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OLGU SUNUMU CASE RAPORT

Disseminated Mycobacteriosis Caused by *Mycobacterium marinum* in a Beauty Rat Snake (*Elaphe taenuria*): A Case Report

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Abstract: Reptiles are one of the popular companion animals today. The fact that they are ectothermic and require specific care conditions for a healthy life distinguishes these species from other popular species and also creates conditions for reptiles to be cared. When these conditions are not met, diseases can be seen because they cannot develop an adequate immune response. Mycobacterial species can live without infecting a host as long as the reptile has a strong immune system. Therefore, these bacterial strains mostly infect reptiles that have been exposed to poor environmental conditions resulting in high bacterial shedding. Mycobacteriosis agents shed from the bodies of reptiles can survive in the environment for a long time due to their resistance to environmental conditions, and they can be found in living spaces, litters and equipment even if the infected animal is removed from the environment. During contact with reptiles with mycobacteriosis, habitat cleaning and maintenance, mycobacteriosis agents in animals and in the environment can be transmitted to humans through contact and mechanical carriers, and granulomatous lesions may develop in humans. Mycobacteriosis is a very important disease for human health as it is a zoonotic disease. Today, mycobacteriosis remains one of the important reptile diseases due to the lack of effective treatment in animals and the difficulty of antemortem diagnosis. In this article, a case of mycobacteriosis in a yellow rat snake presented. Post-mortem diagnostic methods for mycobacteriosis, histopathological examination method, isolation and identification of the agent is presented, different identification methods and what can be done for ante-mortem diagnosis are mentioned. Reptile mycobacteriosis agents, the predisposing factors, the development of the disease, the importance of reptile and human health were evaluated.

Keywords: Mycobacteriosis, *Mycobacterium marinum*, reptile, snake..

Bir Sarı Sıçan Yılanında (*Elaphe teanuria*) *Mycobacterium marinum* Sebebiyle Gelişen Mikobakteriozis: Olgu Sunumu

Öz: Sürüngenler günümüzde popüler eşlikçi hayvanlardan biri olarak yerini almaktadır. Ektotermik yapıya sahip olmaları ve sağlıklı yaşayabilmek için spesifik bakım koşulları gerektirmeleri bu türleri diğer popüler türlerden ayırmakta ve aynı zamanda sürüngenlerin beslenebilmeleri için şartlar oluşturmaktadır. Bu şartlar sağlanmadığında yeterli immun cevap geliştiremedikleri için hastalıklar görülebilmektedir. Sürüngenler yeterli immun potansiyale sahip oldukları zamanlarda mikobakterileri organizmalarında kendileri etkilenmeden taşıyabildiklerinden, uygun olmayan şartlar karşısında mikobakteriozis gelişebilmekte, bunun sonucunda yüksek miktarda etkeni saçabilmektedirler. Zoonoz bir hastalık olması sebebiyle mikobakteriozis insan sağlığı için de oldukça önemli bir hastalıktır. Sürüngenlerin vücutlarından saçılan mikobakteriozis etkenleri çevre koşullarına dayanıklı olmaları sebebiyle çevrede uzun süre canlılıklarını sürdürebilmekte, infekte hayvan ortamdan uzaklaştırılsa bile yaşam

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alanlarında, altlıklarda ve ekipmanlarda bulunabilmektedirler. Mikobakteriozis gelişmiş sürüngenlerle temas, yaşam alanı temizliği ve bakımları esnasında hayvanlardaki ve ortamdaki ve ekipmanlardaki mikobakteriozis etkenleri insanlara temas ve mekanik taşıyıcılar yoluyla bulaşabilmekte ve insanlarda granülamatoz lezyonlar gelişebilmektedir. Hayvanlarda etkin bir tedavinin olmaması ve antemortem teşhisin oldukça zor olmasından dolayı günümüzde mikobakteriozis önemli sürüngen hastalıklarından biri olarak kalmaktadır. Bu makalede, bir sarı sıçan yılanında görülen mikobakteriozis için uygulanan post-mortem teşhis yöntemleri, histopatolojik inceleme yöntemi, etkenin izolasyonu ve identifikasyonu sunulmuş, farklı identifikasyon yöntemleri ve ante-mortem teşhis için yapılabileceklerden bahsedilmiştir. Mikobakteriozisin etkenleri, hazırlayıcı faktörler, hastalığın gelişimi, sürüngen ve insan sağlığı açısından önemi değerlendirilmiştir.

Anahtar kelimeler: Mikobakteriozis, *Mycobacterium marinum*, sürüngen, yılan.

INTRODUCTION

Mycobacteriosis is a chronic disease caused by the *Mycobacteriaceae* family. Members of this genus are Gram-positive, aerobic, and acid-fast microorganisms (Soldati et al., 2004).

Mycobacteria are the most common cause of granulomatous lesions in people and animals because mycobacteria are intracellular agents, the organism's immunological reaction takes the form of a histiocytic granuloma. A great number of macrophages accumulate in the region of the lesion during its early stages, and central macrophage necrosis can be seen (Frye, 1991). The fibrous connective tissue also serves to limit the lesion by covering the exterior wall. This lesion calcifies in mammals during the chronic period; however, it does not calcify in birds or reptiles. Atypical mycobacteria which developed granulomatous grayish-white nodules in reptiles, are virtually always found in necropsies (Ebani, 2017).

Reptiles are considered resistant to mycobacteria as they can retain these microorganisms in their bodies without developing diseases. Inadequate nutrition, decrease in the quality and hygiene of the living environment, decrease in temperature and the presence of other diseases cause microorganisms to multiply and form the disease. According to a screening study, analysis of fecal samples taken from clinically healthy domesticated reptiles revealed that 16% of these animals were carrying mycobacteria and were shed in their feces (Wolinsky, 1992).

Non-tuberculous mycobacteria (atypical mycobacteria) are commonly found in reptile habitats, soil, water, plants, dust deposits, and feces (Ebani, 2017). Mycobacteria enter the environment through contaminated food, water, and skin sores. The mode of entry defines the sickness and symptoms that will manifest in the organism (Rastogi et al., 2001).

In cold-blooded animals, all nontuberculous mycobacterial agents can produce lesions in the lungs, liver, kidneys, spleen, heart, reproductive organs, nervous system, and joints, as well as septicemia (Ebani et al., 2012). Mycobacteriosis is most commonly encountered in cold-blooded species. Mycobacterial infections have been observed in reptiles; including snakes, tortoises, turtles, crocodiles, and lizards according to case reports (Mitchell, 2012).

CASE HISTORY

An eight-year-old, male, captive-bred Beauty Rat Snake (*Elaphe taeniura*) has been kept in a private zoo in Istanbul, Turkey. The animal was housed in a terrarium with 200*200*150 cm measurements, a constant temperature of 24°–27° C, basking and UV lamps, an adequate amount of wood branches, and a biofiltered small pool for water consumption and humidity. As a bedding, coco fiber mixed with aspen wood chips was used. The animal was fed with commercially acquired, medium-size frozen-thawed rats once a week and always accepted the prey without any problems. The animal was under periodic observation for health and behavioral checks by a zoo veterinarian. Generally, all the checks were uneventful, and the animal had a good appetite and showed no signs of disease or stress.

After a feeding session on March 20 of 2021, the animal showed difficulty swallowing the prey and breathing. A physical examination was carried out in order to find the problem. During a complete physical examination, the body weight of the animal was 2200 g and showed no signs of disease other than excess saliva inside the oral cavity. The animal was taken to quarantine and non-specific therapy for suspected pneumonia with enrofloxacin (10 mg/kg i.m. q24h, Baytril 2.5%), parenteral nutrition (Duphalyte 0.5 ml, SC, q24h), vitamins and amino acids (Aminosol 0.5 ml, SC, q24h) started. More detailed tests, such as x-rays and blood tests, are planned for the next day. However, the animal died before the aforementioned tests could be done, and the necropsy of the animal was carried out immediately.

During necropsy, the animal was inspected for body mass condition, skin pathologies, and oral cavity examination. No external pathologies were observed except excess mucus and foam in the oral cavity and

glottis. In a dorsoventral position, the coelomic cavity was opened from the mouth to the cloaca. Excess mucus and foam were observed also in the trachea, which showed the animal had pneumonia-like symptoms. Whiteish-grayish granulomas were observed on the pericardium, left lung, liver, air sacs, and stomach. On macroscopic examination of the lung, there were multifocal to coalescing, roundish, yellowish granulomas with a diameter of 0.5 to 2 cm (Fig 1A) and the cut surfaces of the lesions showed a central yellowish, dry caseous necrosis surrounded by a thin fibrous tissue. In the liver, lesions appeared as disseminated miliary granulomas with a diameter of 0.1 to 0.3 cm. (Figure 1B). Tissue samples were collected from the lungs and liver for histopathological and microbiological analysis.

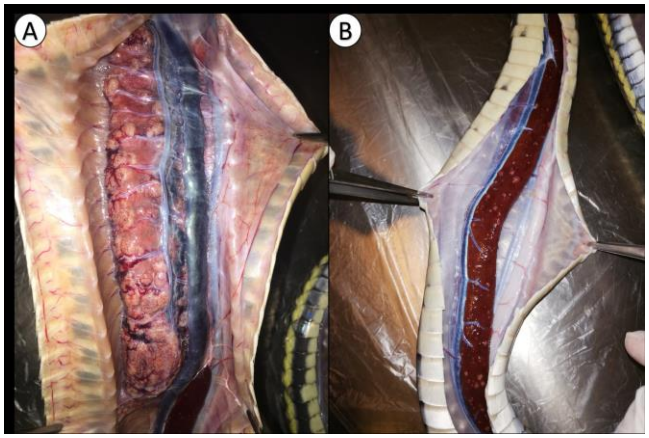

Fig. 1. A. Lung, multifocal to coalescing, roundish, yellowish granulomas 0.5 to 2 cm in diameter. B. Liver, disseminated miliary granulomas 0.1 to 0.3 cm in diameter.

MATERIAL AND METHOD

For histopathological analysis, tissue samples collected from lung and liver were fixed in 10% formaldehyde solution for 24 hours. After the tissue samples were routinely processed, they were embedded in paraffin blocks. Sections of 4 µm of thickness were cut with a rotary microtome and stained by Hematoxylin & Eosin for light microscopy. An additional staining method for the demonstration of Acid-Fast Bacilli (AFB) was performed using Ziehl-Neelsen (ZN) staining kit (GBL, Istanbul, Ref: 5017, LOT: 928).

For microbiological analysis, tissue samples were prepared for inoculation to Lowenstein-Jensen (LJ) media. Firstly, a small amount of tissue with granulomas was taken from the samples and placed in sterile mortar. In order to decontaminate the sample from bacteria other than mycobacteria, NaOH was added in the mortar with twice the amount of the tissue. The sample was homogenized with the help of sterile pestle and the mixture was taken in a sterile test tube. The pH of the mixture was checked with pH strips and adjusted to 7.0 pH using HCl acid. 200 µl of the mixture inoculated into LJ medium. And the inoculated media were incubated at 37°C and 26°C aerobically and checked every day for growth.

For genomic analysis, gene extraction was made using Invitrogen PureLink Genomic DNA kit (Thermofisher, Ref: K182000, Lot: 2070303) with the manufacturer's guidelines. The extracted DNA was sent to a commercial laboratory for internal transcriber spacer (ITS) sequence. Polymerase chain reaction (PCR) and sequence made as described previously by Roth et al. (1998).

FINDINGS AND RESULTS

Histopathological Findings: The histologic examination of the tissue samples collected from lung and liver revealed multifocally distributed granulomas in different sizes, which were predominantly characterized by the infiltration of macrophages and occasional multinucleated giant cells. Most of the granulomas had central caseous necrosis and their outer zones were encapsulated by a thin fibrous connective tissue (Fig. 2A and 2B). In addition, some areas of the lung were characterized by the destruction of bronchi and the intrusion of the necrotic debris and inflammatory cells, including macrophages and heterophils, into the airways. Zhiel-Neelsen staining revealed acid-fast bacilli (AFB) in tissue samples. Multiple clusters of AFB were found in lung by ZN staining method. The AFB were observed as rod-shaped bacilli in the caseous necrosis, intercellular between the macrophage cells and intracytoplasmic of the macrophages (Fig. 3A and 3B).

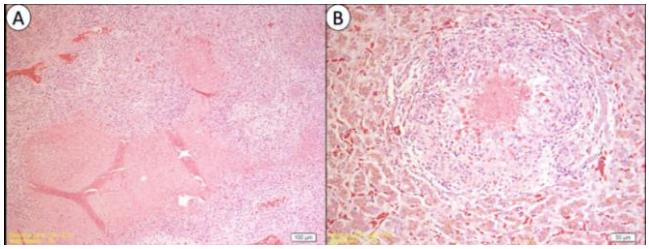

Fig. 2. A. Lung, multifocal to coalescing granulomas with central caseous necrosis surrounded by macrophages and multinucleated giant cells. HE. Bar: 100 µm. **B.** Liver, a solitary granuloma with central caseous necrosis surrounded by demarcation including macrophages and multinucleated giant cells and an encapsulation by thin fibrous tissue.

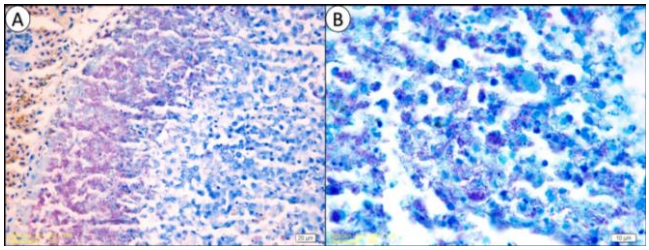

Fig. 3. Lung. **A.** Large numbers of AFB seen intercellular between the macrophage cells or intracytoplasmic of the macrophage cells. ZN Staining. Bar: 20 µm. **B.** Higher magnification of A. ZN Staining. Bar: 10 µm.

Microbiological Findings: For bacterioscopic evaluation of affected tissue samples, a single granulomatous lesion was taken from the tissue and placed between two glass slides and pressure was applied to reveal the content of the lesion. After fixation of the slides, ZN staining was applied. Microscopic evaluation showed acid-fast bacilli in tissue samples.

After incubation of the inoculated LJ media, growth was observed on the sixth and ninth day at 37°C and 26°C respectively. For confirmation of mycobacteria, ZN staining was made from media and acid-fast bacilli were observed.

Genomic sequence result: The sequence results were acquired and BLAST was used for identification of the bacteria. According to BLAST results the bacteria identified as *Mycobacterium marinum* (Accession no: NZ-HG917972.2).

DISCUSSION AND CONCLUSION

Mycobacteriosis caused by atypical mycobacteria is an uncommon disease in reptiles, amphibians, and fish. Because of the zoonotic potential of the disease, it remains an important topic. Even though reptiles are considered resistant to mycobacteria and can harbor the agent without getting sick, a slight disruption in the immune system could result in a localized or disseminated form of mycobacteriosis. Generally, in order to keep and maintain a healthy reptile, keepers must provide specific conditions such as constant heat, humidity, and correct lighting for the species of the reptile and observe if the animal is showing signs of discomfort or disease. Reptiles don't really show a wide variety of symptoms when they get sick. Basically, they present lethargy, loss of appetite, and apathy. As a result of this, various diseases share the same symptoms, so their differentiation and diagnosis could be quite difficult. The form of mycobacteriosis in reptiles can be acute or chronic depending on the species of the microorganism or immune potential of the animal. In the acute form, reptiles may not show any symptoms, and sudden death may be the only observable clue to the disease. (Yoon-Seok et al., 2010). This was the form of the disease in our case. However, if the disease form is chronic, reptiles present nonspecific symptoms such as lethargy, loss of weight, loss of appetite, and pulmonary distress. In addition to these, snakes may show signs of alteration of skin and stomatitis (Mitchell, 2012). Stomatitis symptoms for mycobacteriosis are important for snakes because they can be mixed with mouth rot caused by other microorganisms such as *Pseudomonas aeruginosa,* which shows the importance of isolation and identification of the agent before relying on empirical treatment with wide spectrum antibiotics, if this occurs treatment will not be successful.

Treatment of reptiles with mycobacteriosis is not recommended because of the zoonotic potential of the disease, the length of treatment period, and the lack of successful treatment reports. Usually, when mycobacteriosis is detected while the animals are still alive, the disease is already at an advanced stage, therefore treatment is not recommended and most cases are euthanized because of prognosis and zoonotic potential of the disease (Hernandez-Divers & Shearer, 2002).

Because of the non-specific symptoms and findings, antemortem diagnosis for mycobacteriosis in reptiles still remains rare. Hematologic evaluation can provide useful indicators for inflammatory disease but none of the parameters are considered to be specific for the mycobacteriosis (Muro, 2020). Clinical diagnosis for mycobacteriosis solely relies on isolation and identification of acid-fast bacilli in diagnostic materials. Bacterial culture for the agent is tedious, takes a long time, and demanding. Identification of the bacteria could be done with classic methods, PCR or partial genome sequence (Ullman et al., 2016; Yoon-Seok et al., 2010; Muro, 2020). Histopathology is routinely used to diagnose mycobacteriosis in reptiles. Acid-fast stains are used for the detection of *Mycobacterium* spp. However, isolation and identification of the agent is standard for clinical diagnosis.

Consequently, mycobacteriosis remains an important topic for reptile health also for human health because of its zoonotic potential. Antemortem diagnosis for mycobacteriosis in reptiles is difficult because of non-specific symptoms, however it shouldn’t be overlooked by veterinarians. Even though is not common as other infections, it would be beneficial to bear in mind that patients with non-specific symptoms may have mycobacteriosis.

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