An unusual case of spinal cord restricted mycobacteriosis in a European mink

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Key words
Central nervous system, mink, Mycobacterium avium, spinal cord

Summary
Granulomatous myelitis due to infection with Mycobacterium avium was diagnosed in a 4-year-old male neutered European mink (Mustela lutreola). The causative agent was detected by an acid-fast stain and further characterized by polymerase chain reaction and DNA sequencing of the PCR product. A thorough histological evaluation of the remaining organs revealed no granulomatous lesions or detectable acid-fast organisms. Although minks are generally highly susceptible for mycobacteria, localised infections, especially of the central nervous system, are unusual and may represent an atypical chronic form of the disease.

Case report
Anamnesis
A 4-year-old male neutered European mink was found as a young animal in the wilderness of Sweden and was transferred to a zoo in Germany. Initially, the mink was kept alone and later on a polecat-ferret crossbreed was put to the compound as companion animal. At the age of 4 years the mink was presented with a 4-week history of progressive tetraparesis. Neurological abnormalities were consistent with a right-sided C6-T2 myelopathy including generalised ataxia and mild to moderate tetraparesis with diminished spinal reflexes at the right thoracic limb, a left-sided scoliosis of the neck, and pain on palpation and manipulation of the neck and the right thoracic limb. Due to the poor prognosis the mink was humanely

Introduction
The genus Mycobacterium (M.) includes various pathogens known to cause serious diseases in mammals, amongst them certain mycobacteria are subgrouped into the Mycobacterium tuberculosis complex (MTC) and Mycobacterium avium-intracellulare complex (MAIC). MAIC includes M. intracellulare and four subspecies of M. avium, namely, M. avium ssp. avium, M. avium ssp. hominis-suis, M. avium ssp. silvaticum, and M. avium ssp. paratuberculosis (7, 11). Within the last years, members of the MAIC have emerged as zoonotic pathogens in immunocompromised people, especially in profound immunodeficiency such as late stage of AIDS (1, 2). Pet animals such as dogs are considered as a potential source of mycobacteriosis in a European mink (3, 10, 14).

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Necropsy

A complete necropsy was performed. The mink was in a moderate body condition. On gross examination, there was a segmental grey discoloration of the entire spinal cord between the second and the fifth cervical segment. In addition, the spinal cord showed multifocal subdural hemorrhages at the third cervical segment.

Histopathology and immunohistochemistry

For microscopic examination, tissue samples of skin, lymph nodes, spleen, intestine, bone marrow, heart, lung, liver, kidney, brain, stomach, oesophagus, adrenal gland and thyroid were fixed in 10% neutral buffered formalin, processed by routine methods, embedded in paraffin wax, sectioned at 5 µm and stained with hae-
matoxylin and eosin (HE). Standard special stains including Groc-
cott silver stain, Ziehl-Neelsen acid-fast stain, Gram stain, and Giemsa stain were carried out on liver, spleen, kidney, mesenteric lymph node and selected CNS sections. Immunohistochemistry was performed on liver and CNS sections using murine monoclo-

cals directed against parvovirus, canine distemper virus, feline in-
fecious peritonitis virus, Neospora caninum, and Toxoplasma gondii, no infectious agents were detected within the lesions of liver, brain and spinal cord.

Due to the formaldehyde-pretreatment of the tissue, there was no bacterial growth on culture media. PCR targeting IS1245, virus (Biologo, Kronshagen, Germany) (13), Neospora caninum (VMRD Inc., Pullman, USA), and Toxoplasma gondii (Quartett, Berlin, Germany) and the avidin-biotin-peroxidase-complex (ABC) method (Vector Laboratories Inc., Burlingame, USA).

Microbiological and molecular biological examination

Formalin-fixed samples of the spinal cord lesion were used for bacterial culture and were processed for molecular biological examination to identify the causative agent. DNA from tissue was extracted using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany). Four different PCRs targeting 16S rDNA for the genus Mycobacterium, the insertion sequences IS901 (11), two gene sequences specific for M. avium, and a hypothetical helicase to detect members of the Mycobacterium tuberculosis complex were applied (12). Additionally, DNA sequencing of the generated PCR product was performed using amplification primers (7) and BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany) according to the instructions of the manufacturer. Analysis was done using an ABI Prism 310 genetic analyser (Applied Biosystems, Darmstadt, Germany).

Results

Histopathological examination of the spinal cord revealed a randomly distributed accumulation of inflammatory cells within the grey and white matter and occasionally affecting the meninges. These inflammatory cells consisted of macrophages with abundant foamy cytoplasm, epithelioid cells, lymphocytes, plasma cells and an increased number of glial cells between the second and fifth cervical segment (Fig. 1). Langhans-type multinucleated giant cells were not notable within the lesion. The nervous tissue showed swollen myelin sheaths with few macrophages. Occasionally, intrale-sional cholesterol clefts were evident. Within the white and grey matter of the cerebrum, cerebellum and brain stem, there was a moderate multifocal inflammation characterized by lymphocytes and macrophages with multifocal malacia and astroglisis. The liver revealed mild lymphocytic perportal hepatitis with mild bile duct hyperplasia. The kidneys showed a mild lymphocytic pyelitis. Other organs examined were without significant histopathological changes.

Special stains including Grocott, Giemsa and Ziehl-Neelsen revealed myriads of 3 to 5 µm long slender rod-shaped acid-fast bac-


Fig. 1 Multifocal randomly distributed granulomatous myelitis affecting the grey (white arrow) and white matter (asterix) as well as the meninges (black arrows). Insert: higher magnification of the granulomatous myelitis with swollen myelin sheaths (arrows), HE, bar 200 µm.

Abb. 1 Multifokale, zufällig verteilte, granulomatöse Myelitis in der grauen (weißer Pfeil) und weißen Substanz (Stern) sowie in den Meningen (schwarzer Pfeil). Einschub: stärkere Vergrößerung der granulomatösen Myelitis mit geschwollenen Myelinscheiden (Pfeile), HE, Balken 200 µm.
which is indicative for *M. avium*, revealed a weak but evident PCR product with the characteristic molecular weight of 425 bp which was further substantiated by sequencing of the respective DNA fragment. The PCR targeting IS901, the 16S rDNA and the hypothetical helicase were not successful in generating PCR products of molecular weights of 1108 bp, 1030 bp and 499 bp, respectively.

**Discussion**

Based on histological and molecular findings, a granulomatous myelitis caused by *M. avium* was diagnosed. Failure of bacteriological culture, which represents the gold standard for detection of mycobacterial infections, was not unexpected and due to the formalin fixation of the tissue prior to bacteriological examination. Therefore, molecular methods were the only methods to be applicable to successfully identify the causative agent.

A genome sequence specific for *M. avium* was detected by applying the IS1245-targeting PCR. This insertion sequence is found in the *Mycobacterium avium* ssp. *avium* and *Mycobacterium avium* ssp *hominissuis* (7). The latter represents the species of the MAIC found in the majority of cases of *M. avium* infections in domestic and wild mammals whereas *M. avium* ssp. *avium*, characterized by presence of IS1245 and IS901, is typically infecting birds and poultry (16). In addition, DNA fragments of members of the MTC causing the classic tuberculosis were not detected using PCR technique. However, formalin does not only avoid bacteriological culture, it interferes with the generation of PCR products (18). Thus, the negative outcome of the PCR targeting the 16S rDNA yielding a relatively high molecular PCR product of 1108 bp might be due to damage of the target DNA sequence. Consequently, an exact determination of the respective subspecies was not possible. However, the PCR results combined with the species in which the mycobacteria were detected points towards an infection with *M. avium* ssp. *hominissuis*. Nevertheless, the type of inflammatory reaction in this case was compatible with the alterations commonly seen following infection with MAIC that generally differs from lesions caused by the MTC members in the majority of animal species (17). MAIC infections are characterized by a diffuse granulomatous inflammation consisting of large numbers of epithelioid cells, lack of necrosis, calcification and fibrosis. In addition, prominent multinuclear cells represent a frequent finding, and the lymphocyte response is usually inconspicuous when compared with MTC infections (17).

Although sporadic reports of *M. avium* infection in minks exist (9, 20), a CNS-restricted infection has not been reported yet. Since minks are known to be highly susceptible to *M. avium* infections, which generally cause widespread systemic infections in this species, our case represents an unusual manifestation. However, it should be kept in mind that the existing reports of mycobacterium infections in minks relate to American minks (*Neovision vision*) which are closely related to the European minks (*Mustela lutreola*) but still somewhat different. Therefore, a different susceptibility to *M. avium* might be possible. Nevertheless, similar cases have been reported in humans and other species including cats and dogs, which are thought to be less susceptible to mycobacteriosis than minks (3, 10). For *M. avium*, known to inhabit soil and water, the alimentary route represents a common route of infection followed by a systemic spread throughout the body in susceptible animals (19). In addition, members of the MAIC have been found in invertebrates, such as the common shrew (*Sorex araneus*), and in small rodents, such as the yellow-necked mouse (*Apodemus flavicollis*) (5). Furthermore, in cats and dogs, MAIC represents the most slow-growing opportunistic mycobacterial species isolated from cutaneous wounds (19).

After entering the body, mycobacteria might spread into the CNS by extending directly from an osteomyelitis of the adjacent vertebra or by haematogenous spread (4). Although nothing is known about the molecular mechanisms allowing *M. avium* to enter the CNS, it is suggested that the bacteria might cross the blood-brain barrier by invading the epithelial cells in the choroidal plexus (21). Since no lesions in other organs including the bone adjacent to the CNS were found in the presented case, the route of infection into the CNS remains undetermined. Whether there was an initial mycobacterial infection of the brain that has been eliminated in the further course of the disease remains speculative, since no bacteria were found histologically using special stains. In addition, no other infectious agents including parvovirus, canine distemper virus, feline infectious peritonitis virus, *Neospora caninum* and *Toxoplasma gondii* have been found in the brain. Therefore, the cause of the inflammatory reaction in the brain is undetermined, though it remains a possibility that these changes represent residual lesions of a mycobacterial infection.

The causative agent of the spinal cord lesion was most likely *M. avium* ssp. *hominissuis* which is in line with the histological....
Conclusion for practice

Clinical signs due to central nervous system (CNS) especially spinal cord lesion could be caused by various agents and processes. In the presented case an inflammatory or neoplastic process was considered after first clinical presentation. Inflammation in the CNS of a mink could be due to Aleuten disease, distemper, toxoplasmosis or bacterial infection. In contrast, infection with Mycobacterium avium represents a rather rare cause. Although minks are generally highly susceptible for mycobacteria, localised infections, especially of the central nervous system, are unusual. However, as shown by the present case, they should be considered as potential etiology and a subsequent granulomatous myelitis as a differential diagnosis for CNS disease.

changes and the finding of this M. avium subspecies in other mammalian species even though the genus-specific PCR did not yield a specific PCR product. Furthermore, this report shows that formalin fixed material can be used to further identify and partially characterize mycobacteria in formalin fixed tissues and that CNS restricted infections with acid-fast bacteria may occur in the mink.

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Conflict of interest

The authors confirm that they do not have any conflict of interest.

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