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CASE REPORT

Companion or pet animals

Highly resistant *Mycobacterium abscessus* subsp. *abscessus* infection in a Swiss cat

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Abstract

A 2-year-old European shorthair cat was presented with a nonhealing skin lesion on the right abdominal wall. Repeated surgical excision and antimicrobial treatment with cefovecin, clindamycin, and trimethoprim-sulfamethoxazole did not lead to clinical improvement. Histopathology of the skin lesion showed pyogranulomatous dermatitis and panniculitis. Definitive diagnosis was made by mycobacterial culture and *rpoB* sequencing, revealing *Mycobacterium abscessus* subsp. *abscessus*. To the authors' knowledge, this is the first report of *Mycobacterium abscessus* subsp. *abscessus* infection in a cat in Switzerland and the second case in Europe. The isolated strain carried a functional *erm*(41) gene that confers inducible macrolide resistance. Further phenotypic resistances to doxycycline, minocycline, and imipenem were detected, resulting in little chance of successful antimicrobial treatment. *Mycobacterium abscessus* subsp. *abscessus* is an emerging pathogen in human medicine and poses a non-negligible zoonotic risk for the owners of the cat.

KEYWORDS
antimicrobials, cats, mycobacteria, resistance, skin, zoonoses

BACKGROUND

Infections with non-tuberculous mycobacteria (NTM) pose an increasing health risk in human medicine. Most often these ubiquitously found mycobacteria are the cause of opportunistic infections in immunocompromised patients or patients with lung disease.[1] *Mycobacterium abscessus* subsp. *abscessus* (Mab) is one of the most frequently isolated NTMs, and causes severe diseases of skin and lung in humans, especially in patients with cystic fibrosis.[2,3] Mab infections in veterinary medicine are mentioned in infectious diseases reference books[4,5]; however, only a few documented cases of Mab skin infections have been published so far— one from Europe[6] and a few cases of *Mycobacterium chelonae-abscessus* group infections in cats and dogs from the United States, where the species involved was not characterised.[7] Proper identification at species and subspecies level requires sequencing of *rpoB* or *hsp65* genes.[8]

Cutaneous mycobacteriosis should be suspected following non-responsiveness to otherwise successful antimicrobial treatment of cutaneous or subcutaneous nodules with or without ulcerations or recurrence after surgical excision. Lesions typically involve the panniculus and often occur in the inguinal area, flanks or at the tail base. Skin lesions can be multifocal or single with enlargement of the involved region over time. Sometimes disseminated disease occurs.[9] Infections with Mab are especially challenging, as these mycobacteria are known to be among the most resistant mycobacteria, due to multiple intrinsic resistances.[10] The current case demonstrates the difficulties and limitations in treatment of mycobacterial infections in companion animals and underlines the potential health risk for the owners.

CASE PRESENTATION

A 2-year-old, free-roaming European shorthair cat was presented to a private veterinarian with an abscess on the right abdominal wall in March 2019. Vaccination against feline calicivirus (FCV), feline herpesvirus (FHV)−1, and feline panleukopenia virus (FPV) was regularly performed, and the cat tested negative for feline immunodeficiency virus (FIV) and feline leukaemia virus (FeLV). General physical examination was unremarkable. A bite wound was suspected as the cause of the lesion. Surgical wound care was performed, and the cat was treated with a single, subcutaneous injection of cefovecin (8 mg/kg, Convenia, Zoetis, Switzerland). Re-examination after 2 and 4 months showed recurrence and spreading of the skin lesion near the primary wound. Treatment with cefovecin injection (8 mg/kg) was repeated twice. In December 2019, the

cat was again presented due to relapse of the cutaneous lesion. This time the lesion was more severe, with induration, ulceration and purulent discharge on a surface of 6 × 10 cm.

The recurrence of the lesion and the clinical presentation was very characteristic for an infection with rapidly growing mycobacteria. Surgical wound care was repeated and biopsies were taken for histopathology and bacteriology. Antimicrobial therapy with clindamycin (10 mg/kg PO q12h, Antirobe, Zoetis, Switzerland) was started after surgery. Two biopsies of haired skin were also sent for histopathological investigations. A multinodular to diffuse severe inflammatory infiltrate consisting of numerous macrophages and epithelioid cells with admixed neutrophils and few lymphocytes and plasma cells was observed within the dermis and subcutis. In addition, a mild activation of fibroblasts was present (Figure 1). These findings were consistent with a pyogranulomatous dermatitis and panniculitis and an infectious cause was suspected. However, special stainings for fungi (periodic acid-Schiff [PAS]) and acid-fast bacteria (Ziehl-Neelsen) were negative. Because of this negative result a sterile pyogranulomatous process was a possible differential diagnosis. Microbial standard cultures showed no growth of aerobic and anaerobic bacteria. Based on these results, therapy with prednisolone (1 mg/kg, PO q24h, as a starting dose, Spiricort, Spirig Health Care AG, Switzerland) was started in addition to clindamycin to suppress ongoing inflammation.

During the following weeks, the skin lesion improved significantly, clindamycin therapy was stopped and therapy with prednisolone was continued for another 3 weeks. Two months later, the cutaneous lesion recurred, and another surgical revision was performed. A skin incision to the latissimus-dorsi muscle was performed and the approximal 6 × 2.5 cm large lesion was removed with outer borders of 2-3 cm. Two Penrose drainages were placed to achieve adequate draining of serous fluids. A second histopathological examination revealed deep granulomatous and nodular dermatitis, however, using Kinyoun and PAS staining, no acid-fast bacilli or mycological pathogens could be observed. Since there was no lasting clinical improvement in May 2020 (Figure 2), the treating veterinarian contacted one of the authors for advice. New swab samples and biopsies were submitted for histopathological investigations and bacteriological specialist culture. Histopathologic examination yielded the same results as already seen in December 2019 and March 2020.

LEARNING POINTS/TAKE HOME MESSAGES

- Mycobacterial infections should always be a differential diagnosis in cats with chronic non-healing wounds, draining tracts or nodules, especially in locations rich in subcutaneous fat tissue.
- Appropriate sample collection is essential for detection of mycobacterial infection and should include skin or surgical biopsies with deep subcutaneous fat tissue. The tissue samples should be cut into halves, with one part formalin-fixed for histopathological examination, and one part in sterile saline solution for routine and specific culture. Swabs from draining tracts often identify only secondary bacterial infections. Special stainings in histopathology, including Ziehl-Neelsen, may result in a false-negative even if mycobacterial infections are present.
- Susceptibility testing of the mycobacterial isolate should always be performed, and in some cases genotypic resistance determination is required. Proper identification of a *Mycobacterium* isolate is required for adequate interpretation of susceptibility testing.
- High owner and cat compliance are crucial for successful treatment of mycobacterial infections. The owners should be informed of the costly and lengthy treatment, of up to 12 months, that should include continuous antimicrobial treatment for 1-3 months after complete clinical healing.
- *Mycobacterium abscessus* subsp. *abscessus* is an important human pathogen and therefore a strict hygiene regime must be conducted while handling infected cats. Owners should be informed of the zoonotic risk. Immunosuppressed patients should not be in contact with infected animals.

INVESTIGATIONS

A swab sample from the purulent skin lesion was submitted for mycobacterial culture. The specimen was decontaminated and processed as described before.[11,12] Direct Ziehl-Neelsen staining of the decontaminated material revealed acid-fast bacilli (Figure 3). Three days after inoculation of liquid mycobacterial media (BBL MGIT, Becton Dickinson, Switzerland) supplemented with PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin) antibiotic mixture, and incubation at 30°C, growth was detected. Subcultures on solid media (Middlebrook 7H10) showed rough, whitish colonies. Species identification was performed using MALDI-TOF MS mass spectrometry[12] and revealed the presence of *Mycobacterium abscessus*. Sequencing of the *rpoB* gene[13] allowed further characterization at subspecies level, and the isolate was identified as *Mycobacterium abscessus* subsp. *abscessus* with an identity score of 99% (728/729) compared with *Mycobacterium abscessus* subsp. *abscessus* type strain DSM 44196^T.[14]

Phenotypic susceptibility testing was performed using a commercial broth microdilution method for rapid growing NTM according to the manufacturer's instructions (RAPMYCO Sensititre; TREK Diagnostic Systems, UK). The minimal inhibitory concentration (MIC) was read manually and determined as the lowest concentration of the antibiotic compound showing 100% growth inhibition, and 80% growth inhibition for trimethoprim/sulfamethoxazole, respectively. Clinical breakpoints for rapidly growing mycobacteria were applied according to CLSI M62 Ed1E 2018.[15] Phenotypic resistances were reported for doxycycline, minocycline, tobramycin, and imipenem. MIC testing for ciprofloxacin, moxifloxacin and clarithromycin yielded intermediate resistant results (Table 1). By definition the activity of "intermediate" tested antimicrobial agents are associated with uncertain therapeutic effects[16,17] and were therefore reported as resistant in the present case. The *erm*(41) gene was sequenced

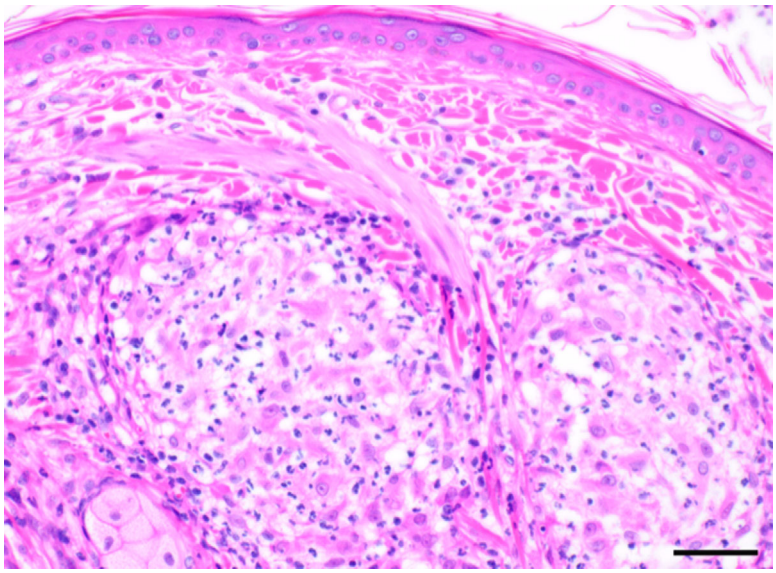

FIGURE 1 Multiple dermal granulomas composed of epithelioid macrophages and neutrophilic granulocytes with a few admixed lymphocytes. HE, bar = 50 μm

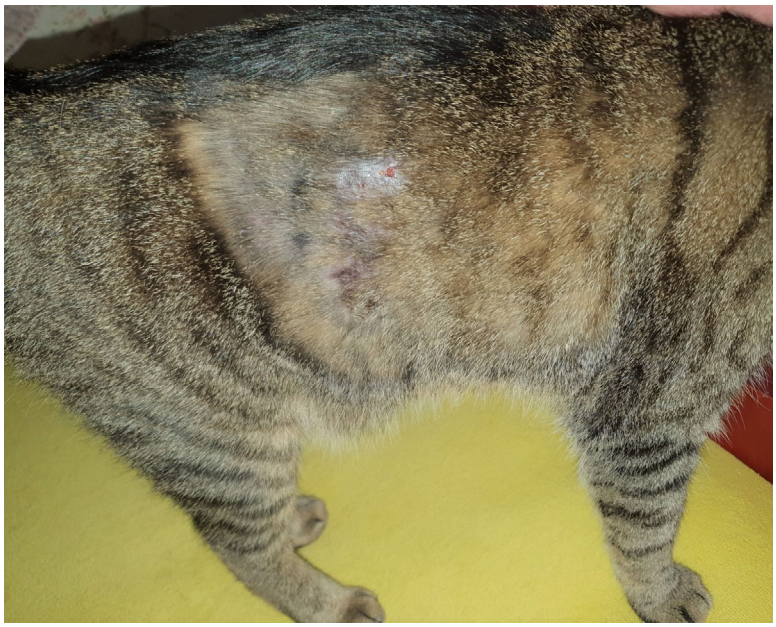

FIGURE 2 In May 2020, the skin lesion was still present with ulceration surrounded by alopecia

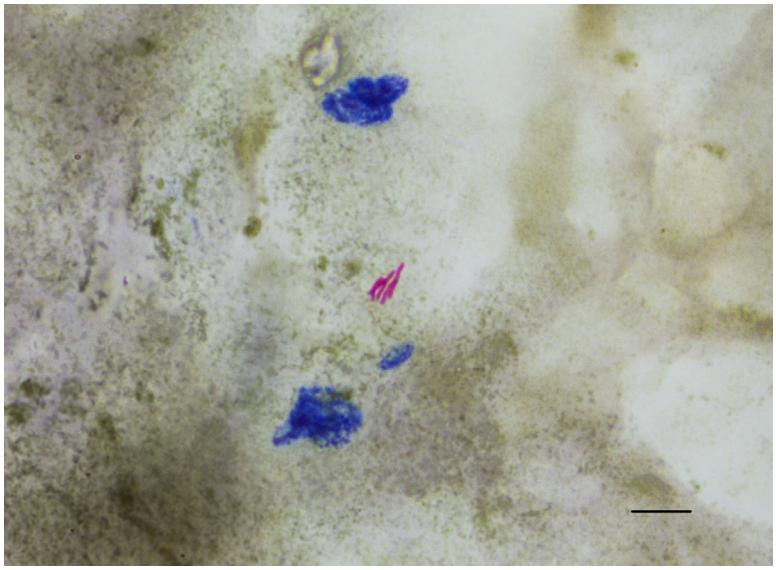

FIGURE 3 Ziehl-Neelsen staining of decontaminated material revealed acid-fast bacilli (bar = 10 μm)

TABLE 1 Antimicrobial susceptibility testing performed on the *M. abscessus* subsp. *abscessus* isolate using the RAPMYCO Sensititre broth microdilution (MIC) method for rapid growing NTM

Antimicrobial	Interpretation[§] μg/mL			MIC values measured	Result
	S	I	R		
Trimethoprim/ sulfamethoxazole	≤2/38	–	≥4/76	2/38	S
Clarithromycin[*]	≤2	4	≥8	4	I
Ciprofloxacin	≤1	2	≥4	2	I
Moxifloxacin	≤1	2	≥4	2	I
Cefoxitin	≤16	32-64	≥128	16	S
Imipenem	≤4	8-16	≥32	64	R
Amikacin	≤16	32	≥64	8	S
Doxycycline	≤1	2-4	≥8	≥16	R
Minocycline	≤1	2-4	≥8	≥8	R
Tobramycin	≤2	4	≥8	8	R
Linezolid	≤8	16	≥32	≤1	S

[§]Minimal inhibitory concentration (MIC) values interpretation for testing rapidly growing mycobacteria according to CLSI M62 Ed1E 2018.
*Extended incubation period of 14 days for clarithromycin MIC testing according to CLSI M24 Ed3 2018.
Abbreviations: S, susceptible; I, intermediate; R, resistant.

according to Brown-Elliott et al[18] and revealed sequevar 6, confirming an inducible macrolide resistance.[19] Histopathologic investigations yielded similar results as already seen in December 2019.

After recurrence of nodular skin lesions 4 months later in September 2020, another specimen was sent for mycobacterial culture and histopathologic examination. Mycobacterial culture yielded the same result, including the susceptibility testing. No histological improvement of the lesions could be observed.

TREATMENT

Following the diagnosis of mycobacterial infection with Mab, and after the susceptibility testing results were available in July 2020, the cat's treatment was modified to trimethoprim-sulfamethoxazole (TMS) monotherapy (5 mg/kg trimethoprim and 25 mg/kg sulfamethoxazole q12h, Nopil, Mepha Pharma AG, Switzerland). After one week of treatment, the owner experienced difficulties with twice daily administration of the medication to the cat and continuous antimicrobial regimen could not be achieved. Different oral application forms, such as powdered and liquid form, were tested, without any improvement. Daily subcutaneous administration by the veterinarian was only possible with fixation of the patient in a cage and was considered too stressful for the animal as a long-term solution. The oral medication was, however, continued for six months at irregular intervals.

OUTCOME AND FOLLOW-UP

After recurrence of the nodular skin lesions in September 2020, another specimen was sent for mycobacterial culture and histopathologic examination. Mycobacterial culture yielded the same result as in May 2020, including the susceptibility testing. No histological improvement of the lesions could be observed.

At the time of writing in February 2021, no signs of improvement have been noticed and the above described lesions are unchanged (Figure 4). Euthanasia was recommended because of animal welfare and the zoonotic risk posed by the cat for the owners living in the same household.

DISCUSSION

Mab is an emerging pathogen responsible for severe respiratory and skin infections in both immunocompetent and immunocompromised humans.[10] Mab belongs to the rapidly growing mycobacteria (RGM) and is a member of the *M. abscessus* complex, comprising further two recently assigned subspecies: *M. abscessus* subsp. *bolletti* and *M. abscessus* subsp. *massiliense*.[20] These two subspecies were previously known as independent species; however, recent phylogenetic studies based on phenotypic and current genomic data revealed close relationship supporting reclassification.[20] Older publications designated Mab as *M. abscessus* or *M. abscessus* sensu stricto, without subspecies definition.[21] Mab infections in animals occur most likely after contact with environmental sources, especially in free-roaming cats. Predisposing trauma,

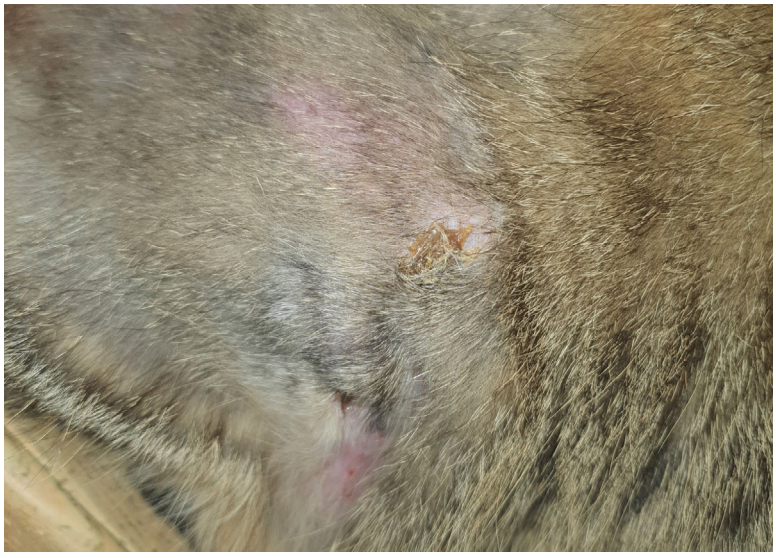

FIGURE 4 In January 2021, the lesion was ulcerated with purulent secretion surrounded by alopecia

such as bite wounds, are commonly reported in animals.[7] Direct or indirect contact with an infected person poses a non-negligible risk for human infections.[10] Reverse zoonosis between pet owners and their companion animals has not been reported so far, however cannot be excluded. The cat was first presented with an abscess on the abdominal wall, most likely caused by a cat fight.

Surgical intervention is described as an important part of the treatment for RGM infections; however, antimicrobial therapy is considered at least as important.[7] Repeated surgical excision of skin lesions did not lead to clinical improvement in the current case, even though wide surgical margins were attempted. Recurrence of skin changes only 1 week after surgical removal was reported in the case report by Jassies-van der Lee et al[6] in a cat with Mab skin lesions. Although the second histopathology in the present case indicated demarcation from healthy tissue, it is not clear whether Mab already penetrated deeper into the tissue or an intraoperative contamination occurred. Concomitant antimicrobial therapy may have prolonged asymptomatic phases after surgical interventions in the present case. However, Relapses occurred approximately 2 months later.

Intrinsic resistances, due to numerous antimicrobial target modifications, and acquired resistances complicate therapy of Mab infections. Clarithromycin is a first-line antimicrobial in treating Mab infections. However, Inducible macrolide resistance, conferred by the *erm*(41) gene, excludes this treatment option in the present case. Due to difficulties in MIC determination for clarithromycin, *erm*(41) sequencing is recommended to predict inducible macrolide resistance.[18] The identified sequevar type 6, should be reported as resistant to macrolides according to CLSI guidelines,[19] despite the rather low clarithromycin MIC of 4 μg/mL observed. This underlines the challenge in macrolide susceptibility testing.

TMS monotherapy was chosen for the treatment of the current case, based on susceptibility results, and it was the only available oral medication. There is scarce literature about the effectiveness of TMS against RGM, especially in Mab treatment.[22] However, A combination therapy with tetracycline, macrolide and TMS is described in initial empirical treatment against RGM in cats.[23] Due to macrolide and tetracycline resistances, dual therapy with amikacin and cefoxitin would have been the most appropriate drug choice.[6] However, oral formulations of both cefoxitin and amikacin are not available in Switzerland. Combination therapy with TMS and linezolid, which both were tested susceptible, was not an option due to the classification of linezolid as last-resort antibiotic in human medicine in Switzerland. Two new tetracycline analogs, eravacycline and omadacycline, are promising drugs for treating Mab infections.[24] However, these antimicrobial agents are currently not available in Switzerland and most likely they will only be approved for treatment of human infections. Thus, limited oral drug availability, as well as difficulties with long-term oral administration of preferably two to three compounds and acquired resistances makes treatment of Mab infections in cats extremely difficult.

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