

Human-to-human and human-to-dog *Mycobacterium tuberculosis* transmission studied by IS6110 RFLP analysis: a case report

M. MORAVKOVA¹, M. SLANY¹, I. TRCKA¹, M. HAVELKOVA², J. SVOBODOVA³,
M. SKORIC⁴, B. HEINIGEOVA⁵, I. PAVLIK¹

¹Veterinary Research Institute, Brno, Czech Republic

²National Institute of Public Health, Prague, Czech Republic

³Regional Institute of Public Health, Brno, Czech Republic

⁴University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic

⁵Hospital Jindrichuv Hradec, Czech Republic

ABSTRACT: This study reports on the transmission of *Mycobacterium tuberculosis* of the same IS6110 RFLP type between two acquaintances with open pulmonary tuberculosis and a five-year-old Doberman bitch. No clinical signs, gross lesions at necropsy or histopathological lesions were observed in the infected lungs and gastrointestinal tract of the dog, although *M. tuberculosis* was directly detected by IS6110 PCR and culture examinations in the respiratory and gastrointestinal tracts. IS6110 PCR positivity in the faeces and blood of the dog poses a risk of *M. tuberculosis* transmission between the dog and humans.

Keywords: zoonosis; human tuberculosis; pet animal

Mycobacterium tuberculosis is primarily a human pathogen, although it can cause tuberculosis in animals that are in close contact with infected humans. Previous cases include monkeys, cattle, parrots, cats, dogs, domestic pigs and other animals (Ocepek et al., 2005; Pavlik et al., 2005; Pavlik, 2006; Peters et al., 2007; Une and Mori, 2007). Generally, it is assumed that dogs are resistant to mycobacterial infection, even though a few studies have been published in which infection with *M. tuberculosis* or other mycobacterial species, e.g., *M. bovis*, *M. avium* and *M. fortuitum* were described in canines (Gay et al., 2000; Horn et al., 2000; Irwin et al., 2000).

Most *M. tuberculosis* infections in dogs run subclinically, with pathological lesions localised mainly

in the lymph nodes, lungs, small intestine, liver, kidneys and spleen. Histopathological examinations show granulomatous inflammation with caseation. Since *M. tuberculosis* infections in dogs usually run subclinically, with a negative tuberculin test, intravital detection of *M. tuberculosis* in dogs is complicated (Bonovska et al., 2005). **When clinical signs are present, they are usually associated with the respiratory tract, although in rare cases they can be localised in other areas depending on the dissemination of the organism (Horn et al., 2000; Hackendahl et al., 2004).**

The current report describes the diagnosis of *M. tuberculosis* in one dog and the tracing of infection by IS6110 RFLP analysis of human and dog isolates.

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Case description

A 42-year old male, who was a strong smoker (60 cigarettes per day), alcoholic and long-term negativistic patient was hospitalized with acute respiratory, circulatory and hepatorenal failure and cachexia. In the anamnestic data, a significant cough (forming “black” sputum) lasting more than one year, long-term high fever (above 38 °C), dyspnoea and significant loss of weight (40 kg in 12 months) were present. A skiagraph of the chest revealed dilatation in the right-side of the heart, lung hypertension and massive lung infiltration with bronchiectasis. Huge amounts of acid-fast bacilli (AFB) were detected in the patient’s sputum after Ziehl-Neelsen (ZN) staining. Mycobacterial isolates obtained by culture were characterised as *M. tuberculosis*. Twenty-six days after hospital admission, the patient (diagnosed with open pulmonary tuberculosis) died of septic shock.

Four months before the man’s hospitalization, a eight-year-old Doberman bitch (D1) belonging to him died of chronic renal failure and was buried in the wood. The same month, the owner bought a five-year old clinically healthy Doberman bitch (D2). On the day of the owner’s hospitalization, the bitch (D2) was housed in the City police station. Eight days later, examination for *M. tuberculosis* was carried out at the police station due to the contact of the canine with workers and two service dogs. The IS6110 DNA locus specific for all members of the *M. tuberculosis* complex (MTC) was detected using a commercial PCR kit (Malamite, Brno, Czech Republic) in both the Doberman’s blood and faecal samples. As a result, the dog was euthanized 10 days later. Gross examination of all organs in the thoracic and abdominal cavities revealed no lesions, except

for one nodule (5 × 8 cm) in the mammary gland, which was later histopathologically diagnosed as a mammary adenocarcinoma. Granulomatous inflammation was not diagnosed by histopathological examination in any of the parenchymatous organs, mesenteric lymph nodes or in the intestinal mucosa after haematoxylin-eosin staining.

AFB were not detected by ZN staining in any of the histopathologically examined tissue samples. IS6110 PCR examination of the tracheobronchial, mediastinal, jejunal and ileal lymph nodes was positive, whereas examination of the liver, spleen, kidney, lungs and intestinal tissues was negative. Culture examinations revealed *M. tuberculosis* only in the IS6110 PCR-positive tissue samples. *M. tuberculosis* detection in this Doberman has been published previously in a paper describing the prevalence of bovine and human tuberculosis in cattle and other animals in central European countries (Pavlik et al., 2005).

The body of the eight-year old Doberman bitch (D1) was exhumed and examined eight months after its death. No signs of gross tuberculous lesions were observed in the lungs and submandibular lymph nodes, liver, spleen, kidney and mesenteric lymph nodes. AFB were not detected by ZN staining in any of the histopathologically examined tissue samples.

To determine the risk that the *M. tuberculosis*-infected Doberman bitch (D2) posed to other humans and animals, ten samples from the environment of the dog’s box in the City police station (soil, dust, water, faeces, the remains of feed and faecal samples of two other dogs) were examined. Three isolates from soil, dust and water samples were identified as *M. fortuitum* using the GenoType MTBC assay (Hain Lifesciences, Nehren, Germany). No other mycobacteria were isolated.

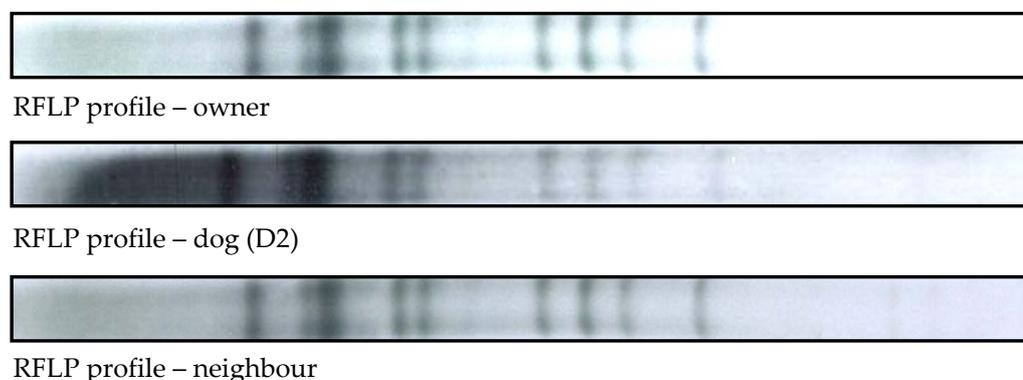


Figure 1. IS6110 RFLP type of *Mycobacterium tuberculosis* isolates from the dog (D2), owner and his neighbour obtained by endonuclease *PvuII* restriction

A detailed epidemiological investigation was conducted. It was found that nine months prior to the owner's hospitalization, his neighbour was diagnosed with open lung tuberculosis caused by *M. tuberculosis*. The two men spent time together on a daily basis especially in the pub. AFB staining of the neighbour's sputum gave a strongly positive result at microscopy, and he was diagnosed with the following: cachexia, chronic obstructive bronchopneumonia, subcompensated *cor pulmonale chronicum* and chronic alcoholism with hepatopatia. The neighbour was hospitalized for 90 days and his tuberculosis was treated with a combination of four antitubercotics (Isoniazide, Rifampicine, Pyrazinamide and Ethambutol). For the subsequent nine months, he was held in a tuberculosis treatment facility and successfully cured with Isoniazide and Rifampicine.

Human and canine isolates were examined by IS6110 restriction fragment length polymorphism analysis (RFLP) according to a previously published method (van Embden et al., 1993). IS6110 RFLP revealed that isolates from both the owner and his neighbour were of the same RFLP type; additionally, the isolate from the dog showed identical RFLP patterns (Figure 1).

DISCUSSION AND CONCLUSION

In our study, IS6110 was detected in both the Doberman's blood and faecal samples although no clinical signs were manifest and no gross or histological lesions were observed. The reason for this could be the short period of time (four months) during which the dog and tuberculosis-infected owner were in close contact. *M. tuberculosis* is usually present for only a short period in the blood and its persistence is associated with HIV/AIDS infection in humans (Chiu et al., 2007). In our case, the presence of *M. tuberculosis* in the blood could be caused by weakened immunity of the dog resulting from the adenocarcinoma, which was histopathologically diagnosed.

Comparative analysis of the *post mortem* results, obtained after cultivation and direct IS6110 PCR of the tissue suggested that PCR is a more suitable and faster method for the detection and identification of *M. tuberculosis* in dogs than cultivation. Similar observations have been made by others (Aranaz et al., 1996; Bonovska et al., 2005).

The localisation of *M. tuberculosis* in intestinal, mediastinal and tracheobronchial lymph nodes

shows an aerogenic and/or oral route of infection. Bonovska et al. (2005) reported that, after *per os* laboratory infection *M. tuberculosis* was later detected in dog lung tissue. Simultaneously, a risk of human infection in poor hygienic conditions can be posed by faecal contamination (Bonovska et al., 2005). In our study, *per os* infection was highly probable because the dog was fed with the owner's food leftovers (salted leftovers caused chronic nephritis in the older bitch). IS6110 RFLP analysis performed on three isolates from the infected dog matched isolates from the sputum of the dog's owner as well as the neighbour.

In conclusion, this and previously published studies suggest that the dogs of patients with open tuberculosis should also be tested for the presence of *M. tuberculosis* in faeces and blood. Canines could potentially be a source of *M. tuberculosis* transmission between other non-infected dogs or other animals through close contact (Bonovska et al., 2005). Whether the infected dogs are a potential source of infection for immunosuppressed humans remains to be elucidated. To our best knowledge, this is the first case of human-to-human and human-to-dog *M. tuberculosis* transmission in the Czech Republic.

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REFERENCES

- Aranaz A, Liebana E, Pickering X, Novoa C, Mateos A, Dominguez L (1996): Use of polymerase chain reaction in the diagnosis of tuberculosis in cats and dogs. *Veterinary Record* 138, 276–280.
- Bonovska M, Tzvetkov Y, Najdenski H, Bachvarova Y (2005): PCR for detection of *Mycobacterium tuberculosis* in experimentally infected dogs. *Journal of Veterinary Medicine Series B, Infectious Diseases and Veterinary Public Health* 52, 165–170.
- Chiu YS, Wang JT, Chang SC, Tang JL, Ku SC, Hung CC, Hsueh PR, Chen YC (2007): *Mycobacterium tuberculosis* bacteremia in HIV-negative patients. *Journal of the Formosan Medical Association* 106, 355–364.

- Gay G, Burbidge HM, Bennett P, Fenwick SG, Dupont C, Murray A, Alley MR (2000): Pulmonary *Mycobacterium bovis* infection in a dog. *New Zealand Veterinary Journal* 48, 78–81.
- Hackendahl NC, Mawby DI, Bemis DA, Beazley SL (2004): Putative transmission of *Mycobacterium tuberculosis* infection from a human to a dog. *Journal of the American Veterinary Medical Association* 225, 1573–1577, 1548.
- Horn B, Forshaw D, Cousins D, Irwin PJ (2000): Disseminated *Mycobacterium avium* infection in a dog with chronic diarrhoea. *Australian Veterinary Journal* 78, 320–325.
- Irwin PJ, Whithear K, Lavelle RB, Parry BW (2000): Acute bronchopneumonia associated with *Mycobacterium fortuitum* infection in a dog. *Australian Veterinary Journal* 78, 254–257.
- Ocepek M, Pate M, Zolnir-Dovc M, Poljak M (2005): Transmission of *Mycobacterium tuberculosis* from human to cattle. *Journal of Clinical Microbiology* 43, 3555–3557.
- Pavlik I (2006): The experience of new European Union Member States concerning the control of bovine tuberculosis. *Veterinary Microbiology* 112, 221–230.
- Pavlik I, Trcka I, Parmova I, Svobodova J, Melicharek I, Nagy G, Cvetnic Z, Ocepek M, Pate M, Lipiec M (2005): Detection of bovine and human tuberculosis in cattle and other animals in six Central European countries during the years 2000–2004. *Veterinarni Medicina* 50, 291–299. <http://www.vri.cz/docs/vetmed/50-7-291.pdf>
- Peters M, Prodinger WM, Gummer H, Hotzel H, Mobius P, Moser I (2007): *Mycobacterium tuberculosis* infection in a blue-fronted amazon parrot (*Amazona aestiva aestiva*). *Veterinary Microbiology* 122, 381–383.
- Une Y, Mori T (2007): Tuberculosis as a zoonosis from a veterinary perspective. *Comparative Immunology Microbiology and Infectious Diseases* 30, 415–425.
- van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, Hermans P, Martin C, McAdam R, Shinnick TM (1993): Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. *Journal of Clinical Microbiology* 31, 406–409.

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Corresponding Author:

Prof. MVDr. Ivo Pavlik, CSc., Veterinary Research Institute, Department of Food and Feed Safety, Hudcova 70, 621 00 Brno, Czech Republic
Tel. +420 533 331 601, Fax +420 541 211 229, E-mail: pavlik@vri.cz
