Case Report

Mycobacterium genavense Infection as a Cause of Disseminated Granulomatous Inflammation in a Horse

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ABSTRACT

A yearling mare (16-month-old) presented with respiratory symptoms and a history of retarded physical development for several weeks. Clinical and laboratory examinations indicated infectious disease. Attempts of antibiotic therapy failed, and the animal was continuously in poor general condition, so it was euthanized due to bad prognosis. Necropsy and microscopical examinations revealed granulomatous inflammation in the spleen, lungs, aortic wall, aortic, mandibular, and inguinal lymph nodes. Intracellular acid-fast bacilli were solely detectable in the spleen, but only in very few numbers. Molecular biological investigations of formalin-fixed tissue samples showed the presence of Mycobacterium genavense DNA in the spleen and the aortic wall. To the best of our knowledge, this is the first report on M. genavense infection in a horse.

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1. Introduction

Mycobacterium genavense belongs to the group of atypical mycobacteria and is considered to be an emerging pathogen in human medicine because it is thought to be ubiquitous and mainly affects immunocompromised individuals [1–5]. Besides, M. genavense has been shown to be pathogenic for a variety of species, including dog, cat, rabbit, ferret, mouse, chinchilla, grizzled giant squirrel (*Ratufa macroura*), brown lemur (*Lemur macaco mayottensis*), and several bird species, which account for the vast majority of the cases reported in the literature [4,6–17]. Recently, M. genavense has also been detected in raw milk samples of cattle [18]. Despite this wide range of avian and mammalian hosts, M. genavense infections of odd-toed ungulates (*Perissodactyla*) and especially of horses have not been reported to date.

2. Case Report

A 16-month-old Thoroughbred mare kept in a group of yearlings in a professional horse husbandry in northern Germany was presented with respiratory symptoms and a history of retarded physical development for several weeks. On clinical examination, the animal was subfebrile, and hematological investigations revealed moderate-to-marked leukocytosis. Blood gas analyses showed moderate-to-marked depression of arterial partial pressure of oxygen. Tracheobronchial fluid was collected endoscopically; numerous neutrophil granulocytes and moderate numbers of macrophages as well as some multinucleated giant cells were detected by microscopical examination. By means of ultrasonography of the lung, so-called "comet tail echoes" could be seen, indicating irregularities of the pleural surface. X-ray showed mottled lung shadows and a marked bronchial pattern. Altogether, the findings indicated bacterial

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bronchopneumonia. Antibiotic therapy with ceftiofur (2.2 mg/kg q24-hour IM) accompanied by administration of dembrexin (0.3 mg/kg q12-hour PO) and clenbuterol (0.8 mg/kg q12-hour PO) was unsuccessful, and the animal was continuously in a poor general condition, so the owners elected euthanasia after several weeks of disease.

Remarkable necropsy findings occurred mainly in the spleen and the lungs. The spleen exhibited numerous well-demarcated gray–white to yellowish firm nodules of 1 to 2 mm in diameter that partly coalesced to areas of up to 20 mm in diameter (Fig. 1). The lung parenchyma was congested and had a mottled appearance; the pleural surface showed some firm gray–white nodules measuring up to 4 mm in diameter. Moreover, the ascending aorta exhibited some thickenings of the adventitial tissue and the adjacent aortic lymph node as well as the inguinal and mandibular lymph nodes appeared enlarged and had an inhomogeneous cut surface. Representative samples of these organs were fixed in 10% neutral-buffered formalin and routinely processed for microscopical investigation.

Histopathology revealed gradually variable granulomatous inflammation of the spleen (Fig. 2), lung and pleura, adventitia of the ascending aorta, the adjacent aortic lymph node, as well as of the inguinal and mandibular lymph nodes. The granulomas were of the tuberculoid type and consisted of macrophages and epithelioid cells, some Langhans giant cells as well as lymphocytes, plasma cells, and fibroblasts/fibrocytes. Neither areas of necrosis nor calcification were found within the histologic slides. Ziehl–Neelsen and Grocott methenamine silver stain were performed on all specimens. Acid-fast bacilli were only detectable in the cytoplasm of macrophages and giant cells in the spleen (Fig. 3). Notably, only very few bacterial organisms were visible (paucibacillary type of granulomatous inflammation). Grocott stain did not reveal any fungal structures.

Genomic DNA was isolated with the Qiagen Blood and Tissue kit from specimens of all formalin-fixed tissue samples. Polymerase chain reaction using the mycobacteria-specific 16S rDNA primers 246 and 264 [19] revealed the presence of *Mycobacterium* sp. DNA in splenic and aortic tissue. Sequencing of the 1030-bp amplicons lead to
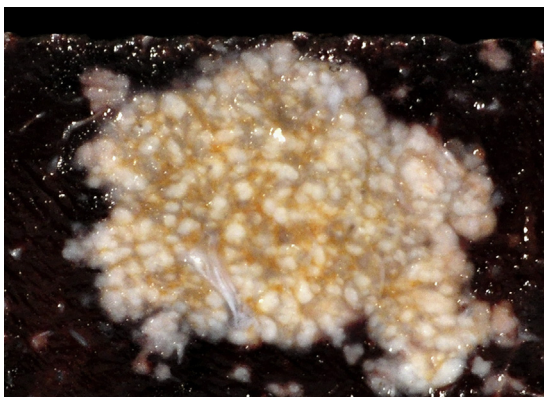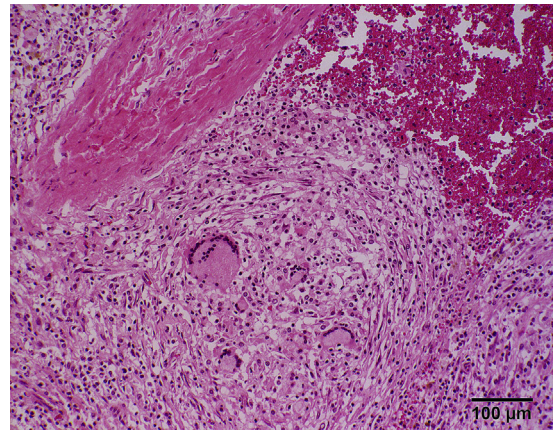

Fig. 2. Section of spleen. Granulomatous inflammation consisting of macrophages, lymphocytes, fibroblasts, and some Langhans giant cells. Hemalum-eosin stain.

identification of *M. genavense* DNA (GenBank accession number: KM820764).

3. Discussion

M. genavense infections of animals are known to occur mainly in birds [4,14–17]. Besides, various mammalian species have been reported to be susceptible to *M. genavense* infection, with domestic mammals including cattle [18] as well as animals kept as pets, that is a dog, a cat, a dwarf rabbit, two ferrets, and a chinchilla [6–9,11]. To our knowledge, *M. genavense* infections of horses have not been reported yet.

In humans, immunosuppression (often due to primary human immunodeficiency virus infection) is known to be a predisposing factor for *M. genavense*–associated disease [2]. In veterinary medicine, knowledge about predisposing factors is frequently limited, not least because appropriate investigations are not carried out [13], often due to cost restrictions. However, Hughes et al [7] reported on a cat with disseminated *M. genavense* infection secondary to
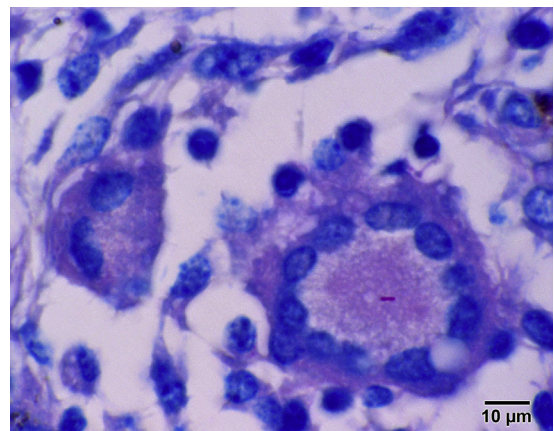

Fig. 1. Spleen, cut surface. Numerous coalescing nodular lesions are present throughout the parenchyma.

Fig. 3. Section of spleen. A single acid-fast rod-shaped bacterium is visible in the cytoplasm of a Langhans giant cell. Ziehl–Neelsen stain.