# Lung tuberculosis in a horse caused by *Mycobacterium avium* subsp. *avium* of serotype 2: a case report

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**ABSTRACT**: Interstitial pneumonia (2/3 of the lungs were affected) and diffusely enlarged bronchial and mediastinal lymph nodes were diagnosed by gross examination of a dead 16-year-old mare. Based on histopathological examination and the detection of acid-fast rods after staining by the Ziehl-Neelsen technique, tuberculosis was suspected. *Mycobacterium avium* subsp. *avium* of serotype 2 and IS*901*+/IS*1245*+ genotype was isolated from the pulmonary lymphnode after five-week incubation at 37°C. Due to the fact that horses have a naturally high resistance to mycobacterial infections, the high age of the mare most likely contributed to the development of the disease.

**Keywords**: avian mycobacteriosis; avian tuberculosis; *Mycobacterium avium* complex; zoonosis

Mycobacterial infections are very rare in horses. In the first part of 20th century mycobacterial infections in horses were mainly caused by *Mycobacterium bovis*. The situation has changed since the second part of 20th century, and the most common cause of mycobacterial infections in horses are now members of the *M. avium* complex (*MAC*). Generally, mycobacterial infections in horses arise via ingestion, though primary respiratory infection does occur. The prevailing clinical signs are chronic weight loss, diarrhoea, pyrexia, chronic pneumonia, septic arthritis and blindness in both eyes; abortions have also been documented, as analysed in a published review by Pavlik et al. (2004).

In the Czech Republic and in other Central European countries, bovine tuberculosis is one of the eradicated infectious diseases of domestic animals (Pavlik, 2006), wild animals (Trcka et al., 2006) and humans (Pavlik, 2006). The Czech Republic belongs (according to the Commission Decision No. 2004/320/EC of March 31, 2004) to those European countries officially free of bovine tuberculosis. The causative agent of human tuberculosis, *M. tuberculosis,* has been found only occasionally in domestic and wild animals in Central European countries during these years (Pavlik, 2006); nevertheless, potential infection with this pathogen cannot be precluded (Pavlik et al., 2004).

On the other hand, the description of *MAC* infection in different animal species is more common. *M. avium* subsp. *hominissuis* (*M. a. hominissuis*) of IS*901*–/IS*1245*+ genotype and serotypes 4–6, 8–11 and 21 causes infection mainly in pigs (Matlova et al., 2004; Pate et al., 2004; Pavlik et al., 2005). It is commonly isolated from the environment and from immunosupressed patients (Dvorska et al., 2002; Matlova et al., 2003; Reed et al., 2006). *M. avium* subsp. *avium* (*M. a. avium*) of IS*901*+/IS*1245*+ genotype and serotypes 1–3 is the agent of avian

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tuberculosis of birds; but it is also occasionally isolated from other animals such as pigs or cattle (Pavlik et al., 2000; Pate et al., 2004; Moravkova et al., 2007; Shitaye et al., 2007).

The diagnosis of lung tuberculosis in a horse used for teaching at the University of Veterinary and Pharmaceutical Sciences in Brno was serious; therefore, the purpose of the present study was to describe the clinical signs, gross findings and the specific identification of the mycobacterial isolate in a 16-year-old mare.

## Case history

**Clinical examination**. Inappetence and a febrile state not exceeding 39.5°C was detected in a 16-year-old warm-blooded mare from the property of the Faculty of Veterinary Medicine, Brno (Czech Republic).

Clinical examination of the mare did not show any pathology except for an increased breath sound over the entire lung fields. Endoscopic examination of the airways revealed secretion in the trachea. No lesions were found by examination of the upper respiratory tract. Haematological examination results were within reference range. Alveolar macrophages and a small amount of neutrophils were detected by cytological examination of the tracheal secretion, collected by transtracheal aspiration. Bacteria were not detected in the sample, either by cytological examination or bacteriological culture.

**Therapy and further examinations**. The mare was administered Procaine penicillin G (Biotika, Slovenska Lupca, Slovak Republic) in the dose of 10 000 IU/kg b.w. for three days. Due to the fact that the clinical state did not improve during that time and the auscultatory finding in the chest persisted, a latero-lateral radiogram of the chest was performed, which showed a nodular interstitial and alveolar pattern (Figure 1). During the following four days, gradual deterioration of the clinical state occurred. The inappetence and febrile state persisted despite the fact that the mare was administered Sulfadoxin-Trimethoprim (Borgal, Hoechst, Germany) in the dose of 15 mg/kg b.w. twice a day. Bioptic sample of lung tissue was collected. Due to the injury of a large blood vessel, pulmonary bleeding occurred, which could not be stopped. The mare died about 15 min after the biopsy.

## *Post mortem* findings

**Necropsy and histopathological examination**. At necropsy, a serofibrinous bloodstained effusion in the thoracic cavity and in the collapsed lung were revealed. Coarse confluent nodular lesions of lardaceous appearance affecting 2/3 of the caudodorsal part of diaphragmatic lobes of lung grossly resembled a sarcoma. Pulmonary pleura above the area of lung parenchyma consolidation showed diffused granular thickenings. The pattern of pale homogeneous tissue caused enlargement and effacement of bronchial lymph nodes with a loss of corticomedullary distinction. No other marked gross lesions were seen except heart hypertrophy and visceral organ congestion.

During the necropsy, samples of the lungs, bronchial lymph nodes, liver, spleen and kidneys were collected for bacteriological and histopathological examinations. Samples were fixed in 10% buffered formalin and routinely processed by the paraffin technique. The thickness of histological sections was 5 μm. After deparaffinisation, these were stained with haematoxylin and eosin. Samples from lung tissue and lung lymph nodes were stained by the Ziehl-Neelsen (Z-N) technique for the acid-fast rods (AFR) detection (Vacek, 1972).

Histopathological changes of lesioned lung tissue were characterised by the obliteration of both alveolar and interalveolar spaces by macrophages, epithelioid cells, lymphocytes and a few giant polynuclear cells. In the central zone of the granulomatous inflammation, the proliferative fibrous tissues with lymphocytes and macrophages, infiltration was observed. Caseation was occasional and of a small extension. No calcification was observed. The Z-N staining method showed only sporadic single AFR.

**Basic bacteriological examination**. The tissue samples from lung lymph nodes and lung tissue were bacteriologically examined for the presence of pathogenic aerobic and anaerobic bacteria. Aseptically collected samples were cultured on Columbia agar (Oxoid, CM331) enriched with 5% sheep blood in sodium citrate and MacConkey agar No. 3 (Oxoid, CM115). Seeded agars were incubated at 37°C. The basic bacteriological examination of pulmonary tissue and pulmonary lymphnodes was negative.

**Mycobacteriological examination**. Two samples (tuberculous lesioned lung tissue and lung lymph node) were also examined by mycobacte-

rial culture examination. About 1 g of the sample was homogenised, treated with 1 mol/l HCl and 2 mol/l NaOH and 40 µl was pipetted on solid egg media according to Stonebrink, two solid Herrold egg yolk media without Mycobactin J and antibiotics. Decontaminated samples were also inoculated onto two liquid serum media according to Sula. The growth of mycobacteria was monitored at 37°C after the first week and then every two weeks for a period of three months (Matlova et al., 2003). One mycobacterial isolate originating only from the lung tissue with tuberculous lesions was detected by the Z-N staining technique after six weeks of incubation.

**PCR identification**. The AFR positive isolate was examined by the PCR method for the detection of 1 030 bp fragments of the 16S rRNA gene specific for the genus *Mycobacterium* (Wilton and Cousins, 1992). For identification of the *M. tuberculosis* complex (*MTC*) and *M. avium* subsp. *paratuberculosis*, the IS*6110* PCR and IS*900* PCR methods were used (Bartos et al., 2006). For differentiation of *M. a. avium* and *M. a. hominissuis* the PCR method for the specific insertion elements IS*901* and IS*1245* was used Bartos et al. (2006). This isolate was identified as *M. a. avium* of IS*901*+ and IS*1245*+ genotype. Molecular analysis of the isolate by restriction fragment length polymorphism (RFLP) determined the profile Q-A14, when restriction endonucleases *Pvu* II and *Pst* I were used as published previously (Dvorska et al., 2003).

**Serotyping and the study of virulence**. The serotype of the *M. a. avium* isolate was identified by serotyping (Sussland and Hrdinova, 1976). The examined isolate was of serotype 2. Virulence of one primo isolate was studied after intramuscular administration to a chicken by a method previously described (Pavlik et al., 2000). The primo isolate was virulent (causing miliary tuberculosis of the liver and spleen) in the biological experiment on a chicken.

## DISCUSSION

Clinical signs of pulmonary tuberculosis in the mare were non-specific. The only data in history giving evidence of disease were the febrile state and inappetence. Clinical examination revealed only an increased breath sounds over the lung fields and increased amount mucus in the trachea. The transtracheal aspiration sample contained a small amount of alveolar macrophages and minute amounts of neutrophils, which pointed to a lower respiratory tract inflammation. No pathogenic microorganisms were detected by bacteriological examination of the aspirated fluid. Haematological findings were also within physiological ranges. It was only radiographic examination (Figure 1) that confirmed a nodular interstitial and alveolar pattern that has often been concurrent to pulmonary tuberculosis in horses (Muser and Nassal, 1962).

When the clinical state gradually worsened, interstitial pneumonia was suspected. Due to the fact that the clinical state deteriorated despite the initiated antibiotic therapy, prognosis was unfavourable. Intravital diagnosis of mycobacterial infections in horses is very difficult because of the diversity of the clinical signs and the fact that available intravital

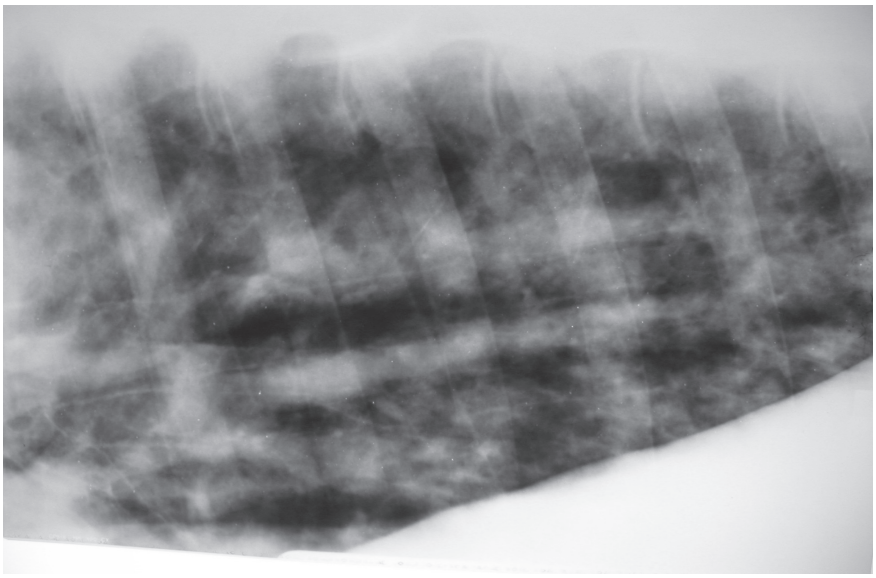

Figure 1. Radiogram of the chest (Z. Zert)

diagnostic methods are scarce (Konyha and Kreier, 1971). Besides radiographic examination, diagnosis is based on microscopy and culture of respiratory tract secretions (tracheal lavage fluid), nasal swabs or biopsied liver or lung tissue (Lofstedt and Jakowski, 1989; Mair and Jenkins, 1990). In the case of suspected intestinal tuberculosis Pearson and Heidel (1998), recommended to perform a rectal or distal colon biopsy. The biopsied tissue could also be examined by histology and molecular biological methods (particularly nested PCR methods) as performed previously (Mair et al., 1986; Monreal et al., 2001). The intradermal tuberculin test that is used for the testing of cattle is unreliable in horses (Muser and Nassal, 1962).

The present study concerning the mare's health state was diagnosed on the basis of histopathological and culture examinations of necropsy material for the presence of mycobacteria. Due to the fact that tuberculous lesions in pulmonary tissue were detected by necropsy, infection caused by in particular *MTC* members was considered a risk to students' health. *M. bovis* and *M. tuberculosis* species have been isolated from both animals and humans in Central Europe, including the Czech Republic (Pavlik, 2006).

As revealed by gross examination, the findings in advanced pulmonary tuberculosis are different for horses than for other animal species because horses are highly resistant to mycobacterial infection (Luke, 1958). That was also confirmed in the present case, where gross lesions in pulmonary tissue more probably resembled sarcomatous proliferation. Histopathological examination revealed a tuberculous granulomatous inflammatory reaction with occasional AFR.

Due to the fact that the causative agent of avian tuberculosis *M. a. avium* was subsequently detected by culture, the safety measures (i.e. prohibition of the use of the other horses previously housed together with the infected mare for teaching the students) were cancelled.

After detection of specific IS*901,* a biological experiment on pullets confirmed the assumed virulence of the isolate for birds (Pavlik et al., 2000). This identification was not surprising with regard to the frequent detection of this *MAC* member in the Czech Republic after bovine tuberculosis in cattle and other domestic animals has been brought under control. Among other intracellular parasites found in the tuberculous lesions of pigs, cattle and horses, *Rhodococcus equi* (in young horses), in particular, prevails (Skalka, 1987; Dvorska et al., 1999; Pavlik et al., 2005). However, that was not detected in the present case.

The source of the *M. a. avium* infection can be only hypothesised with respect to the age of the mare. The animal may have been infected throughout its entire life, and the infection was only manifested after the weakening of the immune system due to age or another disease, similarly as it does in other infected organisms (Brown et al., 1994). The results of RFLP analysis (Dvorska et al., 2003) and comparison of the isolate from the mare with RFLP profiles of isolates from other hosts showed that *M. a. avium* with the same profile has also been detected in pigs in previous studies (Dvorska et al., 2003; Moravkova et al., 2007). Therefore, we suppose that *M. a. avium* with this profile is common in the environment and accordingly, the mare has probably been infected through contact with infected animals or birds (Pavlik et al., 2000; Dvorska et al., 2007). There is a risk represented by a contaminated stable environment (Matlova et al., 2003); sawdust or shavings used for bedding can also not be excluded (Matlova et al. 2004). However, in this case there was no anamnestic data available that could confirm or reject this hypothesis.

Due to the fact that advanced tuberculosis was diagnosed in the lung (Figure 1), we can assume that *M. a. avium* was excreted through sputum, or after swallowing pulmonary excretions, also through faeces. In a more advanced stage, the infection of the digestive tract with the causative agent of avian tuberculosis can not be ruled out; that infection has been described previously in a horse (Merrit et al., 1975). Accordingly, the question of potential transmission of infection from the mare to the other housed horses, keepers, teachers and students remains open.

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