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REFERENCES

1 Coker R, Bell A, Pitman R, *et al.* Tuberculosis screening in migrants in selected European countries shows wide disparities. *Eur Respir J* 2006; 27: 801–807.
2 Story A, Murad S, Verheyen M, *et al.* Tuberculosis in London – the importance of homelessness, problem drug use and prison. *Thorax* 2007; 62: 667–671.
3 World Health Organisation. Western Pacific Region. Assessing tuberculosis prevalence through population-based surveys. Geneva, World Health Organization, 2007.
4 Balabanova Y, Coker R, Fedorin I, *et al.* Variability in interpretation of chest radiographs among Russian clinicians and implications for screening programmes: observational study. *BMJ* 2005; 331: 379–382.
5 Shaw NJ, Hendry M, Eden OB. Inter-observer variation in interpretation of chest X-rays. *Scott Med J* 1990; 35: 140–141.
6 Nyboe J. Results of the international study on x-ray classification. *Bull Int Union Tuberc* 1968; 41: 115–124.
7 den Boon S, White NW, van Lill SW, *et al.* An evaluation of symptom and chest radiographic screening in tuberculosis prevalence surveys. *Int J Tuberc Lung Dis* 2006; 10: 876–882.
8 den Boon S, Bateman ED, Enarson DA, *et al.* Development and evaluation of a new chest radiograph reading and recording system for epidemiological surveys of tuberculosis and lung disease. *Int J Tuberc Lung Dis* 2005; 9: 1088–1096.
9 Graham S, Das GK, Hidvegi RJ, *et al.* Chest radiograph abnormalities associated with tuberculosis: reproducibility and yield of active cases. *Int J Tuberc Lung Dis* 2002; 6: 137–142.
10 Zellweger JP, Heinzer R, Touray M, *et al.* Intra-observer and overall agreement in the radiological assessment of tuberculosis. *Int J Tuberc Lung Dis* 2006; 10: 1123–1126.
11 de Vries G, van Hest RA, Richardus JH. Impact of mobile radiographic screening on tuberculosis among drug users and homeless persons. *Am J Respir Crit Care Med* 2007; 176: 201–207.
12 Crofts JP, Gelb D, Andrews N, *et al.* Investigating tuberculosis trends in England. *Public Health* 2008; 122: 1302–1310.
13 CDC. Instructions to panel physicians for completing chest x-ray and classification worksheet (DS-3024). www.cdc.gov/ncidod/dq/dsforms/3024.htm Date last updated: August 9, 2006. Date last accessed: June 4, 2008.
14 Nakamura K, Ohmi A, Kurihara T, *et al.* [Studies on the diagnostic value of 70 mm radiophotograms by mirror camera and the reading ability of physicians.] *Kekkaku* 1970; 45: 121–128.
15 Frieden T. Toman's Tuberculosis. Case Detection, Treatment and Monitoring. Geneva, World Health Organisation, 2004; pp. 92–95.
16 Dasgupta K, Menzies D. Cost-effectiveness of tuberculosis control strategies among immigrants and refugees. *Eur Respir J* 2005; 25: 1107–1116.
17 Tulsky JP, Hahn JA, Long HL, *et al.* Can the poor adhere? Incentives for adherence to TB prevention in homeless adults. *Int J Tuberc Lung Dis* 2004; 8: 83–91.

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Importance of identifying *Mycobacterium bovis* as a causative agent of human tuberculosis

To the Editors:

The case study described hereafter emphasises the potentially vital importance of *Mycobacterium bovis* identification for appropriate tuberculosis patient management.

M. bovis, the classical causative agent of bovine tuberculosis, can be responsible for human TB, which makes this bacterium an important zoonotic species. In developed countries, the introduction of pasteurisation, preventing contamination from milk of infected cows, and eradication programmes for infected herds have considerably reduced the prevalence of human disease due to the bovine TB bacillus, but have not completely eradicated it [1–3]. Nevertheless, in many developed countries, the possibility of TB due to *M. bovis* infection, instead of *Mycobacterium tuberculosis*, is considered unlikely or even disregarded by microbiologists and clinicians.

Distinction of *M. bovis* from *M. tuberculosis* has significant relevance to patient management. In contrast to the other members of the *M. tuberculosis* complex, *M. bovis* is intrinsically resistant to pyrazinamide [4]. Beyond its use for specific *M. bovis* identification, this natural resistance is particularly important to consider. Pyrazinamide is usually given in the classical first-line TB treatment, as it is an effective sterilising drug that helps to shorten TB therapy due to its synergistic effect with rifampicin [5]. Thus, in case of *M. bovis* infection, pyrazinamide would be ineffective if implemented in a patient's anti-TB regimen. Unfortunately, results of *in vitro* susceptibility assays to pyrazinamide by conventional methods are not systematically correlated to *in vivo* activity. Susceptibility is, therefore, not often routinely assayed in laboratories. In addition, TB due to *M. bovis* is clinically, radiologically and microscopically indistinguishable from disease caused by *M. tuberculosis*. After culture on solid media, *M. bovis* displays specific characteristics like dysgonic colonies, and negative biochemical test results for nitrate reduction and niacin accumulation. However, clinical laboratories are increasingly using automated liquid culture systems followed by molecular tests for faster isolation and identification. Classical biochemical tests to identify *M. bovis* are not applicable using such liquid media, and most classical molecular identification methods based on targets like IS*6110*, 16S rDNA, 23S rDNA or ITS, do not distinguish between *M. bovis* and the other members of the *M. tuberculosis* complex. Other genetic markers [6] and the single commercial test (GenoType Mycobacterium, Hain, Nehren, Germany) allowing distinction between *M. tuberculosis* complex members are not widely used.

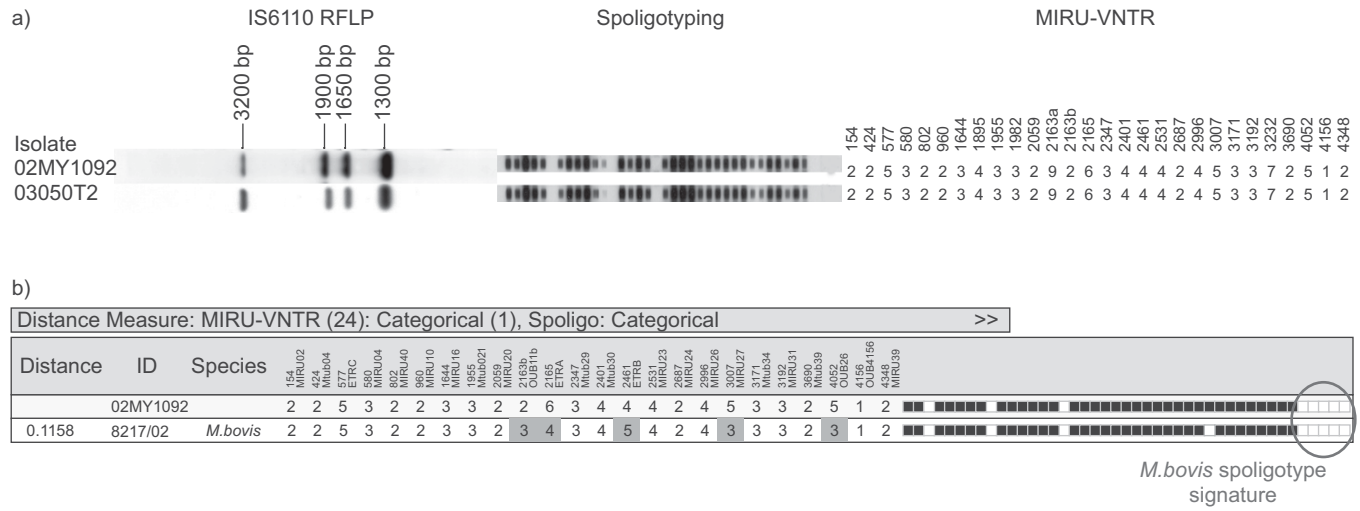

FIGURE 1. *Mycobacterium bovis* strain genotypes and identification. a) Comparison of human (above) and bovine (below) isolate genotypes. b) *M. bovis* identification based on MIRU-VNTR*plus* best match analysis. Grey-shaded boxes indicate variant mycobacterial interspersed repetitive unit variable-number tandem repeat (MIRU-VNTR) alleles between the human isolate and an *M. bovis* reference strain. Please note that identification in MIRU-VNTR*plus* is based on the set of 24 MIRU-VNTR loci standardised for *Mycobacterium tuberculosis* [8, 11]. According to standardisation, MIRU-VNTR markers are designated by their position in kbp on the *M. tuberculosis* H37Rv chromosome. RFLP: restriction fragment length polymorphism.

Here, we report a case of a 70-yr-old female living on a farm in Belgium. She was admitted in May 2001 with altered health condition and important weight loss, with a weight of only 32 kg. TB was diagnosed, and considered to be a reactivation of an infection contracted previously, as pulmonary lesions were already documented in 1994. At the time of hospitalisation, her sputa were strongly smear positive and the mycobacteria obtained from culture were identified as *M. tuberculosis* complex using a routine in-house PCR test based on IS*6110* insertion sequence. Susceptibility testing for three first-line drugs (isoniazid, rifampicin and ethambutol) was performed, and isolates were susceptible to these three drugs. The patient received a classical tritherapy comprising isoniazid, rifampicin and pyrazinamide (in Belgium, ethambutol is normally prescribed only for patients originating from high TB incidence countries). She did not tolerate *per os* medication and received intravenous regimen. However, she developed drug hepatitis and the dosage was adjusted. Cavitary lesions on chest radiography initially enlarged during hospitalisation and sputa became negative only 6 weeks later. The patient gained 4.5 kg of weight (36.5 kg) and left the hospital with the same regimen of antituberculous drugs, two months after her admission.

5 months later, in December 2001, the patient, who was still on standard isoniazid and rifampicin follow-up regimen, was readmitted with increasing cough and dyspnoea, and a weight of 34.5 kg. There was wheezing on both lungs with a sort of lapping at the upper left lung lobe. Thorax radiography showed that this lobe was largely damaged and replaced by a large cavity. Although culture was negative after 4 weeks, sputum was smear positive.

During this second admission, transaminases suddenly increased, and gastroenterology examination suggested a novel episode of isoniazid-related hepatitis. This sudden recurrence of hepatitis was thought to result from lack of compliance of the patient at home and restored adherence to her anti-TB treatment after readmission. Therefore, isoniazid was replaced by pyrazinamide in addition to ethambutol in the follow-up regimen. The patient left the hospital after 3 weeks. She died from respiratory insufficiency 2 months later.

The mycobacterial strain infecting this patient was subsequently identified as *M. bovis*, independently from the above clinical history as follows. In 2002, the veterinary inspection service detected TB lesions on a cow at the slaughterhouse and subsequently a TB outbreak in cattle due to *M. bovis* on a farm. Epidemiological inquiry mentioned a case of human TB in the farmer's family, namely the patient described above. Consequently, the veterinary public health service alerted the medical public health service. Mycobacterial interspersed repetitive unit variable-number tandem repeat (MIRU-VNTR) typing based on 28 loci [7, 8], spoligotyping [9] and IS*6110* restriction fragment length polymorphism [10] typing concordantly revealed fully matching genotypes for human and bovine isolates and disclosed, at the same time, the *M. bovis* identification of the patient's isolate (fig. 1) [11].

The two *M. bovis* isolates displayed a rare four-IS*6110* band fingerprint observed in only 3% of Belgian herds [7]. This observation rendered even more likely the conclusion that both isolates represented infection by the same *M. bovis* strain, based on the concordant results of the three genotyping techniques. Although the genotype identity of the human and bovine isolates does suggest zoonotic infection acquired on this farm, a definitive scenario for the transmission route could not be established. No culture was available to confirm the patient's suspected previous TB episode dating back to 1994, and thus to confirm or exclude subsequent reactivation of such infection. Moreover, retrospective investigation of available veterinary inspection data did not allow us to establish when the TB outbreak precisely started in this herd.

Regardless and more importantly, the initial absence of *M. bovis* identification of this patient's isolate compromised the efficiency of her treatments, and plausibly influenced the final fatal outcome. Because of this lack of specific identification, pyrazinamide was inadequately administered in the 2 months of induction chemotherapy and, worst, was inappropriately used to replace isoniazid in the follow-up treatment because of toxic hepatitis.

If the pneumologist had initially known that *M. bovis* was the causative agent, a treatment with isoniazid, rifampicin, and ethambutol, instead of pyrazinamide, would probably have been started. For the second episode, the pneumologist would probably have added a quinolone, again instead of pyrazinamide, in addition to ethambutol and rifampicin. In this context, nonutilisation of pyrazinamide would additionally have avoided hepatotoxic effects also known to be associated with this drug, for a patient prone to anti-TB drug-related hepatitis.

In conclusion, our report shows that absence of specific identification of *M. bovis* may have adverse consequences for TB patient management. We believe that even in high-income countries, human TB due to *M. bovis* is underestimated, because of frequent use of identification techniques that do not specifically distinguish *M. bovis* from the rest of the *M. tuberculosis* complex, and because susceptibility to pyrazinamide is not systematically tested. Therefore, clinical laboratories should routinely use molecular tests to differentiate *M. bovis* from *M. tuberculosis* and/or systematically check resistance to pyrazinamide. In addition, our report constitutes an additional example of the persistent significance of *M. bovis* as a zoonotic pathogen [1, 2], even in countries such as Belgium, which has been declared officially free of cattle TB according to the European Commission decision 2003/467/EC. Finally, it illustrates that molecular-guided cooperation between human and veterinary health services can improve detection of zoonoses.

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REFERENCES

1 Evans JT, Smith EG, Banerjee A, *et al.* Cluster of human tuberculosis caused by *Mycobacterium bovis*: evidence for person-to-person transmission in the UK. *Lancet* 2007; 369: 1270–1276.
2 LoBue PA, Moser KS. Treatment of *Mycobacterium bovis* infected tuberculosis patients: San Diego County, California, United States, 1994-2003. *Int J Tuberc Lung Dis* 2005; 9: 333–338.
3 Rivero A, Marquez M, Santos J, *et al.* High rate of tuberculosis reinfection during a nosocomial outbreak of multidrug-resistant tuberculosis caused by *Mycobacterium bovis* strain B. *Clin Infect Dis* 2001; 32: 159–161.
4 O'Donohue WJ Jr, Bedi S, Bittner MJ, *et al.* Short-course chemotherapy for pulmonary infection due to *Mycobacterium bovis*. *Arch Intern Med* 1985; 145: 703–705.
5 Blumberg HM, Burman WJ, Chaisson RE, *et al.* American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. *Am J Respir Crit Care Med* 2003; 167: 603–662.
6 Brosch R, Gordon SV, Marmiesse M, *et al.* A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. *Proc Natl Acad Sci USA* 2002; 99: 3684–3689.
7 Allix C, Walravens K, Saegerman C, *et al.* Evaluation of the epidemiological relevance of variable-number tandem-repeat genotyping of *Mycobacterium bovis* and comparison of the method with IS6110 restriction fragment length polymorphism analysis and spoligotyping. *J Clin Microbiol* 2006; 44: 1951–1962.
8 Supply P, Allix C, Lesjean S, *et al.* Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. *J Clin Microbiol* 2006; 44: 4498–4510.
9 Kamerbeek J, Schouls L, Kolk A, *et al.* Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *J Clin Microbiol* 1997; 35: 907–914.
10 van Embden JD, Cave MD, Crawford JT, *et al.* Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. *J Clin Microbiol* 1993; 31: 406–409.
11 Allix-Beguec C, Harmsen D, Weniger T, *et al.* Evaluation and strategy for use of MIRU-VNTRplus, a multifunctional database for online analysis of genotyping data and phylogenetic identification of *Mycobacterium tuberculosis* complex isolates. *J Clin Microbiol* 2008; 46: 2692–2699.

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