

Pre-Multidrug-Resistant *Mycobacterium tuberculosis* Beijing Strain Associated with Disseminated Tuberculosis in a Pet Dog

Ana Botelho,^a João Perdigão,^b Ana Canto,^a Teresa Albuquerque,^a Nuno Leal,^c Rita Macedo,^d Isabel Portugal,^b Mónica V. Cunha^a

INIAV, I.P.—Instituto Nacional de Investigação Agrária e Veterinária, Unidade Estratégica de Produção e Saúde Animal, Lisbon, Portugal^a; Centro de Patogénese Molecular, URIA, Faculdade de Farmácia da Universidade de Lisboa, Lisbon, Portugal^b; Hospital Veterinário do Oeste, Lourinhã, Portugal^c; Laboratório de Saúde Pública: Micobacteriologia/Tuberculose, Departamento de Saúde Pública, Administração Regional de Saúde de Lisboa e Vale do Tejo, I.P., Lisbon, Portugal^d

Resistance to isoniazid, ethambutol, and streptomycin was detected in a *Mycobacterium tuberculosis* strain, belonging to the Beijing family lineage, isolated from two nodule exudates of a Yorkshire terrier with generalized tuberculosis. This report alerts medical practitioners to the risk of dissemination of pre-multidrug-resistant tuberculosis (preMDR-TB) through exposure to *M. tuberculosis*-shedding pets.

CASE REPORT

An 18-month-old Yorkshire male dog presenting symptoms of weight loss, cough, prostration, diarrhea, and hyperthermia (40°C) was clinically evaluated in a private veterinary practice. The dog was treated with metronidazole and cefixime without improvement. Subsequent clinical inspection detected two cutaneous nodules, in the right hind limb and scapula, from which exudates were collected for bacteriological analysis. Hematology tests revealed anemia, neutrophilia, hypoalbuminemia, and high gamma-glutamyltransferase and high serum alkaline phosphatase activities. Thoracic and abdominal radiographs together with abdominal echography revealed hepatomegaly, slight mediastinal and mesenteric lymph node enlargement, and abdominal effusion. Cytological preparations from lymph nodes, using May-Grunwald-Giemsa (MGG) staining, revealed pyogranulomatous inflammation, with abundant negative-staining rods in the macrophage cytoplasm, suggestive of generalized tuberculosis (TB) disease with hematogenous spread. Exudate samples, collected from the two nodules, were processed and decontaminated for bacteriological analysis, according to World Organisation for Animal Health (OIE) manual standard procedures (1), and inoculated onto Bactec 9000 liquid medium and Stonebrink, Lowenstein-Jensen, Lowenstein-Jensen with thiophen-2-carboxylic acid hydrazide, and Lowenstein-Jensen with pyruvate solid media. The isolate (number 1527) was identified as *Mycobacterium tuberculosis*/*Mycobacterium africanum* type II by an in-house PCR-restriction endonuclease analysis system (PCR-REA), based on *gyrB* gene amplification, followed by hydrolysis with *RsaI* and *SacII* restriction enzymes, as reported previously (2).

The identification of *M. tuberculosis*, rather than other animal-associated members of the *Mycobacterium tuberculosis* complex (MTBC), as the causative agent of extrapulmonary tuberculosis (TB), prompted us to investigate the genetic relatedness of this animal isolate with clinical isolates recovered from human TB cases. Spoligotyping and 24-locus mycobacterial interspersed repetitive-unit-variable-number tandem-repeat (MIRU-VNTR) analysis were performed to genotype the *M. tuberculosis* isolate. *M. tuberculosis* H37Ra was used as a positive control. For spoligotyping, the existing spacer regions in the direct repeat (DR) locus were amplified using GoFlexiTaq polymerase (Promega) and 20 ng of genomic DNA, as reported earlier (3). Detection was carried out

by reverse hybridization on a membrane with amino-linked immobilized probes for the standard set of 43 spacer regions, using an ECL chemiluminescence detection system (GE Healthcare), following the manufacturer's instructions. Lineage, clade, and shared international type (SIT) assignments of spoligotyping profiles were done using the SITVIT WEB international database (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/index.jsp) (4). The isolate was classified as the most frequent collected SIT, SIT 1, which is associated with the Beijing family (Table 1).

MIRU-VNTR amplification was done as described before (5) in triplex amplification reactions. Amplicon sizing was performed by capillary electrophoresis in an ABI 3130XL platform (Applied Biosystems), using a custom 1,200-bp ROX-labeled MapMarker molecular mass marker. Complementary genotyping, based on 24-locus MIRU-VNTR analysis, generated the profile displayed on Table 1.

A combined comparison of the 24-locus MIRU-VNTR and spoligotyping profiles with the profiles from 186 characterized strains deposited in the MIRU-VNTRplus database (<http://www.miru-vntrplus.org>) revealed that the most closely related strain was a Beijing lineage isolate recovered from the former Soviet Union, also classified as SIT 1 but differing on two MIRU-VNTR loci (loci 802 and 2165). Based on the same molecular markers, the genetic comparison of the animal strain with human TB isolates recovered from the Lisbon area failed to find any matching profiles (6, 7).

Given the importance of *M. tuberculosis* as an ecotype specifically adapted to human TB, the isolate was tested, under standardized conditions, for susceptibility to the first-line drugs used in the treatment of human TB and in the evaluation of multidrug resistance in human isolates. Drug susceptibility testing for isoniazid, rifampin, streptomycin, ethambutol, and pyrazinamide was performed using the automated fluorimetric Bactec MGIT960 system

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Address correspondence to Ana Botelho, ana.botelho@iniav.pt.

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TABLE 1 Molecular typing data and drug susceptibility profile of the *Mycobacterium tuberculosis* isolate

| Isolate | Host | DST ^a | Spoligotype | | No. of MIRU-VNTR repeats at indicated locus | | | | | | | | | | | | | | | | | | | | | | | |
|---------|------|------------------|-----------------|-----|---|-----|-----|-----|-----|-----|------|------|------|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | | | Octal | SIT | 154 | 424 | 577 | 580 | 802 | 960 | 1644 | 1955 | 2059 | 2163b | 2165 | 2347 | 2401 | 2461 | 2531 | 2687 | 2996 | 3007 | 3171 | 3192 | 3690 | 4052 | 4156 | 4348 |
| 1527 | Dog | ISE | 000000000003771 | 1 | 2 | 4 | 4 | 2 | 1 | 3 | 3 | 6 | 2 | 6 | 3 | 4 | 4 | 2 | 5 | 1 | 5 | 3 | 3 | 5 | 3 | 8 | 2 | 3 |

^a DST, drug susceptibility profile (ISE, resistant to isoniazid, streptomycin, and ethambutol).

(BD Diagnostics). Standardized drug critical concentrations and data interpretation followed the manufacturer instructions (BD Diagnostics). The isolate was found to be resistant to isoniazid, ethambutol, and streptomycin (Table 1) and can be considered a pre-multidrug-resistant strain.

The notion that *M. tuberculosis* lineages are almost exclusively associated with human TB has been progressively challenged with expanding descriptions in the literature of *M. tuberculosis* infections in domestic animals (8, 9) and wild animals (10–12). However, data gathered so far suggest that most *M. tuberculosis*-infected animals probably represent accidental hosts. In captive settings, a few cases of animal *M. tuberculosis* infection with a human origin have been reported (13). Thus, humans suffering from active TB are believed to represent the main source of *M. tuberculosis* lineages in animals, including cattle (14). *Mycobacterium tuberculosis* infection in dogs is rarely reported and has not been previously documented in Portugal. Recently, disseminated *Mycobacterium tuberculosis* infection, which was apparently caused by contact with infected owner, was also described in a pet dog in Brazil (15), although molecular typing was not performed to confirm this hypothesis. In our study, genotyping revealed that the dog strain represented the most frequent shared international type (SIT 1) among humans and belonged to a widespread *M. tuberculosis* genetic clade (the Beijing family). Pulmonary infections in dogs due to Beijing strains have been reported previously in TB high-risk settings (17, 18). No 24-locus MIRU-VNTR type similar to that of the dog isolate was found among the characterized clinical strains recovered from TB patients in the Lisbon area. Its susceptibility profile was also uncommon; it was found to be resistant to isoniazid, ethambutol, and streptomycin and can be considered a pre-multidrug-resistant TB strain. Analysis of the published laboratory data on human TB from a 6-year period in Lisbon showed that this resistance profile was reported only once, back in 2005 (7). The recovery, from animals that live in proximity with humans, of a strain resistant to the first-line drugs used in TB treatment may represent an increased risk for the dissemination of multidrug-resistant tuberculosis (MDR-TB). To our knowledge, this is the first report of an *M. tuberculosis* animal infection involving a drug-resistant strain. Since the owners had no clinical symptoms consistent with TB and the pet had no contact with other animals, the infection could possibly have occurred in the first few months after birth, while the animal was still with the breeder. However, this hypothesis could not be further confirmed since, after TB diagnosis, the animal owners were uncooperative and poorly adherent to clinical recommendations. Because of public health concerns, which were aggravated by the worsening physical condition of the dog, euthanasia of the animal was performed 2 months after the first symptoms.

Exposure to *M. tuberculosis*-shedding pets and captive wild animals raises public health concerns, particularly because such animal

TB cases are caused by an ecotype specifically adapted to human infection. Cases of tuberculosis of pets have been scarcely reported in Portugal, but it is possible that they go unnoticed, so the true impact of these situations in public health is yet to be clarified.

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