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Human *Mycobacterium bovis* Infections in London and Southeast England

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Variable-number tandem repeat (VNTR) and spoligotyping analyses were used to assess transmission of *Mycobacterium bovis* between humans. VNTR was more discriminatory than spoligotyping. Low case numbers, despite a substantial animal reservoir, and resolution of all isolates provided no evidence of recent human-to-human transmission or recent significant infection from animals.

Mycobacterium bovis is the causative agent of the majority of cases of bovine tuberculosis (TB) and is also associated with disease for a range of domestic and wild animals as well as humans. Only a small proportion (0.2 to 7.2%) of culture-confirmed cases of human TB in industrialized countries is caused by *M. bovis*, but it remains an important cause of TB in nonindustrialized countries (1). While an increase in the incidence of bovine TB in the United Kingdom has been observed since the early 1990s, primarily in southwest England and Wales, no increase in human *M. bovis* TB has been observed (1). Human *M. bovis* infection in the United Kingdom has remained at approximately 0.5% of culture-confirmed cases of human TB since the year 2000 (7, 8). The principal mode of transmission to humans is believed to be consumption of contaminated unpasteurized dairy products (12). Additional routes of transmission include close contact with infected animals or their carcasses. Human-to-human transmission may also play a role, although it remains rare (3). The proportion of cases due to *M. bovis* continues to be very low, suggesting that the epidemic in bovine and nonbovine animal species is not expanding into the human population to any significant degree. Spoligotyping and variable-number tandem repeat (VNTR) typing have been used to distinguish *M. bovis* isolates of both human and bovine origins. Based on these genotyping techniques we describe the genetic diversity of human *M. bovis* isolates from individuals in London and southeast England.

The study population comprised all culture-confirmed cases of human *M. bovis* infection reported from London and southeast England between January 2005 and December 2010. A case was defined as an individual who was culture positive for *M. bovis*. Positive cultures were identified using the GenoType MTBC DNAstrip (Hain Lifesciences, Nehren, Germany). Spoligotyping was performed as previously described, and spoligotypes were compared with the *M. bovis* specific database (www.mbovis.org) curated by the Veterinary Laboratories Agency (10). VNTR typing was performed as previously described using the loci indicated in Table 1 (4, 6, 13, 15). The resulting spoligotypes and VNTR profiles were analyzed using Bionumerics software (Applied Maths, St. Marten-Latem, Belgium). Clustering was done using a categorical index and the unweighted pair group method with arithmetic mean. The Hunter-Gaston index (HGI) was calculated to determine the discriminatory power of each method and for individual VNTR loci (9).

The study population contained 39 culture-confirmed human cases of *M. bovis*. The mean number of cases per year was 6.5 (range, 6 to 10). The majority of cases (59%) presented in London, with the remainder in surrounding Health Protection districts of Sussex and Surrey (15.4%), Kent (15.4%), and Thames Valley (10.3%). The mean age of cases was 49 years (range, 16 to 88 years). Seventeen of the 39 cases (43.6%) were reported in individuals born before 1960, i.e., of an age where childhood milk consumption might have occurred prior to comprehensive and complete milk pasteurization in the United Kingdom. When the site of disease was known (32 of 39 cases), 47% and 54% of cases were associated with pulmonary infection in those born before and after 1960, respectively. Extrapulmonary infection was associated with 53% and 46% of cases in those born before and after 1960, respectively. The proportions of human *M. bovis* cases in London and southeast England were 0.11% and 0.45%, respectively, over the period 2005 to 2009. These proportions were marginally lower than those reported over the period 2000 to 2005 (0.2% in the London region and 0.5% in the southeast region). Overall, the mean proportion of *M. bovis* cases in London and southeast England over the period 2005 to 2009 (0.17%) was lower than the national figure of 0.5%. This may have been due to a larger number of *M. bovis* cases in rural areas, including northeast, east, and southwest England, as observed in the period 2000 to 2005, possibly reflecting zoonotic infection (7). Surprisingly, given that 84% of tuberculosis cases in London are in patients that were born abroad, predominantly in countries where there are no effective bovine TB control programs, *M. bovis* accounts for a lower proportion of tuberculosis cases in London than the surrounding rural areas (8). For future prospective studies, country of birth data will be collected.

Spoligotypes were obtained for the 33 available isolates, producing 28 individual spoligotypes (HGI, 0.9886). Six isolates were unavailable for testing, of which 5 were from London and 3 were born before 1960. The largest cluster, comprising 3 isolates, belonged to international type SB0140, while the smaller clusters, each containing two isolates, belonged to international types

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TABLE 1 VNTR sets used to genotype human *M. bovis* isolates

Locus or summary HGI for VNTR set	MIRU-VNTR set[a]		HGI for locus
	VNTR-8	VNTR-24	
ETR-A	X	X	0.752
ETR-B	X	X	0.653
ETR-C	X	X	0.534
ETR-D	X	X	0.176
ETR-E	X	X	0.117
ETR-F	X		0
MIRU-2		X	0
MIRU-10		X	0
MIRU-16		X	0.511
MIRU-20		X	0.117
MIRU-23		X	0.171
MIRU-24		X	0.402
MIRU-26		X	0.443
MIRU-27		X	0.232
MIRU-39		X	0
MIRU-40		X	0
VNTR-424		X	0.171
VNTR-1955		X	0.373
VNTR-2163b	X	X	0.674
VNTR-2347		X	0.117
VNTR-2401		X	0.117
VNTR-3171		X	0.061
VNTR-3232	X		0.861
VNTR-3690		X	0.061
VNTR-4052		X	0.688
VNTR-4156		X	0
HGI (VNTR only)	0.998	0.998	
HGI (VNTR + spoligotyping)	1.0	1.0	

[a] MIRU, mycobacterial interspersed repetitive unit. The VNTR-8 set included eight loci and the VNTR-24 set included 24 loci. The HGI column shows the index for each locus, while the summary HGI values at the bottom of the table reflect the discriminatory power the MIRU-VNTR group collectively. VNTR-8 loci were identified from references 4, 13, and 15; VNTR-24 loci were identified from reference 6.

SB0121, SB0134, and SB0944. International type SB0140 is the most frequently seen spoligotype of *M. bovis* (>30% of all isolates) isolated from cattle and has a wide geographical range in the United Kingdom (2). Incidentally, the human isolates belonging to SB0140 were cultured from individuals born prior to 1960. One of the cases had extrapulmonary disease. Spoligotypes SB0121, SB0134, and SB0944 have been detected across the globe.

The VNTR analyses were more discriminatory than spoligotyping, as VNTR-8 and VNTR-24 analyses both revealed 31 different profiles (HGI, 0.9981). When the results of spoligotyping were combined with VNTR-8 or VNTR-24, all 33 isolates were resolved. False-positive clustering was observed with each method, as demonstrated by clustering rates (n/N) of 27.3%, 6%, and 6% for spoligotyping, VNTR-8, and VNTR-24, respectively. Among the 26 VNTR loci in panels VNTR-8 and VNTR-24, 5 were highly discriminatory (HGI, >0.6), 5 were moderately discriminatory (HGI, 0.3 to 0.6), 10 showed low discrimination (HGI, <0.3), and the remaining 6 loci were monomorphic within this population (14). From the data a minimal set of 5 VNTR loci (ETR-A, ETR-B, ETR-C, VNTR 2163b, and VNTR 3232) was identified that provided an HGI of 0.9981 and resolved the isolates into 31 unique profiles, as observed with the VNTR-8 and VNTR-24 panels.

This study demonstrates the effectiveness of VNTR analysis for typing human *M. bovis* isolates. Several studies have investigated the relevance of VNTR typing for *M. bovis* and various sets of loci have been proposed for the optimal resolution of isolates of animal origin, but studies on the performance of VNTR analysis for human *M. bovis* isolates are limited (5, 11). In our setting, the standard VNTR-24 analysis proved useful for discrimination of human *M. bovis* isolates. The level of discrimination observed was further improved when VNTR-24 analysis was combined with spoligotyping, which resulted in the resolution of all isolates into unique types, i.e., there was no evidence of recent human-to-human transmission of *M. bovis* seen in the population in London and southeast England. This is also the first report whereby a panel of VNTR loci (VNTR-8) previously identified as useful for the discrimination of animal *M. bovis* isolates has also been shown to be useful for the differentiation of human *M. bovis* isolates (15). Although the results of this study do not rule out the possibility of human-to-human transmission in a patient's foreign country of origin, it seems likely that locally acquired disease is more likely due to animal exposure/consumption of unpasteurized dairy products.

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