Vertebral Spondylodiscitis With *Mycobacterium bovis*: Failure of Detection by PCR-Based IS6110 Analysis

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(ABSTRACT: max. 50 words)

*Mycobacterium bovis* is responsible for a zoonosis originating in cattle. We report a case of a man with vertebral spondylodiscitis caused by *Mycobacterium bovis*. Diagnosis was complicated because of the lack of IS6110. These strains are rare, but microbiologists should be aware of their existence.

**CASE REPORT**

In May 2009, a 72-year-old man, born in Belgium, was seen in the orthopaedic department because of back pain with right sided radiation since a few weeks. This back pain progressively decreased his ability to perform daily activities. He had no pulmonary or cardiac complaints. He had already received antibiotics (first amoxicillin-clavulanic acid, then ciprofloxacin and oxacillin) by his general practitioner, for a presumptive diagnosis of bacterial discitis.

His previous medical history was unremarkable. The patient was a diplomat and ambassador, and had lived in almost every continent. His carrier started in Kinshasa in 1965 and ended in Canberra in
2002. He actively competed in running competitions. Relevant history included a 7-year stay in Asia, including Pakistan, Malaysia, and Thailand, returning 10 years prior to hospitalisation. The patient had consumed dairy products and non-pasteurised milk in Asia, and also had close contact with wild boar.

During his hospitalisation, a magnetic resonance imaging study was performed which showed the presence of a spondylodiscitis involving D9 and D10 vertebral bodies and a beginning paravertebral abscess. Biological testing showed a white blood count of 7130/mm³, a C-reactive protein level of 3.90 mg/dl, and an erythrocyte sedimentation rate of 17 mm.

Culture of a biopsy of the involved disc was negative. Blood cultures were taken, but remained negative. Mycobacterial culture was not performed. He was treated with intravenous antibiotics amoxicillin-clavulanic acid 1g four times a day and vancomycin 1g twice daily. The patient was discharged with oral amoxicillin-clavulanic acid 875 mg three times a day.

A few weeks later, the patient was rehospitalised for a follow-up NMR. This showed spondylodiscitis of D8, D9 and D10 and a progression of the paravertebral abscess (Fig. 1A). At that time, a tuberculin skin test (TST) was performed and was positive, with an induration of 30x40 mm. Taking the diagnosis to be a Pott’s abscess, another biopsy of the involved region was performed and an anti-tuberculosis drug regimen was initiated, consisting of 300 mg rifampicin (RMP) twice daily, 300 mg isoniazid (INH) once daily, and 500 mg pyrazinamide (PZA) three times a day.

Ziehl-Neelsen stain of the biopsy was positive for acid-fast bacilli, however PCR testing for M. tuberculosis complex based on IS6110 was negative. Subsequently, clarithromycin 500 mg twice daily was associated to the triple therapy, because atypical mycobacteria could not be excluded at that time.

After 5 weeks, acid fast bacilli were grown on Löwenstein-Jensen (37°C). PCR for M. tuberculosis complex based on IS6110 was again negative but 16S rDNA sequencing showed an 100% homology with M. tuberculosis complex. The strain was niacin and nitrate reductase negative -which pointed
towards M. bovis and was sent to the National Reference Laboratory for Mycobacteria for confirmation and subtyping: another PCR based on the IS6110 element (using other primers and probes) was also negative and genotyping by IS6110-RFLP showed no bands. A multiplex PCR based on the deletion of a 12.7-kb DNA fragment in the genome of M. bovis compared to M. tuberculosis (1) identified the strain as M. bovis. A subsequent PCR revealed the presence of the RD1 region in the DNA (15) confirming that the isolated strain was M. bovis and not M. bovis BCG. Identification was also confirmed by GenoType MTBC (Hain). Drug susceptibility testing showed susceptibility to rifampicin, ethambutol and isoniazid but resistance to pyrazinamid with the specific C169G M. bovis mutation in the pncA gene (2). Spoligotyping revealed the octal code 616600000017600 and the MIRU-VNTR pattern 22532233235345423332512. A strain with a similar pattern had never been observed before in Belgium. Contact tracing found no incidence of TB among the patient’s relatives or colleagues.

Approximately one month later, the patient developed a drug-induced hepatotoxicity, probably due to INH and/or RMP. Both drugs were discontinued, as well as PZA after the confirmation of the etiologic agent being M. bovis (naturally resistant to PZA). Ethambutol (EMB) 15mg/kg once daily was started and levofloxacin 500 mg twice daily was associated once the liver enzymes normalized. Patient developed temporarily an opticus neuropathy caused by EMB. Magnetic resonance imaging was performed 3 months after the end of treatment, which showed no abscess but calcification around the involved discs and regression of the lesions (Fig. 1B). Symptoms and clinical findings showed excellent response so that the patient could participate again in running competitions with equally good results.
A few cases of possible symptomatic primary \textit{M. bovis} infection have been described in the literature \textsuperscript{(2, 3, 4, 5, 6, 7)}. Our case is unusual because the strain does not contain a copy of IS\textit{6110}. To our knowledge, this is the first isolation of a \textit{M. bovis} strain lacking IS\textit{6110} to be reported in Europe.

\textit{M. bovis}, a bacterial species of the \textit{M. tuberculosis} (MTB) complex, is a pathogen that primarily infects cattle. However, humans also can become infected, most commonly through consumption of unpasteurized dairy products or close contact with infected animals. Therefore, these infections are most often suspected of being of zoonotic origin \textsuperscript{(8)}. A few studies have estimated the proportion of \textit{M. bovis} infection in human tuberculosis cases to be in the range of 0.3 to 1.5\% in developed countries \textsuperscript{(7, 9, 10, 11, 12)}. Cases of interhuman transmission have only rarely been documented \textsuperscript{(6, 11, 13, 14)}. However, the exact source of infection remains undetermined in most cases. Although human disease caused by \textit{M. bovis} and other species of \textit{M. tuberculosis} complex are similar, the anatomic site of \textit{M. bovis} disease is more often extrapulmonary.

Genetically, all the members of \textit{M. tuberculosis} complex are extremely similar; having identical 16S rRNA sequences and 99.9\% genomical similarity at the nucleotide level \textsuperscript{(15)}. The list of its members \textit{(M. tuberculosis, M. bovis, M. bovis BCG, M. microti, M. canetti, M. africanum, M. caprae, M. pinnipedii, M. mungi, M. orygis)} is increasing.

The insertion sequence IS\textit{6110} is specific for the members of the \textit{M. tuberculosis} complex, and the difference in the location and number of copies of this IS\textit{6110} in the genome is a source of polymorphism between isolates. The first technique used to observe such polymorphisms was the IS\textit{6110} based restriction fragment length polymorphism (IS\textit{6110}–RFLP) \textsuperscript{(16)}. In contrast to \textit{Mycobacterium tuberculosis}, this technique provides only limited discrimination among \textit{M. bovis} isolates, since \textit{M. bovis} strains usually only have a single or a few copies of the IS\textit{6110} element.

Alternative markers used for genetic typing were both the DR element and the GC-rich repetitive
element (17, 18). The PCR-based genotyping techniques now currently used, spoligotyping (19) and MIRU-VNTR (20), have both shown a good discriminatory power on \textit{M. bovis} (8).

PCR has the potential to provide a more rapid, sensitive, and specific detection of \textit{M. tuberculosis} complex in clinical specimens. Most (commercial) assays are based on IS\textsubscript{6110} because of specificity for MTB-complex and the multi-copy number. However in Asia MTB without IS\textsubscript{6110} is described, and according to the literature, would occur more frequently in the group of Asian patients/travellers (8, 18, 21, 22, 23). Because of the much higher incidence of tuberculosis among recent immigrants compared to the general population it is likely that in the majority of these cases, the disease was contracted in the country of origin, thus increasing the likelihood of strains lacking IS\textsubscript{6110}. While the most immediate concern is the treatment of these patients, implementing the use of alternative genetic markers is important for identifying any spread of these strains within local communities and farther afield. This case report demonstrates that not all strains can be detected with primers directed towards IS\textsubscript{6110}. Because this can lead to false-negative results, multi-targeted testing in molecular assays should be rule.

In conclusion, although rare, primary \textit{M. bovis} infection should be considered in travellers and immigrants returning from an endemic zone, particularly in the case of a history of exposure to risk factors. The absence of IS\textsubscript{6110} is rare, but must be kept in mind, and should give drive to multi-targeted molecular testing.

REFERENCES


FIG. 1. Magnetic resonance imaging scans of the lumbar spine. (A) before treatment, arrow indicates the position of the paravertebral abscess around the involved discs. (B) follow-up NMR 3 months after the end of treatment.