



Case report

A rare case of spinal tuberculosis due to *Mycobacterium bovis*. Is zoonotic tuberculosis underdiagnosed?

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ABSTRACT

We report a rare presentation of Pott's disease caused by *M. bovis*, suggesting transmission from infected cattle, and only the second case described so far in scientific reports. Noteworthy of this case was that the strain was only isolated on Stonebrink medium, a sodium pyruvate-containing culture medium for the isolation of mycobacteria. This medium is frequently ignored in diagnostic laboratories and in the laboratory manuals of most international health organizations. In general laboratories use a culture medium that contains glycerol, a carbon substrate considered inhibitory for the growth of *M. bovis*. The use of glycerol-containing medium therefore likely contributes towards underestimating zoonotic tuberculosis. Our case suggests that, in order to improved surveillance efforts for zoonotic TB and increase the notification rate for *M. bovis* to human TB, the use of pyruvate-containing media should be promoted, particularly in developing countries with a high prevalence of bovine TB, but also through the World Health Organization' (WHO) *End TB Strategy* and the *Roadmap for Zoonotic TB*

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Introduction

Mycobacterium bovis, the causative agent of tuberculosis (TB) in cattle, is a member of the *Mycobacterium tuberculosis* complex (Mtb-complex). It can cross the species barrier and is the second most common cause of TB in humans after *M. tuberculosis*. Infection takes place via the consumption of unpasteurized, contaminated dairy products and their derivatives, or via direct contact with infected animals. Both *M. bovis* and *M. tuberculosis* share genetic homology, with no significant variation between their DNA sequences (<0.05 %), although differences exist in phenotypical characteristics and biochemical properties. [1] For example, *M. bovis* colonies appear on solid medium as smooth while *M. tuberculosis* strains have a rough or granular morphology. Also, the absence of the enzyme nitrate reductase in *M. bovis* is a characteristic used to differentiate between them in the microbiology laboratory. Another important distinction between the two species, especially for the isolation of *M. bovis* in culture medium, is

the ability to use carbon sources. The glycolysis in *M. bovis* is disrupted because of a single nucleotide polymorphism (SNP) in *pykA*, the gene that encodes a pyruvate kinase [2]. This mutation upsets its ability to utilize glycerol, the preferred carbon source of many mycobacterial species, including *M. tuberculosis*. Glycerol is the only carbon source present in most (commercial) culture media used for the isolation of mycobacteria. On solid medium containing glycerol as the sole carbon source, the growth of *M. bovis* is therefore 'dysgonic' or sparse and slow and colonies only appear after extended culturing. However, *M. bovis* grows readily on medium with pyruvate or Tween 80 as a carbon source [3].

Solid egg-based media are the mainstay of TB diagnosis in most countries, particularly in resource-constrained settings or regional laboratories. The diagnosis should include two different slopes: one with glycerol (Lowenstein-Jensen (LJ) medium) and another with pyruvate (Stonebrink medium). The use of two different carbon sources permits the isolation of both types of the Mtb-complex bacteria- the human and the bovine types- and provides an optimum yield of positive cultures.

Here we present a rare case of a *M. bovis* infection of the spinal cord. We were able to diagnose this special case because the laboratory uses both L-J and the pyruvate-containing Stonebrink

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medium on a routine basis for all clinical samples. Without the Stonebrink medium, the diagnosis of this *M. bovis* infection could have been delayed or completely missed. Consent for publication in print and electronically has been obtained from the patient

Case report

A 62-year-old female patient, who resides in Caracas, Venezuela, and had no previous relevant medical history was seen in January 2019, because of a lower back pain and a sciatica leg pain affecting both lower limbs. The straight leg raise test (Lasègue test) was positive, producing a radiating pain in both extremities. Other physical examinations, including a neurological examination, biochemical and cellular blood examinations, a chest X-ray and an electrocardiogram, were normal. An MRI (Magnetic Resonance Imaging) scan of the lumbar spine showed evidence of a fracture and pseudoarthrosis of L2-L3-L4 and lysis of discs L2-L3 and L3-L4. The patient was hospitalized with a differential diagnosis of infectious spondylodiscitis versus a space-occupying lesion (Fig. 1). A percutaneous transpedicular biopsy of the spine was cultivated on egg-based solid Lowenstein-Jensen and Stonebrink media. On Stonebrink medium, a confluent growth of smooth colonies was observed after 3 weeks of incubation. After 7 weeks of incubation (one week longer than the regular incubation time for cultures in most TB laboratories), dysgonic growth of a few small colonies of acid-fast bacilli were observed in L-J medium. Due to this dysgonic growth on said medium and the appearance of smooth colonies and atypical rough colonies of *M. tuberculosis*, the laboratory further characterized the Mtb-complex strain for the presence of regions of difference (RD), a molecular technique used to differentiate between the members of the MTBC based on variable regions among the genomes of the MTBC, which are results of insertion-deletion events. This genetic analysis revealed an RD9 and RD4 deletion and the presence of the region RD1, characteristic for a *M. bovis* strain [4]. Given that *M. bovis* is naturally resistant to pyrazinamide, the patient was treated with a standard TB treatment replacing pyrazinamide with moxifloxacin. A control MRI scan was performed after six months of treatment and showed a radiological improvement compared to the earlier imaging studies. The patient is now on the waiting list for a bilateral laminectomy of the L3, L4, and L5.

Discussion

Generally speaking, tuberculous spondylitis or spinal TB represents between 1 and 5% of the tuberculosis case load and is, in developed countries, a rare form of extra-pulmonary TB. The incidence of spinal TB in the US is low: in 2011, the rate was 1 case per 2 million people [5]. In the Netherlands, between 1993 and 2001, bone and joint TB, which includes spinal tuberculosis, accounted for 3.5 % of all tuberculosis cases [6] and depended on the geographic origin of the patients (0.2–1.1 % in patients of European origin, and 2.3–6.3 % in patients of non-European origin).

The diagnostic work-up of spinal TB is not easy and needs to be based on clinical symptoms, epidemiological factors, imaging tests such as CT and MRI, and a clinical sample for bacteriological, pathological or molecular confirmation [7]. The definitive diagnosis of spinal TB requires the isolation and identification of a causal agent in a biological sample. In the available scientific literature, spinal tuberculosis is generally caused by *M. tuberculosis* and indicates human to human transmission. An unusual cause of tuberculous spondylitis is infection with the vaccine strain, *Mycobacterium bovis* BCG, used for the immunotherapy of superficial transitional cell carcinoma of the urinary bladder. A recent review revealed 16 registered cases of *M. bovis* BCG vertebral osteomyelitis, secondary to this immunotherapy [8]. It is important to mention that *M. bovis* BCG can metabolize glycerol and will grow on glycerol-containing media due to a reversal of the mutation in the pyruvate kinase that happened during the serial passage of this vaccine strain in the attenuation process [2] and thus it will grow on L-J medium. Tuberculous spondylitis due to *M. bovis*, the pyruvate-dependent cattle strain, has been reported only once before in English scientific literature, namely in 2009 when a 72-year-old male patient from Belgium developed spinal tuberculosis [9].

Our strain was isolated on solid Stonebrink medium after 3 weeks of incubation and was easily recognized as different to *M. tuberculosis* by the smooth morphological characteristics of the acid-fast colonies. The same sample inoculated in L-J medium showed no growth after 6 weeks of incubation, the usual incubation time for the mycobacteriology laboratory. Failure to culture *M. bovis* due to unsuitable selective media has been recognized in several other publications [10–12]. In low-income

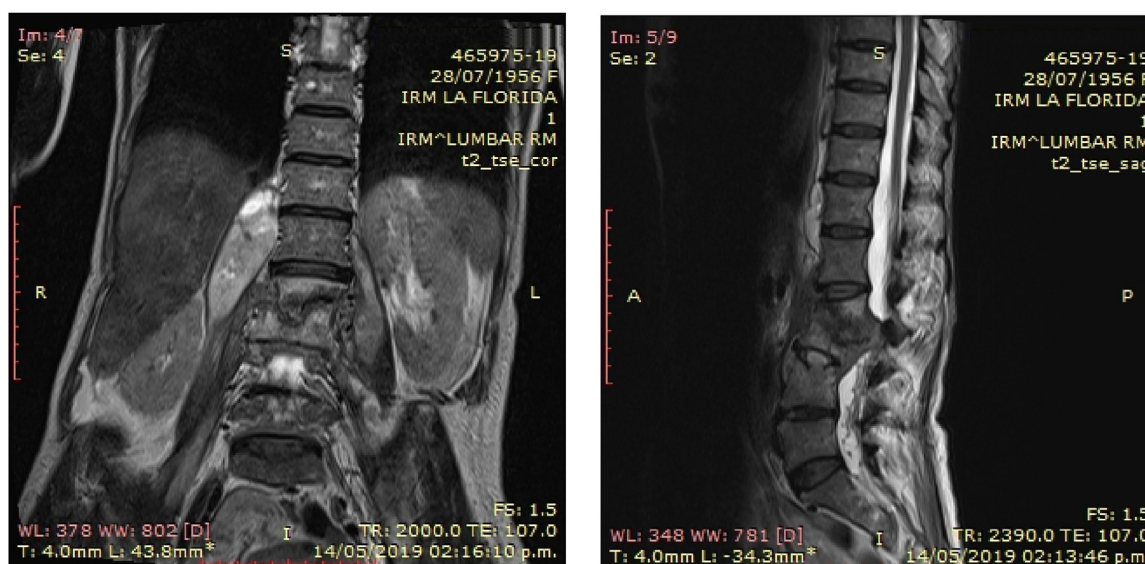


Fig. 1. Magnetic Resonance Imaging (MRI) scan of the lumbar spinal showing the collapse of L2-L3-L4 vertebral bodies.

and/or resource-constrained settings but also in developed countries, laboratory technicians generally use only L-J medium for TB culture. Solid culture media that support the growth of *M. bovis* are not often used [12] and are not even mentioned in the laboratory manuals endorsed by the WHO or the European Centre for Disease Prevention and Control (ECDC) [13,14]. Furthermore, “The roadmap for zoonotic tuberculosis”, a joint project by the WHO, FAO, IUATLD and OIE whose aim is to increase awareness for zoonotic TB, makes no mention of the special culture medium requirements for the isolation of *M. bovis* [15]. Therefore, the low coverage of culture methods, especially of those including pyruvate-containing media, which are appropriate for isolating *M. bovis*, will most probably contribute towards an underestimate of the zoonotic problem of TB [10–12].

To obtain reliable data on *M. bovis* infection, we strongly recommend the incorporation of a culture medium with pyruvate in the diagnosis of TB. Both *M. tuberculosis* and *M. bovis* will grow with pyruvate, while glycerol in the culture medium is an impediment for the isolation of *M. bovis*. Special attention should also be given to samples of extra-pulmonary origin, since tuberculosis caused by *M. bovis* infection is mainly extra-pulmonary [16]. Moreover, the limitations of molecular diagnosis for the diagnosing *M. bovis* infection should be taken into account, as the current commercially available molecular assays identify mycobacteria only to the complex level and are unable to differentiate *M. tuberculosis* from the closely related *M. bovis* and *M. bovis* BCG.

Authors' contribution

FECA and LADN did all the laboratory work, searched for relevant literature and wrote the first draft. AM and ME were the patient's internal medicine physicians and responsible for the patient's care, did a literature search and assist in writing the first draft. JHdW supervised the diagnostic procedures, helped to write the first draft, did literature research and wrote and submitted the final manuscript. All authors read and approved the final manuscript. Both FECA and LADN can be considered as first author.

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Declaration of Competing Interest

None declared.

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