	3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 7		ST11/26
42	12	Ea03	72	F	A	01/2011			x	3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 7		ST11/26
43	12	Ea18	27	M	A	09/2011		x		3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 7		ST11/26
44	12	Ea31	36	M	A	12/2011	x			3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 7		ST11/26

Preliminary cluster, strain no.†	Cluster	Isolate	Age, y	Sex	Residential area	Registration month/year	Epidemiologic link: Linked	Epidemiologic link: Possibly linked	Epidemiologic link: Not linked	VNTR profile of $24_{Beijing}$-VNTR‡	SLV	*M. tuberculosis* lineage
45	12	Ea33	30	F	A	07/2011			x	3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 7		ST11/26
46	12	Fa25	36	M	A	07/2012			x	3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 7		ST11/26
47	12	Ga06	32	M	A	03/2013			x	3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 7		ST11/26
48	12	Ga08	57	F	A	03/2013	x			3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 7		ST11/26
49	12	Ga18	77	M	A	04/2013			x	3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 7		ST11/26
50	12	Ib16	39	M	B	09/2015		x		3 4 2 2 3 3 4 5 4 4 7 4 3 8 4 3 3 2 14 10 8 12 9 7		ST11/26
51	–	Ia21	53	F	A	01/2015			x	3 4 2 2 3 3 4 5 4 4 7 4 3 9 4 3 3 2 14 10 8 12 9 7	x	ST11/26
13												
52	13	Eb02	32	F	B	01/2011			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 7 10 8 10 12 9		ST25/19
53	–	Ea08	79	F	A	02/2011			x	3 4 2 3 3 4 3 7 4 4 6 5 3 2 5 4 3 5 7 10 8 10 12 12	x	ST25/19
54	13	Ea32	86	F	A	12/2011			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 7 10 8 10 12 9		ST25/19
55	13	Fb11	59	M	B	12/2012			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 7 10 8 10 12 9		ST25/19
56	–	Fa33	83	M	A	11/2012			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 7 10 8 12 12 12	x	ST25/19
57	–	Hb05	54	M	B	03/2014			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 7 13 8 10 12 12	x	ST25/19
58	–	Ha31	87	F	A	12/2014			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 7 10 8 10 12 12	x	ST25/19
14												
59	14	Cb07	83	M	B	04/2009	x			4 4 2 3 1 3 3 6 4 4 5 5 4 8 5 2 4 4 7 10 9 24 14 12	x	ST3
60	14	Eb15	85	F	B	04/2011	x			4 4 2 3 1 3 3 6 4 4 5 5 4 8 5 2 4 4 7 10 9 22 14 12	x	ST3
15												
61	15	Fa01	43	F	A	01/2012			x	2 3 2 1 3 3 1 4 3 2 5 3 5 3 3 3 2 4 12 5 2 5 9 2		Non-Beijing
62	15	Ga33	84	F	A	08/2013			x	2 3 2 1 3 3 1 4 3 2 5 3 5 3 3 3 2 4 12 5 2 5 9 2		Non-Beijing
16												
63	16	Db02	86	F	B	02/2010			x	4 4 2 3 1 3 3 7 4 4 7 5 3 8 5 2 4 4 7 10 9 16 14 13		ST3
64	16	Fa03	68	F	A	01/2012			x	4 4 2 3 1 3 3 7 4 4 7 5 3 8 5 2 4 4 7 10 9 16 14 13		ST3
17												
65	17	Fa04	82	M	A	02/2012		x		4 4 2 3 3 3 4 6 4 4 7 4 3 8 3 3 1 4 7 8 8 13 13 4		Modern Beijing
66	17	Ga04	37	F	A	02/2013		x		4 4 2 3 3 3 4 6 4 4 7 4 3 8 3 3 1 4 7 8 8 13 13 4		Modern Beijing
18												
67	18	Dc13	83	M	C	12/2010			x	2 4 2 1 3 3 1 4 3 2 5 3 5 5 3 3 2 4 12 5 2 4 5 2		Non-Beijing
68	–	Fa07	80	M	A	04/2012			x	2 4 2 1 3 3 1 4 3 2 5 3 5 5 3 3 2 4 12 5 2 3 5 2	x	Non-Beijing
69	18	Fa26	72	F	A	07/2012			x	2 4 2 1 3 3 1 4 3 2 5 3 5 5 3 3 2 4 12 5 2 4 5 2		Non-Beijing
19												
70	–	Da18	93	F	A	09/2010			x	2 4 2 1 2 3 1 4 3 2 5 3 7 4 3 3 2 4 10 5 2 5 5 2	x	Non-Beijing
71	–	Fa09	81	F	A	05/2012			x	2 4 2 1 2 3 1 4 3 2 5 3 5 4 3 3 2 4 10 5 2 5 5 2	x	Non-Beijing
72	–	Ha26	93	F	A	11/2014			x	2 4 2 1 2 3 1 4 3 2 4 3 5 4 3 3 2 4 10 5 2 5 5 2	x	Non-Beijing

Preliminary cluster, strain no.†	Cluster	Isolate	Age, y	Sex	Residential area	Registration month/year	Epidemiologic link: Linked	Epidemiologic link: Possibly linked	Epidemiologic link: Not linked	VNTR profile of $24_{Beijing}$-VNTR‡	SLV	*M. tuberculosis* lineage
20												
73	–	Da24	61	F	A	01/2010			x	4 4 2 3 1 3 3 7 4 4 12 4 3 8 5 2 4 4 7 9 9 13 14 9	x	ST3
74	–	Da27	78	F	A	11/2010			x	4 4 2 3 1 3 3 7 4 4 8 4 3 8 5 2 4 4 7 9 9 13 14 9	x	ST3
75	–	Fa16	85	M	A	04/2012			x	4 4 2 3 1 3 3 7 4 4 11 4 3 8 5 2 4 4 7 9 9 13 14 9	x	ST3
21												
76	19	Db05	79	M	B	04/2010			x	4 4 2 3 1 3 3 6 4 4 7 5 3 8 5 2 4 4 7 10 9 16 14 12		ST3
77	–	Eb07	86	M	B	07/2011			x	4 4 2 3 1 3 3 6 4 4 7 5 3 8 5 2 2 4 7 10 9 16 14 12	x	ST3
78	19	Ea21	85	M	A	01/2011			x	4 4 2 3 1 3 3 6 4 4 7 5 3 8 5 2 4 4 7 10 9 16 14 12		ST3
22												
79	–	Cb04	81	F	B	03/2009			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 7 10 13 9 12 11	x	ST25/19
80	–	Da09	82	M	A	04/2010			x	3 4 2 3 3 4 3 6 4 4 6 5 3 2 5 4 3 5 7 10 8 10 12 11	x	ST25/19
81	20	Da28	93	F	A	11/2010			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 7 10 8 9 12 11		ST25/19
82	20	Ga11	67	M	A	01/2013			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 7 10 8 9 12 11		ST25/19
83	–	Gb18	41	M	B	07/2013			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 7 10 8 13 12 11	x	ST25/19
84	20	Gc16	78	M	C	12/2013			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 7 10 8 9 12 11		ST25/19
85	–	Ha13	88	F	A	06/2014			x	3 4 2 3 3 4 3 6 4 4 7 5 3 2 5 4 3 5 7 10 8 10 12 11	x	ST25/19
86	–	Ia04	89	M	A	01/2015			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 7 10 8 14 12 11	x	ST25/19
87	–	Ib10	77	M	B	06/2015			x	3 4 2 3 3 4 3 6 4 4 7 5 3 2 5 4 3 5 7 10 8 9 12 11	x	ST25/19
23												
88	21	Ca37	32	F	A	12/2009	x			4 4 2 3 3 3 4 5 4 4 8 5 3 10 3 3 3 4 4 8 5 14 14 9		Modern Beijing
89	21	Fa21	39	F	A	09/2012	x			4 4 2 3 3 3 4 5 4 4 8 5 3 10 3 3 3 4 4 8 5 14 14 9		Modern Beijing
90	21	Fa38	44	M	A	12/2012	x			4 4 2 3 3 3 4 5 4 4 8 5 3 10 3 3 3 4 4 8 5 14 14 9		Modern Beijing
24												
91	–	Ea01	90	M	A	02/2011			x	2 4 2 4 2 3 2 3 3 2 5 3 3 8 3 4 2 4 7 5 >20 13 5 4	x	Non-Beijing
92	–	Fa22	80	F	A	06/2012			x	2 4 2 3 2 3 2 3 3/4 2 5 3 3 8 3 4 2 4 7 5 >20 13 5 4	x	Non-Beijing
25												
93	22	Fa23	38	F	A	06/2012			x	4 4 4 3 3 3 3 3 4 2 7 5 3 3 4 3 3 4 7 8 8 12 17 18		STK
94	22	Fa27	85	F	A	07/2012			x	4 4 4 3 3 3 3 3 4 2 7 5 3 3 4 3 3 4 7 8 8 12 17 18		STK
26												
95	–	Ca33	78	M	A	01/2009			x	4 4 2 3 3 3 3 6 4 4 7 5 3 10 5 2 3 4 7 10 >20 >20 12 8	x	ST25/19
96	–	Fa24	79	F	A	07/2012			x	4 4 2 3 3 3 3 6 4 4 7 5 3 10 5 2 3 4 7 10 >20 20 12 8	x	ST25/19
27												
97	23	Eb04	79	M	B	05/2011			x	3 4 1 2 5 1 2 2 3 4 1 3 3 8 4 1 5 2 15 6 >20 5 7 4		Non-Beijing
98	23	Fb04	72	F	B	01/2012			x	3 4 1 2 5 1 2 2 3 4 1 3 3 8 4 1 5 2 15 6 >20 5 7 4		Non-Beijing
28												

Preliminary cluster, strain no.†	Cluster	Isolate	Age, y	Sex	Residential area	Registration month/year	Epidemiologic link			VNTR profile of $24_{Beijing}$-VNTR‡	SLV	*M. tuberculosis* lineage
							Linked	Possibly linked	Not linked			
99	24	Fb07	43	F	B	07/2012			x	1 4 2 3 3 3 3 7 4 4 7 5 3 8 5 3 3 4 7 10 8 8 10 5		ST25/19
100	24	Fb08	76	M	B	09/2012			x	1 4 2 3 3 3 3 7 4 4 7 5 3 8 5 3 3 4 7 10 8 8 10 5		ST25/19
29												
101	25	Fb06	80	F	B	01/2012			x	4 4 4 2 2 2 0 7 3 2 5 3 1 6 3 3 2 4 16 0 5 7 3 4		Non-Beijing
102	25	Gb01	79	M	B	02/2013			x	4 4 4 2 2 2 0 7 3 2 5 3 1 6 3 3 2 4 16 0 5 7 3 4		Non-Beijing
30												
103	26	Fb05	26	M	B	11/2012	x			3 4 1 3 5 1 2 2 3 4 1 3 3 9 4 1 3 2 12 7 >20 6 9 4		Non-Beijing
104	26	Gb02	65	M	B	02/2013		x		3 4 1 3 5 1 2 2 3 4 1 3 3 9 4 1 3 2 12 7 >20 6 9 4		Non-Beijing
105	26	Gb04	49	F	B	01/2013	x			3 4 1 3 5 1 2 2 3 4 1 3 3 9 4 1 3 2 12 7 >20 6 9 4		Non-Beijing
106	26	Gb07	78	M	B	03/2013			x	3 4 1 3 5 1 2 2 3 4 1 3 3 9 4 1 3 2 12 7 >20 6 9 4		Non-Beijing
107	26	Gb08	70	M	B	04/2013			x	3 4 1 3 5 1 2 2 3 4 1 3 3 9 4 1 3 2 12 7 >20 6 9 4		Non-Beijing
108	26	Gb11	66	M	B	07/2013		x		3 4 1 3 5 1 2 2 3 4 1 3 3 9 4 1 3 2 12 7 >20 6 9 4		Non-Beijing
109	26	Gb17	36	F	B	07/2013	x			3 4 1 3 5 1 2 2 3 4 1 3 3 9 4 1 3 2 12 7 >20 6 9 4		Non-Beijing
110	26	Gb24	42	F	B	08/2013	x			3 4 1 3 5 1 2 2 3 4 1 3 3 9 4 1 3 2 12 7 >20 6 9 4		Non-Beijing
111	26	Gb26	30	M	B	09/2013			x	3 4 1 3 5 1 2 2 3 4 1 3 3 9 4 1 3 2 12 7 >20 6 9 4		Non-Beijing
112	26	Hb07	27	M	B	04/2014	x			3 4 1 3 5 1 2 2 3 4 1 3 3 6/9 4 1 3 2 12 7 >20 6 9 4	x	Non-Beijing
113	26	Hb21	51	F	B	11/2014			x	3 4 1 3 5 1 2 2 3 4 1 3 3 9 4 1 3 2 12 7 >20 6 9 4		Non-Beijing
114	26	Ib06	23	M	B	03/2015			x	3 4 1 3 5 1 2 2 3 4 1 3 3 9 4 1 3 2 12 7 >20 6 9 4		Non-Beijing
115	26	Ib11	19	M	B	07/2015	x			3 4 1 3 5 1 2 2 3 4 1 3 3 9 4 1 3 2 12 7 >20 6 9 4		Non-Beijing
116	26	Ib14	57	F	B	07/2015	x			3 4 1 3 5 1 2 2 3 4 1 3 3 9 4 1 3 2 12 7 >20 6 9 4		Non-Beijing
31												
117	–	Db16	64	M	B	11/2010			x	2 4 1 2 3 1 2 2 3 4 1 3 3 7 4 1 5 2 10 6 >20 5 6 5	x	Non-Beijing
118	27	Fb13	79	M	B	12/2012			x	2 4 1 2 3 1 2 2 3 4 1 3 3 7 4 1 5 2 13 6 >20 5 6 5		Non-Beijing
119	27	Ib07	78	M	B	03/2015			x	2 4 1 2 3 1 2 2 3 4 1 3 3 7 4 1 5 2 13 6 >20 5 6 5		Non-Beijing
32												
120	28	Eb01	86	M	B	01/2011			x	4 4 2 3 1 3 3 6 4 4 6 5 3 8 5 2 4 4 4 10 7 16 14 13		ST3
121	28	Ga14	80	F	A	03/2013			x	4 4 2 3 1 3 3 6 4 4 6 5 3 8 5 2 4 4 4 10 7 16 14 13		ST3
122	–	Ha21	91	F	A	08/2014			x	4 4 2 3 1 3 3 6 4 4 6 5 3 8 5 2 4 4 4 10 7 16 14 9	x	ST3
33												
123	–	Gc04	22	M	C	04/2013			x	4 4 2 3 3 3 4 6 4 4 7 5 3 8 3 3 3 4 7 8 8 14 12 10	x	Modern Beijing
124	29	Gb09	44	M	B	05/2013	x			4 4 2 3 3 3 4 6 4 4 7 5 3 8 3 3 3 4 7 8 8 14 15 10		Modern Beijing
125	29	Gb23	18	M	B	09/2013	x			4 4 2 3 3 3 4 6 4 4 7 5 3 8 3 3 3 4 7 8 8 14 15 10		Modern Beijing
34												
126	30	Ea19	83	M	A	09/2011	x			2 4 1 2 5 1 2 2 3 4 1 3 2 8 4 1 3 2 16 6 7 5 8 4		Non-Beijing
127	30	Ga22	80	F	A	05/2013	x			2 4 1 2 5 1 2 2 3 4 1 3 2 8 4 1 3 2 16 6 7 5 8 4		Non-Beijing

Preliminary cluster, strain no.†	Cluster	Isolate	Age, y	Sex	Residential area	Registration month/year	Epidemiologic link: Linked	Epidemiologic link: Possibly linked	Epidemiologic link: Not linked	VNTR profile of $24_{Beijing}$-VNTR‡	SLV	*M. tuberculosis* lineage
35												
128	–	Ca07	86	M	A	02/2009			x	2 4 2 1 3 3 1 2 3 2 5 3 5 5 3 3 2 4 13 5 2 5 5 2	x	Non-Beijing
129	–	Gb13	78	M	B	03/2013			x	2 4 2 1 3 3 1 2 3 2 5 3 5 5 3 3 2 4 10 5 2 5 5 2	x	Non-Beijing
36												
130	31	Gb10	80	M	B	06/2013	x			3 4 1 3 3 4 3 7 4 4 6 5 3 2 5 2 3 5 7 10 8 13 12 14/15	x	ST25/19
131	31	Gb16	79	M	B	08/2013	x			3 4 1 3 3 4 3 7 4 4 6 5 3 2 5 2 3 5 7 10 8 13 12 15		ST25/19
132	31	Hb13	80	F	B	07/2014	x			3 4 1 3 3 4 3 7 4 4 6 5 3 2 5 2 3 5 7 10 8 13 12 15		ST25/19
37												
133	32	Cc03	74	M	C	02/2009			x	4 4 2 3 1 3 3 7 4 4 7 5 3 8 5 2 4 4 7 10 9 14 14 21		ST3
134	32	Ga28	74	F	A	08/2013	x			4 4 2 3 1 3 3 7 4 4 7 5 3 8 5 2 4 4 7 10 9 14 14 21		ST3
135	32	Ga39	82	F	A	10/2013	x			4 4 2 3 1 3 3 7 4 4 7 5 3 8 5 2 4 4 7 10 9 14 14 21		ST3
38												
136	–	Eb09	84	M	B	09/2011			x	5 4 2 3 1 3 3 7 4 4 7 4 3 8 5 2 4 4 7 9 9 16 16 9	x	ST3
137	–	Gc10	66	M	C	08/2013			x	4 4 2 3 1 3 3 7 4 4 7 4 3 8 5 2 4 4 7 9 9 16 16 9	x	ST3
39												
138	33	Fb03	37	M	B	07/2012	x			2 4 2 3 2 3 2 3 3 2 5 3 3 6 3 4 2 4 3 5 >20 14 5 3		Non-Beijing
139	33	Gb21	68	F	B	09/2013	x			2 4 2 3 2 3 2 3 3 2 5 3 3 6 3 4 2 4 3 5 >20 14 5 3		Non-Beijing
140	33	Hb24	26	M	B	11/2014	x			2 4 2 3 2 3 2 3 3 2 5 3 3 6 3 4 2 4 3 5 >20 14 5 3		Non-Beijing
40												
141	–	Eb12	76	F	B	01/2011			x	4 4 2 3 3 3 4 6 4 4 7 5 3 8 3 3 3 4 7 7 8 14 14 10	x	Modern Beijing
142	–	Gd02	23	F	D	01/2013			x	4 4 2 3 3 3 4 6 4 4 7 4 3 8 3 3 3 4 7 7 8 14 14 10	x	Modern Beijing
41												
143	–	Db04	76	M	B	04/2010			x	4 4 2 3 3 3 4 8 4 4 7 5 3 8 3 3 3 4 7 8 8 14 14 9	x	Modern Beijing
144	–	Ga41	25	M	A	11/2013			x	4 4 2 3 3 3 4 9 4 4 7 5 3 8 3 3 3 4 7 8 8 14 14 9	x	Modern Beijing
42												
145	34	Gc07	91	F	C	06/2013	x			4 4 2 3 3 3 4 7 4 4 9 5 3 9 3 3 3 4 7 6 1 12 16 9		Modern Beijing
146	34	Gc09	61	F	C	08/2013	x			4 4 2 3 3 3 4 7 4 4 9 5 3 9 3 3 3 4 7 6 1 12 16 9		Modern Beijing
147	34	Hc02	24	F	C	01/2014	x			4 4 2 3 3 3 4 7 4 4 9 5 3 9 3 3 3 4 7 6 1 12 16 9		Modern Beijing
148	34	Hc03	55	F	C	01/2014	x			4 4 2 3 3 3 4 7 4 4 9 5 3 9 3 3 3 4 7 6 1 12 16 9		Modern Beijing
149	34	Hc22	30	F	C	04/2014	x			4 4 2 3 3 3 4 7 4 4 9 5 3 9 3 3 3 4 7 6 1 12 16 9		Modern Beijing
150	34	Ic03	51	F	C	03/2015	x			4 4 2 3 3 3 4 7 4 4 9 5 3 9 3 3 3 4 7 6 1 12 16 9		Modern Beijing
151	34	Ic05	54	M	C	04/2015	x			4 4 2 3 3 3 4 7 4 4 9 5 3 9 3 3 3 4 7 6 1 12 16 9		Modern Beijing
43												
152	35	Fa05	71	F	A	02/2012		x		4 4 2 3 1 3 3 5 4 4 7 5 3 8 5 2 4 4 7 9 9 18 12 11		ST3
153	35	Ha01	73	F	A	01/2014		x		4 4 2 3 1 3 3 5 4 4 7 5 3 8 5 2 4 4 7 9 9 18 12 11		ST3

Preliminary cluster, strain no.†	Cluster	Isolate	Age, y	Sex	Residential area	Registration month/year	Epidemiologic link			VNTR profile of $24_{Beijing}$-VNTR‡	SLV	*M. tuberculosis* lineage
							Linked	Possibly linked	Not linked			
44												
154	36	Fb10	70	M	B	11/2012	x			4 4 2 3 3 3 4 >20 3 4 7 5 4 8 3 3 3 4 7 8 5 14 14 12		Modern Beijing
155	36	Hb06	28	F	B	03/2014		x		4 4 2 3 3 3 4 >20 3 4 7 5 4 8 3 3 3 4 7 8 5 14 14 12		Modern Beijing
156	36	Hb15	79	M	B	07/2014	x			4 4 2 3 3 3 4 >20 3 4 7 5 4 8 3 3 3 4 7 8 5 14 14 12		Modern Beijing
45												
157	37	Gb27	93	F	B	11/2013			x	3 4 2 2 3 3 4 5 4 4 7 5 3 9 4 3 3 2 10 9 8 12 9 8		ST11/26
158	–	Hc14	62	F	C	05/2014			x	3 4 2 2 3 3 4 5 4 4 7 5 3 9 4 2 3 2 10 9 8 12 9 8	x	ST11/26
159	37	Ia27	29	M	A	01/2015			x	3 4 2 2 3 3 4 5 4 4 7 5 3 9 4 3 3 2 10 9 8 12 9 8		ST11/26
46												
160	38	Fa18	82	F	A	06/2012	x			4 4 4 3 3 3 3 3 4 2 7 5 3 3 4 3 3 4 7 8 8 12 18 17		STK
161	38	Hb18	55	F	B	08/2014	x			4 4 4 3 3 3 3 3 4 2 7 5 3 3 4 3 3 4 7 8 8 12 18 17		STK
47												
162	39	Ga31	83	M	A	09/2013	x			1 4 2 1 3 3 1 4 3 2 4 3 8 5 3 3 2 4 11 4 2 5 3 2		Non-Beijing
163	39	Ha19	82	F	A	07/2014	x			1 4 2 1 3 3 1 4 3 2 4 3 8 5 3 3 2 4 11 4 2 5 3 2		Non-Beijing
48												
164	–	Hc09	83	M	C	02/2014			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 4 10 8 12 12 13	x	ST25/19
165	–	Ha33	81	M	A	04/2014			x	3 4 2 3 3 4 3 7 4 4 7 5 3 2 5 4 3 5 4 10 8 12 12 11	x	ST25/19
49												
166	40	Ca36	85	F	A	12/2009	x			6 4 2 3 1 3 3 7 4 4 8 5 3 7 5 2 5 4 7 10 >20 16 18 13		ST3
167	40	Ia06	92	M	A	03/2015	x			6 4 2 3 1 3 3 7 4 4 8 5 3 7 5 2 5 4 7 10 >20 16 18 13		ST3
50												
168	41	Hb19	89	M	B	09/2014			x	4 4 2 3 3 3 3 3 4 2 6 3 4 7 4 3 3 4 7 8 8 13 18 13		STK
169	41	Ia22	88	M	A	09/2015			x	4 4 2 3 3 3 3 3 4 2 6 3 4 7 4 3 3 4 7 8 8 13 18 13		STK
51												
170	–	Fb01	74	M	B	01/2012			x	4 4 2 3 3 3 3 3 4 2 7 3 4 7 4 3 3 4 7 8 8 13 16 11	x	STK
171	–	Ia23	94	M	A	11/2015			x	4 4 2 3 3 3 3 3 4 2 7 3 4 7 4 3 3 4 7 8 8 13 16 12	x	STK
52												
172	42	Hc26	87	F	C	11/2014			x	3 4 1 2 5 1 2 2 3 4 1 3 3 8 4 1 3 2 13 7 >20 5 9 3		Non-Beijing
173	42	Ia26	87	M	A	12/2015			x	3 4 1 2 5 1 2 2 3 4 1 3 3 8 4 1 3 2 13 7 >20 5 9 3		Non-Beijing

*SLV, single-locus variant; –, the case was not included in a cluster. Underlining indicates the position of the SLV profile.

† Because we defined that a strain can belong to a preliminary cluster, there were cases in which strains of ≥2 loci difference were included in the same preliminary cluster.

‡The 24 digits indicate the VNTR profile according to the following aliases (loci) set order: Mtub04 (424), ETR C (577), MIRU4 (580), MIRU40 (802), MIRU10 (960), MIRU16 (1644), Mtub21 (1955), QUB-11b (2163b), ETR A (2165), Mtub30 (2401), MIRU26 (2996), MIRU31 (3192), Mtub39 (3690), QUB-26 (4052), QUB-4156 (4156), Mtub24 (2074), V2372 (2372), QUB-15 (3155), QUB-3336 (3336), QUB-18 (1982), QUB-11a (2163a), QUB-3232 (3232), V3820 (3820), and V4120 (4120). Additionally, as for the copy number represented by ">20," we confirmed the concordance of PCR product size within the cluster by using agarose electrophoresis.

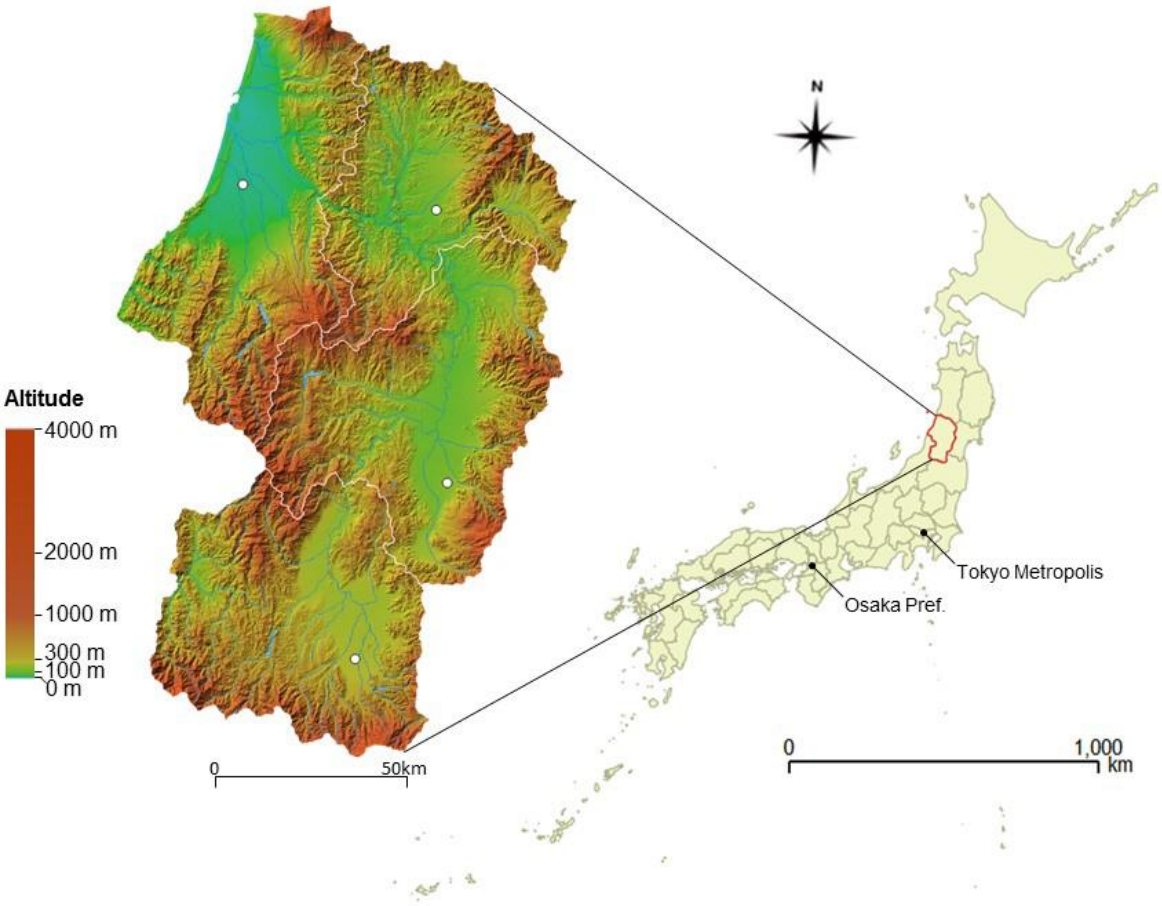
Technical Appendix 1 **Table 2.** Characteristics of three large tuberculosis clusters in Yamagata Prefecture, Japan, 2009–2015

Characteristics of the source case-patient	Cluster 12, N = 35	Cluster 26, N = 14	Cluster 34, N = 8
Isolate number	Aa08	Fb05	Not acquired
Age, y	20s	20s	90s
Sex	Male	Male	Female
Site of disease	Pulmonary (sputum smear positive)	Pulmonary (sputum smear positive)	Lung cancer (TB was not diagnosed)*
Notification date (death date)	November 2007	November 2012	(September 2012)
Time between symptom onset and date of diagnosis (time between symptom onset and date of death)	35 months†	3 months	(21 months)†
Frequently visited places	Two workplaces (changed job once during onset), club team and its events, pachinko parlors‡	A welfare daycare center, pachinko parlors‡	A health clinic (Hospital 1 within cluster 34 in Technical Appendix 1 Figure 2), A hospital admitted (Hospital 2 within cluster 34 in Technical Appendix 1 Figure 2)
Remarks	The source case-patient had a medical checkup annually after 2003, but his TB was not diagnosed properly.	The source case-patient was mildly mentally handicapped.	The probable source case-patient was suspected of having lung cancer by tumor marker diagnosis in July 2010. Steroid therapy for terminal care was performed at Hospital 2 for 5 days before she died. Suctioning of sputum was applied frequently for the patient without adequate standard precautions of healthcare workers.

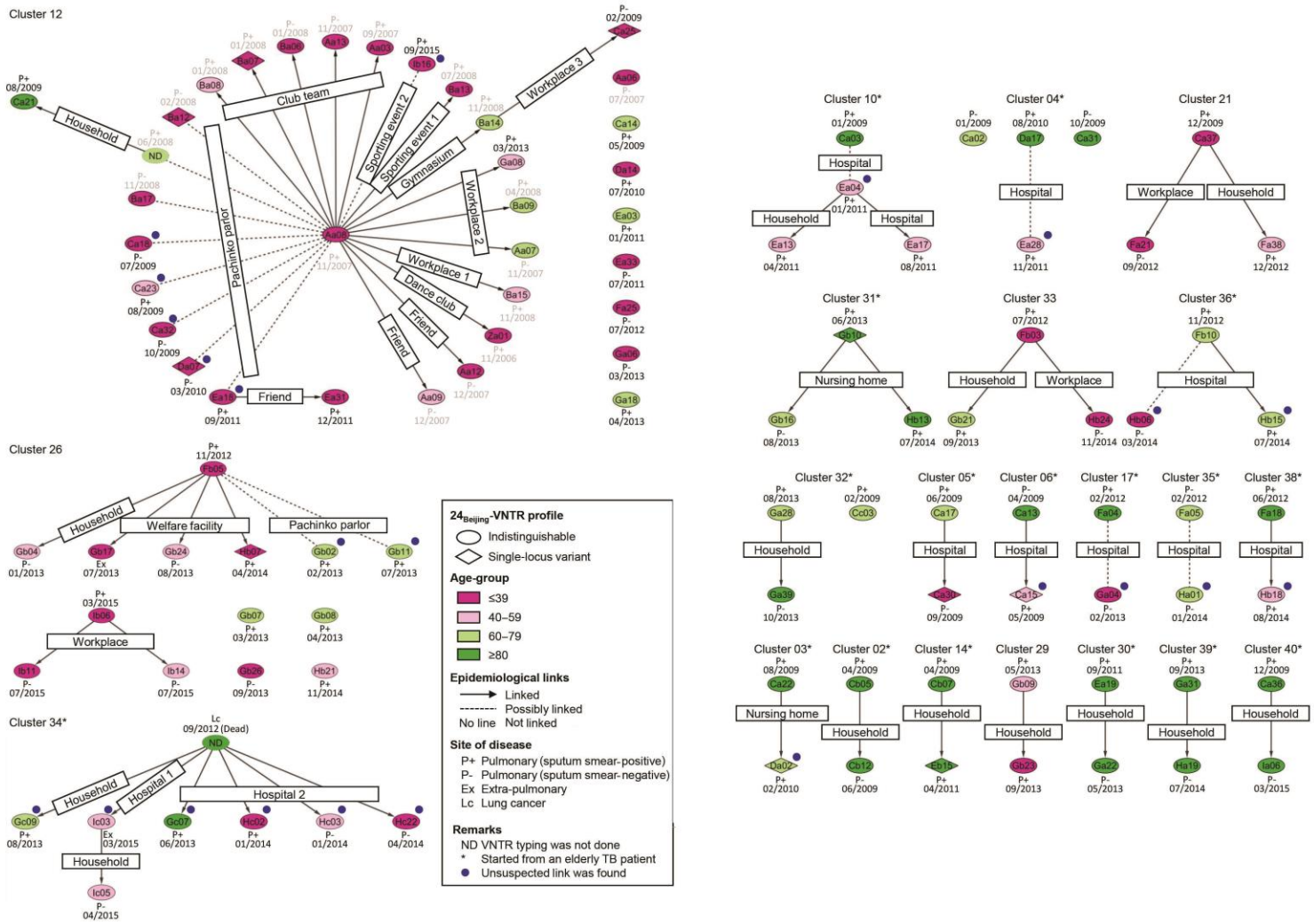
*After $24_{Beijing}$-VNTR typing, a public health center confirmed the case-patient's typical tuberculous cavity in a chest radiograph taken in late August 2012, slightly before the patient's death (data not shown).
VNTR, variable-number tandem-repeat.

†Public health centers estimated the duration of symptoms by retrospective viewing of chest radiographs (data not shown).

‡Pachinko parlors, crowded gambling halls in Japan, are well attended by persons ≥18 years of age.



Technical Appendix 1 **Figure 1.** Topographic map of Yamagata Prefecture, Japan. About 85% of the landforms are mountains. Boundaries among the four areas (white line) are separated mainly by the mountains. White circles show the locations of Public Health Centers in respective areas. Map source: The Geospatial Information Authority of Japan, Ministry of Land, Infrastructure, Transport and Tourism (http://maps.gsi.go.jp/development/ichiran.html)



Technical Appendix 1 **Figure 2.** Twenty-two clusters with epidemiologic links between patients, Yamagata Prefecture, Japan, 2009–2015. In 86 cases depicted, 70 cases are graded as linked or possibly linked; 15 cases are nonlinked; a case (the probable source case of cluster 34) is

excluded from the study because variable-number tandem-repeat (VNTR) typing of the case could not be done. Cluster 12 contains 18 cases that are outside of the study period (shown by gray text). Ovals and diamonds denote individual cases in each cluster; numbers inside symbols are patient identification codes. Profiles of $24_{Beijing}$-VNTR typing, patient age groups, epidemiologic links in each cluster, and patient disease sites are shown in the key. Transmission settings for linked cases are shown within rectangles. Patient disease sites and case notification dates are shown above/below the case symbol. Blue dots signify confirmation of the epidemiologic link by in-depth contact tracings after $24_{Beijing}$-VNTR typing. Asterisks indicate clusters that began with a tuberculosis source patient who was ≥60 years of age.

Article DOI: http://dx.doi.org/10.3201/eid2303.161571

Mycobacterium tuberculosis Transmission among Elderly Persons, Yamagata Prefecture, Japan, 2009–2015

Technical Appendix 2

Public health centers in Yamagata Prefecture, Japan, use 2 versions of a questionnaire (1 long and 1 short) to gather data from tuberculosis case-patients. The long version contains multiple-choice and open-ended questions. The short version contains only the multiple-choice questions from the long questionnaire. Public health centers chose which questionnaire to use, depending on the patient's willingness and ability to respond.

Long Questionnaire

Questionnaire for early findings of tuberculosis

This questionnaire is intended to show us the places where tuberculosis transmissions occur. Please respond with information about your past lifestyle. We would like to use your cooperation to decrease tuberculosis infection in Yamagata.

Please feel free to contact us if you have any questions.

Public health center:_____ Tel.:_____ Person in charge:_____

I. General questions

Q1. Did you know about "tuberculosis" before your hospital stay (or visit)?

Please check the box (□).

□ Yes □ No

Q2. Do you remember any tuberculosis patients around you in the recent past?

□ Yes □ No

➔ If "Yes," please explain a little about that patient(s).

(e.g., my grandfather developed tuberculosis in 2015.)

Q3. Before you were diagnosed with tuberculosis, had you experienced any symptom such as cough, retention of sputum, or fever?

□ Yes □ No

➔ If "Yes," please write the period that you started to have those symptoms.

➔ If "No," please write the period that you were diagnosed with tuberculosis.

Year: _____, Month: _____

II. Questions about your lifestyle of the last two years

Most patients show onset of tuberculosis (or have it discovered) within two years of their infection. In this section, please answer about your lifestyle for the last two years.

Q4. Please check all the facilities that you have visited at least once within the last two years.

□ Convenience store
□ Pachinko parlor
□ Parlors of Japanese chess, mah-jong, or game of go
□ Theater
□ Public bathhouse
□ Drinking spot (e.g., bar, pub)
□ Karaoke bar
□ Culture lesson (e.g., cooking class, karaoke class)
□ Barber shop
□ Religious space (e.g., church, temple)

Continued on next page

□ Internet cafe
□ Amusement arcade
□ Indoor sports facility
□ Academy, cram school
□ Welfare facility (e.g., nursing home, facilities for the disabled)
□ Neighborhood meeting (e.g., neighborhood association, volunteer fire company)
□ Ceremonial function (e.g., bridal, burial)
□ Dormitory for student or office worker
□ Homes of friends, or close relatives
□ Other place ( )

➔ If you checked more than one place, proceed to Q5.
➔ If you visited none of the places above, proceed to Q6.

Q5. Do you remember meeting someone who had a bad cough or a sudden decrease in weight?

□ Yes □ No

➔ If "Yes," please write when and where you met them.

Q6. Please answer the top four places where you have stayed for a long period within the last two years. Select the number of places from "1. Home," "2. Company," "3. School," "4. Amusement facility," and "5. Other place." Please answer separately for weekdays and holidays. In this question, "4. Amusement facility" means a place where you feel amused or relaxed (e.g., drinking spot, karaoke bar, and pachinko parlor).

	Weekday	Holiday
1st		
2nd		
3rd		
4th		

Q7. Within "4. Amusement facility" and "5. Other place" of Q6, please write the top three specific facilities that you have often visited. Please answer separately, divided into weekdays and holidays.

Weekday

	Name of facility	Frequency of visit	Sojourn time per visit, and visited time zone
1st			
2nd			
3rd			

Holiday

	Name of facility	Frequency of visit	Sojourn time per visit, and visited time zone
1st			
2nd			
3rd			

Q8. Please check all the transportation that you have used at least once within the last two years.

□ Family car □ Bus □ Train
□ Taxi □ Chauffeur service □ Plane
□ Company car □ Car of welfare service □ Others ( )

Q9. Please check all the clinical departments where you have visited (including visit the office, attendance, and see someone) at least once within the last two years.

□ Internal medicine □ Surgery □ Dermatology
□ Dentistry □ Ophthalmology □ Urinology
□ Otorhinolaryngology □ Orthopedics □ Plastic surgery
□ Psychiatry □ Radiology □ Psychosomatic medicine
□ Obstetrics and Gynecology □ Others ( )

Q10. Did you change your residence within the last two years?

□ Yes □ No

Q11. Have you stayed more than one month at a facility other than your home (e.g., nursing home, training institute) within the last two years?

□ Yes □ No

➔ If "Yes," please write the place(s) and the period(s) that you have stayed.

( )

Q12. Please check all the regions you have visited at least once within the last two years.

Areas of Yamagata other than the residence area: □ Murayama, □ Mogami, □ Oitama, □ Shonai

□ Miyagi Pref. □ Fukushima Pref. □ Akita Pref.
□ Aomori Pref. □ Iwate Pref. □ Hokkaido
□ Kanto region □ Chubu region □ Kinki region
□ Chugoku region □ Shikoku region □ Kyushu region

➔ If you checked more than one region, proceed to Q13.

➔ If you have not visited any region above, proceed to Q14.

Q13. Within the regions you checked at Q12, please provide details (when and how long did you visit, etc.) of the top three places that you can recall.

	Place	Details of visit
1st		
2nd		
3rd		

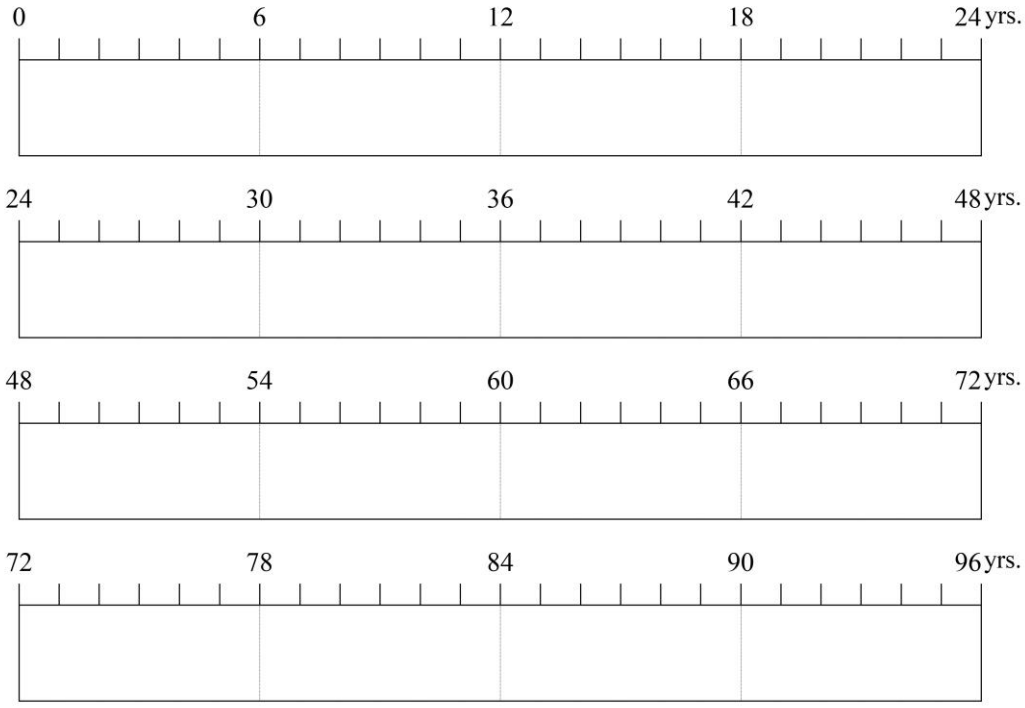
III. Questions about your lifestyle since you were born

Some people show onset of tuberculosis after a lapse of more than two years (sometimes after many decades) following an earlier tuberculosis infection. For tuberculosis control, it is very important for us to consider the places of tuberculosis infection. In this section, please answer about your lifestyle since you were born.

Q14. Where did you live after you were born? Please mark the undernoted figure after reading the following notes.

- Separate the figure by a vertical line, and write the name of your city (e.g., Tendo, Yamagata).
- If you have stayed at foreign countries, write the name of the province (e.g., Yunnan, China).
- If you have stayed at a facility such as a nursing home, write the facility address.

Current age _____ years old



Q15. Please check all the workplaces at which you have worked.

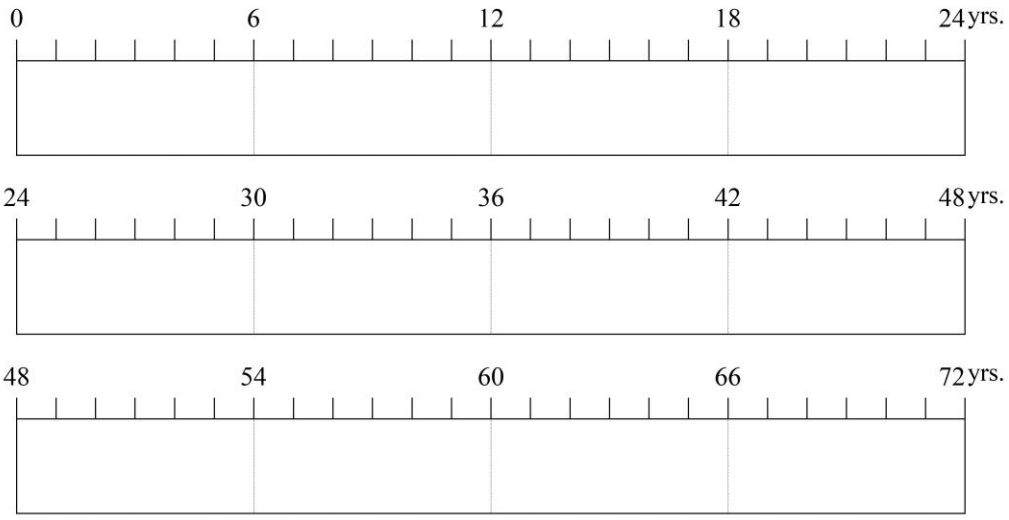
□ Medical agency	□ Welfare facility	□ Restaurant, drinking spot
□ Pachinko parlor	□ School	□ Cram school
□ Construction industry	□ Delivery business	□ Hotel business

Q16. Have you ever gone abroad?

□ Yes □ No

➔ If "Yes," please provide some details about when and where you went abroad.

Q17. Please write the schools and workplaces that you attended (or worked). Please turn off an undernoted figure separating the figure by a vertical line. Write the specific names of schools and workplaces (e.g., X kindergarten, Y corporation).



The questionnaire is finished. Thank you for your cooperation. We would appreciate your further cooperation with any additional questions.

WE WILL END TB

WHO, 2016

Short Questionnaire

Questionnaire for early findings of tuberculosis

This questionnaire is intended to show us the places where tuberculosis transmissions occur. Please respond with information about your past lifestyle. We would like to use your cooperation to decrease tuberculosis infection in Yamagata.

Please feel free to contact us if you have any questions.

Public health center:_____ Tel.:_____ Person in charge:_____

I. Questions about your lifestyle of the last two years

Most patients show onset of tuberculosis (or have it discovered) within two years of their infection. In this section, please answer about your lifestyle for the last two years.

Q1. Please check the box (□) to all the facilities that you have visited at least once within the last two years.

- □ Convenience store
- □ Pachinko parlor
- □ Parlors of Japanese chess, mah-jong, or game of go
- □ Theater
- □ Public bathhouse
- □ Internet cafe
- □ Indoor sports facility
- □ Welfare facility (e.g., nursing home, facilities for the disabled)
- □ Ceremonial function (e.g., bridal, burial)
- □ Homes of friends, or close relatives
- □ Drinking spot (e.g., bar, pub)
- □ Karaoke bar
- □ Culture lesson (e.g., cooking class, karaoke class)
- □ Barber shop
- □ Religious space (e.g., church, temple)
- □ Amusement arcade
- □ Academy, cram school
- □ Neighborhood meeting (e.g., neighborhood association, volunteer fire company)
- □ Dormitory for student or office worker
- □ Other place ( )

Q2. Did you change your residence within the last two years?

□ Yes □ No

Q3. Have you stayed more than one month at a facility other than your home (e.g., nursing home, training institute) within the last two years?

□ Yes □ No

Q4. Please check all the transportation that you have used at least once within the last two years.

- □ Family car
- □ Bus
- □ Train
- □ Taxi
- □ Chauffeur service
- □ Plane
- □ Company car
- □ Car of welfare service
- □ Others ( )

Q5. Please check all the clinical departments where you have visited (including visit the office, attendance, and see someone) at least once within the last two years.

□ Internal medicine	□ Surgery	□ Dermatology
□ Dentistry	□ Ophthalmology	□ Urinology
□ Otorhinolaryngology	□ Orthopedics	□ Plastic surgery
□ Psychiatry	□ Radiology	□ Psychosomatic medicine
□ Obstetrics and Gynecology	□ Others ( )	

II. Questions about your lifestyle since you were born

Some people show onset of tuberculosis after a lapse of more than two years (sometimes after many decades) following an earlier tuberculosis infection. For tuberculosis control, it is very important for us to consider the places of tuberculosis infection. In this section, please answer about your lifestyle since you were born.

Q6. Please check all the workplaces at which you have worked.

□ Medical agency	□ Welfare facility	□ Restaurant, drinking spot
□ Pachinko parlor	□ School	□ Cram school
□ Construction industry	□ Delivery business	□ Hotel business

Q7. Have you ever gone abroad?

□ Yes □ No

Q8. Do you remember any tuberculosis patients around you in the recent past?
(e.g., my grandfather developed tuberculosis)

□ Yes □ No

The questionnaire is finished. Thank you for your cooperation. We would appreciate your further cooperation with any additional questions.

WE WILL END TB

WHO, 2016