Mycobacterium avium and Klebsiella pneumoniae co-infection in the domestic ferret (Mustela putorius furo) – Case Report

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▌Abstract

Introduction and Objective. Pets infected with zoonotic pathogens might become a source of infections for their owners, especially those who are immuno-compromised. The aim of this report is to describe a case of chronic, untreatable pneumonia in a domestic ferret.
Materials and method. The subject was a 5-year-old female ferret suffering from recurrent pneumonia. Ante-mortally, swabs from the nasal cavity, alveolus and throat were collected from the animal. Post-mortally, lesioned organ fragments were collected. Standard microbiological testing was performed. Additionally, mycobacterial diagnosis including culture and molecular tests was performed.
Results. The co-infection of Mycobacterium avium and Klebsiella pneumoniae was microbiologically confirmed.
Conclusions. This case demonstrates the need to pay attention to the possibility of zoonotic pathogens in ferrets. Veterinarians diagnosing ferrets are potentially exposed to Mycobacteria spp. infections and other pathogens.

▌Key words

Klebsiella pneumoniae, zoonosis, domestic ferret, Mycobacterium avium

INTRODUCTION AND OBJECTIVE

The domestic ferret (*Mustela putorius furo*), belonging to the family Mustelidae, was domesticated over a thousand years ago. Nowadays, they are important laboratory animals [1–4], and are becoming more and more popular as pets in both Europe and North America [5].

Like every pet, domestic ferrets can be a source of zoonotic pathogens for humans [6]. They are susceptible to microorganisms that can be potentially transmitted to humans. The pathogen currently receiving the most attention is SARS-CoV-2 [7–9]. A case of natural infection was described in Slovenia: SARS-CoV-2 was transmitted from an owner to a pet ferret. Whole-genome sequencing of the virus isolated from samples from the owner and the ferret revealed a difference of only merely two nucleotides [10]. Further examples of pathogens that can be potentially transmitted from ferrets to humans are bacteria from the genera *Leptospira* [11] or *Mycobacterium* [12].

Mycobacterial diseases are important in ferrets [12]. The species found in ferrets are mainly *Mycobacterium bovis* [13–15], *Mycobacterium microti* [16], *Mycobacterium avium* complex (MAC) [17, 18], *Mycobacterium triplex* [13], *Mycobacterium abscessus* [19], *Mycobacterium xenopi* [20] and *Mycobacterium genavense* [21, 22]. MAC includes mycobacteria which are widespread in the environment. Initially, MAC includes two species: pathogenic *M. avium* and non-pathogenic for birds *M. intracellulare.* Currently, the proposed taxonomic division classified as MAC: *M. avium*, *M. intracellulare*, *M. bouchedurhonense*, *M. chimaera*, *M. colombiense*, *M. ituriense*, *M. lepraemurium*, *M. marseillense*, *M. paraintracellulare*, *M. scrofulaceum*, *M. timonense*, *M. lukis*, and *M. yongonense* [23].

Ferrets can act as reservoirs of mycobacteria, which also place their owners at risk [24]. This is especially dangerous for owners with immuno-deficiencies, such as those infected with human immunodeficiency virus (HIV). As an example, disseminated infection by the opportunistic zoonotic bacteria *Mycobacterium avium* subsp. *hominissuis* was noted in a household where both owners were HIV-positive [17]. The transmission of *Mycobacterium chelonae* to humans through a ferret bite has been described [25], as well as recurrent *M. bovis* infection caused the same way [26].

The aim of this study was to report a case of recurrent pneumonia with a complex diagnosis in a domestic ferret.

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CASE REPORT

An interview with the owner showed that at the beginning of 2022, a 5-year-old female ferret weighing 0.790 kg began to suffer from recurrent pneumonia. At the same time, the household member was diagnosed with SARS-Cov-2 infection. A lung X-ray revealed interstitial reactions and oedema indicated pneumonia. The ferret was given steroid drugs in inhalation and antibiotics. After a few weeks, the animal's condition improved, but shortness of breath remained. Then, the teeth of the right arcade of the mandible became infected and extraction was conducted. The right mandibular lymph node shrank over time. In fine-needle aspiration biopsy, the granulomatous lymphadenitis with numerous bacteria that did not stain with the Diff Quick method were found.

The animal was diagnosed in our facility at the beginning of March 2023. Ante-mortally, swabs from the nasal cavity, alveolus, and throat were collected. At the end of March, the animal was euthanized because of its deteriorating condition. Post-mortally, lesioned organ fragments were collected: sections of the lungs, liver, spleen, and mandibular and retropharyngeal lymph nodes.

Standard bacteriology. The swabs were transferred to Columbia agar supplemented with 5% defibrinated sheep blood (CAB) (Graso Biotech, Starogard Gdański, Poland) and MacConkey agar plates, and cultured for 24 h at 37 °C under aerobic conditions. After incubation, mono-cultures of isolates were prepared by streaking single colonies onto the CAB. The isolates were identified based on the cell morphology (Gram staining), growth features, and biochemical characteristics. Catalase and oxidase production were investigated with conventional bacteriological methods. A single colony from the pure culture was suspended in 5 mL of API Suspension Medium (BioMérieux, Craponne, France), and an API 20E kit (BioMérieux) was used according to the manufacturer's instructions to identify of the isolate recovered.

MYCOBACTERIAL DIAGNOSIS

Decontamination. Materials from swabs were centrifuged at 1,500 g for 10 min. Tissue samples from the lungs, spleen, liver, and mandibular and retropharyngeal lymph nodes were minced using sterile scissors and a mortar, and were decontaminated using 5% oxalic acid.

Bacterioscopy. The obtained sample deposit was placed on a degreased slide and fixed; fluorescence staining was carried out with organic dyes absorbing ultraviolet light (auramine dye). The preparations were viewed using a fluorescence microscope with LED lighting. In the case of the presence of mycobacteria in an auramine-stained smear, the slide was stained by the Ziehl–Neelsen method and subsequently evaluated using a light microscope.

Culture. After decontamination, the material from the resuspended pellet was plated in triplicate onto Stonebrink (Becton Dickinson, Franklin Lakes, NJ, USA) and Löwenstein–Jensen (Becton Dickinson) media and incubated at 37 °C. Growth on solid media was assessed every 7 days for 10 weeks. If rough white to light yellow colonies were observed, they were considered *Mycobacterium* sp. After decontamination, the material from the resuspended pellet was also inoculated into liquid Middlebrook 7H9 medium. The tubes were placed in a BACTEC™ MGIT™ (Mycobacteria growth indicator tube) 960 Mycobacterial Detection System (Becton Dickinson), and incubated at 37 °C for 56 days. Fluorescence readings were taken continuously for 56 days by a UV transilluminator.

Genetic tests. For direct detection of mycobacteria, GeneXpert MTB/RIF (*Mycobacterium tuberculosis* presence and rifampicin resistance) Ultra tests (Cepheid, Sunnyvale, CA, USA) were used according to the manufacturer's manual. Briefly, a sample reagent was added to the sample tube and incubated for 15 minutes at room temperature. Next, 2 mL of inactivated sample was pipetted into the test cartridge, which started the test.

DNA isolation and molecular identification. Before further analysis, material from the liquid media was centrifuged at 13,000 rpm for 10 min and material from solid media was used directly. Isolation of DNA was performed as described previously [27]. Briefly, a Genolyse isolation kit (Hain Lifescience, Nehren, Germany) was used to isolate DNA. The strain was differentiated using the GenoType® *Mycobacterium* CM (common mycobacterium) test (Hain Lifescience). All tests were performed in accordance with the manufacturer's instructions.

RESULTS

Standard bacteriology. Bacterial cultures from pharyngeal swabs yielded growth of a species identified as *Klebsiella pneumoniae* by API 20E.

Mycobacterial diagnostics. Bacterioscopy was positive for mycobacteria in all materials, both swabs and tissues. *Mycobacterium tuberculosis* complex DNA was not detected using GeneXpert in either swabs or tissue. The microbiological results were positive for all samples in the Ziehl–Neelsen method and culture. On both types of solid media, the bacteria were manifested as rough, yellowish colonies. The species was classified as *Mycobacterium avium* using the GenoType® *Mycobacterium* CM test.

Necropsy. Necropsy revealed tuberculous granulomas in the lungs (Fig. 1) and liver (Fig. 2).

DISCUSSION

The case is presented of a ferret with generalized mycobacteriosis caused by *Mycobacterium avium* and *Klebsiella pneumoniae* co-infection. Based on the post-mortem lesions, it could be concluded that the main cause of the animal's chronic pneumonia was atypical mycobacteria. However, *K. pneumoniae* is also not a commensal bacterium of the oral cavity in ferrets. It was suspected that there was a superinfection as a result of the animal being weakened by the mycobacterial infection.

Mycobacterium spp. infections should be included in the differential diagnosis for ocular, respiratory, and

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Figure 1. Tuberculous granulomas in the lungs of a pet ferret infected with Mycobacterium avium.

Figure 2. Tuberculous granulomas in the liver of a pet ferret infected with Mycobacterium avium

gastrointestinal diseases in ferrets [18]. Clinical signs noted in Mycobacteria-infected ferrets included enlarged lymph nodes, organomegaly in the liver and spleen, weight loss, and diarrhoea. The particular signs which were present correlated with the location of granulomas [12]. In ferrets, mycobacterial infections most commonly affect the gastrointestinal tract, and for this reason the most frequent clinical signs are anorexia, emaciation, and diarrhoea [12]. In the presented case, the main clinical signs included shortness of breath and weight loss, andthe post-mortem lesions of tuberculous granulomas were localized not in the gastrointestinal tract, but in the liver and lungs.

Fifteen *K. pneumoniae* strains were isolated from a mink (*Neovison vison*) with respiratory distress in China. After performing a genetic analysis of those strains, it was concluded that a potential threat existed of the transmission of *K. pneumoniae* to humans [28]. Being a member of the same family as mink, pet ferrets infected with *K. pneumoniae* can be a potential threat to owners and veterinarians. In another pet, in this instance a cat, *K. pneumoniae* producing extended-spectrum β-lactamases was confirmed [29]. These examples show that there is a potential risk to owners of *K. pneumoniae*-infected ferrets.

The limitation of this study was the non-conducting of SARS-CoV-2 tests. It would have been reasonable to test the ferret because there was a case of COVID-19 in the household. SARS-CoV-2 infections have been described in farmed mink and resulted in increased mortality, inflicting loss on farmers [30], but such infections are also a zoonotic threat to pet ferret owners [10].

The presented case shows the need to pay attention to the ferret as a potential source of zoonotic pathogens. The zoonotic potential of *Mycobacterium* spp. has been described in more than one publication [31–34]. *Mycobacterium avium* complex infections usually occur in people with compromised immunity [35]; however, there are also other risk factors, such as low body mass index, vitamin D deficiency, gastro-oesophageal reflux disease, or rheumatoid arthritis [36]. Disease can also develop in immunocompetent people without specific predispositions [37, 38]. Infection is usually through inhalation or injured skin [39], which is consistent with the most common form of MAC infection being a pulmonary disease [40], at least in adults. In children, however, a relatively common form of the disease caused by MAC is peripheral lymphadenopathy [41]. The continuing debate about the possible participation of *Mycobacterium avium* sp. *paratuberculosis* in the pathogenesis of Crohn's disease also cannot be forgotten [41–43]. For these reasons, monitoring mycobacteria in pets is highly important.

CONCLUSIONS

This case has shown that ferrets can pose a potential health risk to their owners. Veterinarians diagnosing and treating ferrets are potentially exposed to *Mycobacteria* spp. infections and other pathogens. Therefore, they should apply standard preventive measures, such as appropriate hygiene and sanitation, and should educate pet owners.

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