Intrafamilial Cluster of Pulmonary Tuberculosis Due to *Mycobacterium bovis* of the African 1 Clonal Complex

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A new clonal complex of *Mycobacterium bovis* present at high frequency in cattle from west central African countries has been described as the African 1 (Af1) clonal complex. Here, the first intrafamilial cluster of human tuberculosis cases due to *M. bovis* Af1 clonal complex strains is reported. We discuss hypotheses regarding modes of transmission.

**CASE REPORT**

**Case 1.** In February 2002, a 3-year-old girl born in Chad who had arrived in France a year ago was admitted to the Infectious Pediatric Disease Department of the Montpellier University hospital for suspicion of pulmonary tuberculosis. Clinical manifestations were a cough and a fever (38.5°C). The tuberculin skin test (TST) reaction was phlyctenular. Chest X rays (CXR) were normal. Three gastric aspirations were negative for acid-fast bacilli (AFB), but cultures yielded secondarily were normal. Three pleural effusions were diagnosed. The TST was considered to be reactive. Three sputum specimens, obtained subsequently, were later identified as *M. bovis* with a commercial multiplex line probe assay, GenoType MTBC (Hain Lifescience). Therapy consisted of a combination of INH, RMP, and EMB. EMB was discontinued after 3 months; INH and RMP were continued for a total period of 9 months. Patients in case 1 and case 2 were cured upon completion of the treatment regimen. To determine the infectious link between cases 1 and 2, the 6 *M. bovis* strains isolated (3 from the mother and 3 from the daughter) were genotyped by spoligotyping and mycobacterial interspersed repetitive-unit–variable-number tandem-repeat (MIRU-VNTR) analysis (Fig. 1) (1). The genetic link among the six isolates could be confirmed since the isolates presented identical spoligopatterns (SB1025 [www.Mbovis.org]) and the same MIRU-VNTR profile based on the analysis of 21 loci (Fig. 1).

In Chad, *M. bovis* strains with the SB1025 spoligotype are endemic in cattle, and they correspond to the *M. bovis* group recently described by Muller et al. as belonging to the African 1 (Af1) clonal complex (12). Our investigations have confirmed that these six human strains also belonged to the Af1 clonal complex since they presented 4 criteria of this group: (i) the presence of an Af1-specific deletion (RDAf1) in the genome (data not shown); (ii) the absence of spacer 30 in the spoligotype (Fig. 1); (iii) a spoligopattern regarded as deriving from the progenitor SB0944, which is the case for SB1025; and (iv) isolation from patients or animals originating from an area where this clonal complex is highly endemic, like Cameroon, Nigeria, and as in these cases, Chad (12).

In order to test the hypothesis of zoonotic exposure to *M. bovis* Af1 for cases 1 and 2, MIRU-VNTR patterns based on the analysis of loci ETR-A to ETR-E of SB1025 cattle strains included in the study by Muller et al. describing the Af1 clonal complex (12) were compared to the pattern of the six human strains described in our report. As shown in Fig. 1, SB1025 cattle strains have MIRU-VNTR profiles almost identical to that of the patient isolates except for ETR-D, for which the pleural effusion. The TST was considered to be reactive. Three sputum specimens, obtained subsequently, were negative for AFB. Cultures obtained from sputum samples were later identified as *M. bovis* with a commercial multiplex line probe assay, GenoType MTBC (Hain Lifescience). Therapy consisted of a combination of INH, RMP, and EMB. EMB was discontinued after 3 months; INH and RMP were continued for a total period of 9 months. Patients in case 1 and case 2 were cured upon completion of the treatment regimen. To determine the infectious link between cases 1 and 2, the 6 *M. bovis* strains isolated (3 from the mother and 3 from the daughter) were genotyped by spoligotyping and mycobacterial interspersed repetitive-unit–variable-number tandem-repeat (MIRU-VNTR) analysis (Fig. 1) (1). The genetic link among the six isolates could be confirmed since the isolates presented identical spoligopatterns (SB1025 [www.Mbovis.org]) and the same MIRU-VNTR profile based on the analysis of 21 loci (Fig. 1).

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In sub-Saharan Africa, bovine tuberculosis (bTB) represents a potential health hazard both for animals and for humans. Indeed, the majority of the human population live in close contact with cattle, in which the disease is highly prevalent and/or only imperfectly controlled (2). The WHO estimated that 70% of humans with tuberculosis (TB) and HIV coinfections live in sub-Saharan Africa (5). HIV is a major factor responsible not only for the progression of TB infection to active TB disease but also for increased risk of acquiring zoonotic TB in this area (5). The situation is quite different in developed countries. The systematic slaughter of positive-tuberculin-test cattle, the main measure of bTB control programs, has dramatically decreased bTB incidence in cattle herds and thus direct transmission to humans. The pasteurization of milk is a widespread measure that almost completely interrupted food-borne transmission of TB from cattle to the human population (11). As a result of these expensive control efforts, the risk of contracting zoonotic TB has become extremely low in developed countries (11). Consequently, most human cases of M. bovis tuberculosis in developed countries are nowadays found in people born before the introduction of the above-mentioned control measures or originating from countries where these measures are not applied (8). The local geographical dominance of specific clonal complexes of M. bovis, characterized by specific spoligopatterns, seems to be a feature of the global population structure of this pathogen (7). The M. bovis Af1 complex is the clonal complex present at high frequency in the cattle populations from several sub-Saharan west central African countries (12), but other clonal complexes have also been characterized recently (4). Indeed, Smith et al. have described an Eu1 clonal complex dominant in the British Isles but also in countries directly or indirectly linked to the United Kingdom by cattle trading (15). Furthermore, Berg et al. have recently described a new clonal complex in Africa, the Af2 clonal complex, which is dominant in countries of East Africa (Uganda, Burundi, Tanzania, and Ethiopia) (4; N. H. Smith et al., unpublished data). The Eu1, Af1, and Af2 clonal complexes display specific genetic signatures that permit differentiation from one another (4, 12, 15).

We have reported here the first intrafamilial cluster of human tuberculosis cases due to M. bovis African 1 clonal complex strains.

DNA fingerprinting using MIRU-VNTR analysis plus spoligotyping proves to be a practical discriminatory method to confirm molecular epidemiological links and extend our understanding of dynamic of transmission of M. tuberculosis or M. bovis (1). These molecular tools have been used thoroughly to explore the hypothesis of genetic links between interhuman cases and to compare human to cattle strains (6, 8, 9, 14, 16, 17). As spoligotype SB1025 is endemic in cattle in Chad, this suggests that the patients’ infection occurred in their country of origin. In the first hypothesis, the mother and the daughter could have been contaminated by a common human, animal, or food-borne source in Chad, with subsequent TB reactivation in the daughter and the immunocompromised mother in France (Fig. 2). Indeed, human TB due to M. bovis could be acquired by inhalation of cough aerosols from infected cattle or consumption of contaminated milk (8, 9, 16). The rate of positive detection of AFB by sputum smear examination is low, owing to the paucibacillary nature of pulmonary TB, particularly in children and in HIV-infected individuals (3, 10). However, Behr et al. have demonstrated that patients who are smear negative and culture positive for TB contribute significantly to the transmission of TB (3). This is why an intrafamilial TB transmission hypothesis can also be raised in this case. Person-to-person transmission of M. bovis from HIV-infected individuals is possible, and it occurs relatively frequently (8, 9, 16). In conclusion, our findings help to strengthen the hypothesis that intrafamilial transmission of M. bovis can occur, which could explain the clustering of tuberculosis cases described in this report.
and noninfected patients has been reported previously (6, 8, 17). In the second hypothesis, the daughter could have been infected in Chad, with subsequent TB reactivation in the daughter and daughter-to-mother transmission in France (Fig. 2). Daughter-to-mother transmission seems to be the most likely hypothesis because at the time of TB diagnosis for the daughter (the patient in case 1), contact investigations excluded TB in the mother (the patient in case 2), as she presented a negative TST and a normal CXR. But in HIV-infected patients, the diagnostic difficulties of TB are increased by the presence of nonspecific clinical and radiographic signs and by the low sensitivity of the TST due to the deficient cellular immune response (13). Interestingly, at the time of contact investigation for the daughter’s TB case (case 1), the mother (the patient in case 2) had a CD4+ cell count above 300 cells/µl and was not yet under antiretroviral treatment. In the third hypothesis, the mother could have been infected in Chad, with subsequent TB reactivation undiagnosed (TST and smear results were negative and M. tuberculosis cultures were not done) in the mother and mother-to-daughter transmission occurring in France (Fig. 2).

In conclusion, although M. bovis remains a rare cause of human TB cases in industrialized countries, it has to be searched for in symptomatic immigrants from countries where M. bovis is more prevalent in both cattle and humans. As illustrated in this report, despite the high discriminatory power of the molecular epidemiology tools, i.e., spoligotyping and MIRU-VNTR typing, intrafamilial transmission could not be demonstrated definitely and the source of contamination remains unknown. Studies using molecular methods remain necessary, however, to better understand the transmission dynamics and the circulation of the Afl clonal complex of M. bovis within and between the different animal and human compartments.

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