

Infection with *Mycobacterium avium* subspecies *avium* in a 10 year old Freiburger mare

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Summary

In this case report a 10 year old Freiburger mare with a *Mycobacterium avium* subsp. *avium* infection is presented. This infection leads to a tuberculosis like disease with granulomatous alterations particularly of the intestines and lungs and is only sporadically reported in horses of Central Europe. Diarrhoea, mastitis and neck stiffness as well as dyspnoea and chronic cough are more specific symptoms of the infection, while weight loss, weakness and lethargy are nonspecific signs. As these clinical signs can occur in many other diseases, the diagnosis of mycobacterial infection is difficult and consists of rectum or distal colon biopsies and staining for acid-fast bacilli and bacteriological culture of granulomatous lesions. Classification of *M. avium* subsp. *avium* was achieved by PCR-RFLP. Even though an infection with *Mycobacterium avium* subsp. *avium* is rare, it belongs to the differential diagnosis of granulomatous diseases.

Keywords: horse, *Mycobacterium avium* subsp. *avium*, PCR, RFLP

Infektion einer 10-jährigen Freiburgerstute mit *Mycobacterium avium* subspecies *avium*

In diesem Bericht wird eine 10-jährige Freiburgerstute mit einer *Mykobakterium avium* subsp. *avium* Infektion vorgestellt. Diese Infektion führt zu einer tuberkuloseartigen Erkrankung mit granulomatösen Veränderungen insbesondere des Verdauungstraktes und der Lunge und wird bei Pferden nur noch sporadisch in Zentraleuropa gesehen. Diarrhoe, Mastitis und Genicksteifheit sowie Dyspnoe und chronischer Husten gehören zu den organspezifischen Anzeichen, während Abmagerung, Schwäche und Lethargie unspezifisch sind. Da diese Symptome auch durch andere Krankheiten bedingt sein können, ist die Diagnose einer Mykobakterieninfektion schwierig und bedarf neben der Vorgeschichte und der klinischen Untersuchung einer Biopsie des Rektums oder des distalen Colons sowie des kulturellen Nachweises von Mykobakterien aus dem granulomatös veränderten Gewebe. Mittels PCR-RFLP Analyse, konnten bei der Freiburgerstute die Mykobakterienisolate aus Darm und Lunge genau klassifiziert werden. Obwohl eine Infektion mit Mykobakterien selten ist, darf sie bei granulomatösen Erkrankungen als Differentialdiagnose nicht fehlen.

Schlüsselwörter: *Mykobakterium avium* subsp. *avium*, PCR, RFLP, Pferd

Introduction

Infections with *M. avium* subsp. *avium* in horses are very rare and only occasionally seen in Central Europe. There are no recent data about the prevalence of tuberculosis in horses. An older publication (Anonymous, 1961) refers to a prevalence of 0.3% in slaughter horses in West Germany in 1959 and in the Czech Republic from 1956 to 1958 a prevalence of 2.3% has been reported (Krejci, 1958). The risk of transmission of tuberculous infections caused by members of the *M. avium* complex from horses to humans is very low; horses do not constitute a reservoir host (Pavlik et al., 2004). The infection leads to proliferative enteritis mainly in the small intestine re-

sembling paratuberculosis of cattle. If the large intestine is affected, the appearance is like tuberculous ulcerations (Dungworth, 1993). Miliary granuloma formations are found after haematogenous dissemination in the lungs, liver, spleen and serosa (Peel, 1983). Skin affection and abortion are other findings described (Sevilla et al., 1995; Helie and Higgins, 1996). Clinical signs in horses depend on the organs affected. Anorexia, weight loss, stiff gait, weakness and lethargy as well as tachycardia and dyspnoea are frequently found. Other signs described are diarrhoea, mastitis, subcutaneous oedema, pyrexia, and chronic cough of a terminal lung-affection (Peel, 1983; Beech and Sweeney, 1991). Reported laboratory changes include anaemia, leukocytosis, hypoalbuminemia and increased activity of all liver enzymes.

444 Fallbericht

Due to the diversity of clinical signs as well as to low sensitivities and specificities of the available diagnostic tests, diagnosis of equine *M. avium* complex or *M. tuberculosis* complex infection is difficult (Muser and Nassal, 1962; Konyha and Kreier, 1971). Recommended tests to confirm diagnosis in case of suspected intestinal tuberculosis are rectum and distal colon biopsies (Pearson and Heidel, 1998), with microscopy for acid-fast bacilli (Ziehl-Neelsen staining) and culturing the microbacterial agent. In tuberculous lesions, mycobacteria are sometimes detected after chemical staining, and granulomatous reactions with epithelioid cells, giant cells, lymphocytes and fibroblasts are present (Rooney and Robertson, 1996). For exact classification of species of the *M. avium* complex a panel of molecular biology techniques like genetic sequencing and phylogenetic analysis, restriction fragment length polymorphism (RFLP) analysis and insertion element investigations can be used (Dvorska et al., 2003; Tortoli et al. 2004).

Case history

Clinical findings

A 10 year old Freiburger mare was presented to the equine hospital of the University of Zurich. She had a history of inappetence, fever and dyspnoea, with no response to trimethoprim-sulfomethoxazol (25mg/kg SID; Vétoquinol AG, Ittigen, Switzerland) and flunixin meglumine (1.1mg/kg SID; Berna Veterinärprodukte AG, Switzerland). A blood analysis revealed anaemia 28% (30–50%), lymphopenia $0.4 \times 10^3/\mu\text{l}$ ($1.5–4.0 \times 10^3/\mu\text{l}$), leucopenia $4.3 \times 10^3/\mu\text{l}$ ($5–10 \times 10^3/\mu\text{l}$) and hypoalbuminemia 23.1 g/l (25–54 g/l). Blood tests for *Babesia caballi*, *Theileria equi* and *Anaplasma phagocytophilum* were negative as well as the parasitological examination of the faeces.

At the time of presentation at the clinic the mare moved in a stiff manner, had a stretched position of the neck and dyspnoea. Her body surface was moist and cold. The body condition was good, but weight loss had been reported. The heart and respiratory rate were 88bpm and 64bpm, respectively, and the rectal temperature was 38.1 °C. The oral mucous membranes were slightly pale, with a normal capillary refill time. Increased respiratory sounds were audible by lung auscultation and thorax-percussion was painful.

Endoscopically an increased amount of mucus in the trachea and a small amount of mucus in the left guttural pouch were visible. Pulmonary ultrasonographic examination showed a slightly roughened pleural surface with some comet-tail artefacts without an increased amount of pleural fluid.

Laboratory findings

No abnormalities were found on rectal examination and nasogastric intubation, but the peritoneal fluid was red-

dish in colour, slightly cloudy with a total solids concentration of 86 g/l and a leukocyte count of 68'000 cells/ μl . Its cytological analysis revealed mainly degenerated leukocytes without bacteria. In haematology erythrocyte sedimentation rate 190 mm/15 min (0–15 mm/15 min), total solids concentration 86 g/l (60–70 g/l) and plasma fibrinogen concentration 12 g/l (1–5 g/l) were increased. Mild hyperglycemia 6.5 mmol/l (4.5–5.9 mmol/l), hyperproteinemia 80 g/l (57–70 g/l), hypoalbuminemia 21 g/l (25–34 g/l), a moderate elevation of BUN 25.5 mg/dl (9.8 mg/dl–19.6 mg/dl), and a severe elevation of the creatinine concentration 385 $\mu\text{mol/l}$ (82–147 $\mu\text{mol/l}$) were evident in blood chemistry. All liver enzymes' activities were slightly increased: ALP 759 U/l (81–183 U/l), GLDH 33.6 U/l (0.5–2.2 U/l), GGT 59 U/l (6–31 U/l), SDH 36.1 U/l (0.1–7.6 U/l) and LDH 873 U/l (369–822 U/l). Serum electrolyte concentrations differed only slightly from standard values.

Diagnosis and treatment

Due to the horse's bad general condition and the findings of the abdominocentesis, the increased heart rate and the missing response on pain reliever, the horse had to undergo an exploratory laparotomy. Intraoperative findings included multifocal tuberculous lesions in the serosa of the dorsal colon and the small intestine and on the facies abdominalis of the diaphragm. Biopsies of these alterations were taken and granulomatous inflammation was found on histology. Bacteria were not found but a bacterial infection was suspected. Diffuse, round, white areas on the liver surface were interpreted as worm granules. The caecum was clotted to the left abdominal wall and one part of the mesentery did adhere to the di-

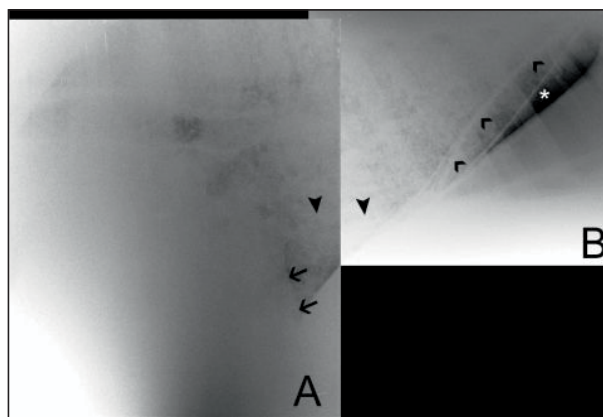


Figure 1 A and B: Right lateral radiographs of the thorax. A: caudoventral projection, B: caudodorsal projection. Note the severe miliary to nodular interstitial lung pattern. The pulmonary vessels, the caudal vena cava (dorsal contour indicated by arrow heads) and the caudal contour of the cardiac silhouette (arrows) are barely visible. The gas (*) within the peritoneal cavity outlining the diaphragm (open arrows) is due to the abdominal surgery performed one day before.

aphragm. During surgery the adhesions were separated and part of the mesentery was resected. During recovery from anaesthesia the horse developed a pulmonary oedema and had to be treated with furosemide (0.5 mg/kg; Veterinaria AG, Zürich, Switzerland) and dexamethasone (0.04 mg/kg; Veterinaria AG, Switzerland). Aftercare consisted of cefquinome (0.04 mg/45 kg BID; Veterinaria AG, Switzerland), flunixin meglumine (1.1 mg/kg BID; Berna Veterinärprodukte AG, Bern) and dalteparinum natrium (1250 IU/kg SID; Pfizer, Zürich). Oxygen was administrated intranasally at a rate of 8 l/min because of persisting dyspnoea, tachycardia and low paO_2 89.3 mmHg (90–100 mmHg).

One and two days after surgery, lateral radiographs of the right thorax were taken (craniodorsal, caudodorsal and caudoventral view; Fig. 1A and B). On both days the opacity of the lung was severely increased and showed a diffuse miliary to nodular interstitial pattern. Therefore, a vasculitis probably due to a toxic condition, miliary metastases or a granulomatous pneumonia were more likely diagnosed. Additionally, an ultrasonographic follow-up examination of the lung was performed. The amount of comet-tail artefacts increased bilaterally as compared to the preoperative examination and a slight amount of anechogenic pleural fluid was visible in the right hemithorax. These findings were consistent with a mild pleuritis with mild pleural effusion. The mare was euthanized because of her poor condition.

Necropsy and bacteriological findings

On necropsy a severe, multifocal, necrotizing pneumonia (Fig. 2–4) with associated necrotizing lymphangitis of the pulmonary lymph nodes was seen. Furthermore, a severe multifocal, chronically active granulomatous and necrotizing intramural and serosal inflammation in the small intestine and cranial parts of the large intestine was found. A severe, chronic multifocally mixed cellular hepatitis with extended fibrosis and a severe diffuse chronic lymphoplasmacellular villous serositis of the facies abdominalis of the diaphragm were recognized, both typical for ascarid larval passage.



Figure 2: Lung lobes were heavy, dark red and contained countless miliary, grey to white, dense nodules of up to 5 mm in diameter visible through the capsule and scattered throughout the parenchyma.

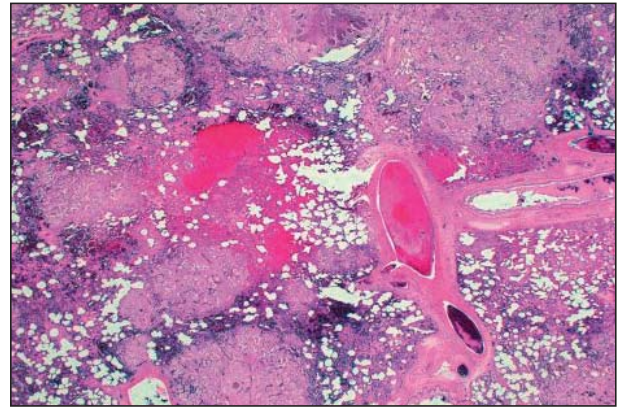


Figure 3: Multiple focal areas of acute necrosis, extensive haemorrhage and alveolar oedema in the lung HE.

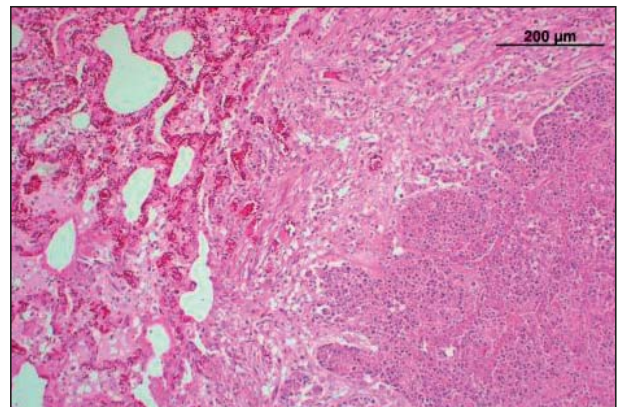


Figure 4: Left side: Extensive hyperemia and alveolar oedema with infiltration of inflammatory cells in the alveoli (mainly macrophages) HE.

Right side: Necrosis consisting of degenerated neutrophilic granulocytes, macrophages and cellular debris, surrounded by a thin layer of fibrous tissue infiltrated by lymphocytes and plasma cells.

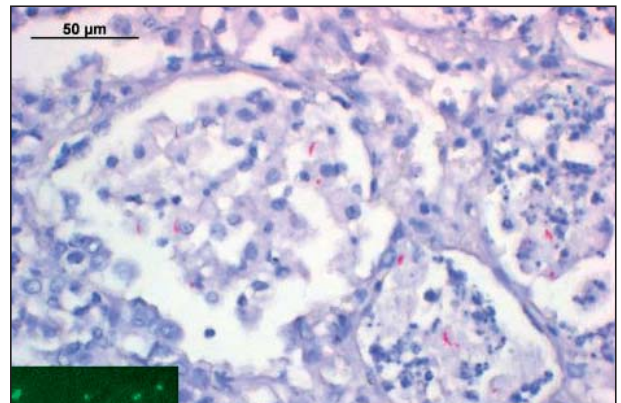


Figure 5: Necrotic areas in the lung with numerous long, slender, acid-fast bacteria. Ziehl-Neelsen (Inset: Auramin stain, fluorescence microscopy).

Microscopically, abundant acid-fast bacilli were detected in Ziehl-Neelsen- and Auramin- stained smears (Fig. 5) from granulomatous pulmonary lesions. Samples from these lesions were processed and inoculated onto Löwenstein-Jensen agar slants (Enclit, Oelzschau, Germany) and Middlebrook 7H9 agar according to standards (OIE, 2004) and yielded luxuriant growth of smooth, opaque colonies of acid-fast bacilli. The slowly, for more than 7 days growing isolate (Shinnik and Good, 1994) was classified as *M. avium* by PCR-coupled DNA sequence analysis of the 5' part of the mycobacterial 16S rRNA gene (Böddinghaus et al., 1990). By PCR-RFLP analysis of the β -subunit of the RNA polymerase gene (*rpoB*), the isolate was identified as *M. avium* subsp. *avium* (Kim et al., 2001). No bacteria were found in the abdominal lesions.

Discussion

This case report describes the clinical, laboratory and pathological findings of a horse with a severe activated chronic necrotizing pneumonia and granulomatous lesions in the intestinal serosa due to *M. avium* subsp. *avium* infection. The reduced defecation and the severe changes in the abdominal fluid were somewhat misleading and complicated the diagnosis. The horse was taken to exploratory laparotomy during which biopsies of the intestinal serosal lesions were taken indicating granulomatous inflammation. However, the radiological findings and the changes in the haematology were indicative of a generalized infectious disease. Final diagnosis was not evident until the bacteriological culture of lung tissue post mortem showed a growth of *M. avium* complex, identified as *M. avium* subsp. *avium*. The necrotizing pulmonary lesions were histologically far more acute than the granulomatous intestinal inflammation. This led to the conclusion that the mycobacterial infection originated in the intestine and subsequently spread via bloodstream into the lung.

This case demonstrates the diagnostic challenge of an infection with *M. avium* complex and shows the clinical signs in a horse with a generalized infection of this pathogen. The severity of clinical signs and the extent of pathologic lesions illustrate the poor prognosis of horses affected with *M. avium*.

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