



A Case of Pulmonary Aspergillosis in a 20-day Old Suckling Lamb

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Abstract

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In this study, a case of pulmonary aspergillosis was described in a 20-day-old suckling lamb. Lamb suffering from high fever, anorexia, weakness and balance disorder was brought to the Department of Pathology of Kafkas University Faculty of Veterinary Medicine for necropsy. According to the anamnesis obtained from the animal owner, an intense and random drug treatment was administered to the animal. Following the systemic necropsy of the animal, various tissue samples were taken from for histopathological and microbiological analysis. On macroscopic examination, a yellowish-white colored focus in a diameter of 2x2 mm was detected in only one area of the animal's lung. Histopathological examinations revealed a granuloma in the lung. In the center of this granuloma, in addition to severe necrosis, neutrophils and fungal hyphae were detected. In the PAS staining of the lung tissue, it was noted that these hyphae took the stain and gave a positive reaction in terms of fungus. Ziehl-Neelsen staining of the lung and liver tissues revealed that these tissues were negative for tuberculosis. As a result of histopathological and microbiological analysis, this case, which is rarely seen in dairy lambs, was diagnosed as pulmonary aspergillosis.

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20 Günlük Emziren Kuzuda Bir Pulmoner Aspergilloz Olgusu

Özet

Bu çalışmada 20 günlük bir süt kuzusunda pulmoner aspergillozis vakası tanımlanmıştır. Yüksek ateş, iştahsızlık, halsizlik ve denge problemleri yaşayan kuzu nekropsisi yapılmak üzere Kafkas Üniversitesi Veteriner Fakültesi Patoloji Anabilim Dalı'na getirildi. Hayvan sahibinden alınan anamnez bilgilerine göre hayvana yoğun ve gelişigüzel bir şekilde ilaç tedavisi yapıldığı kaydedildi. Ardından hayvanın sistemik nekropsisi yapıldı. Histopatolojik ve mikrobiyolojik incelemeler için hayvandan çeşitli doku örnekleri alındı. Makroskopik muayenede hayvanın akciğerinde sadece bir alanda 2*2 mm çapında sarımtırak beyaz renkli bir odak tespit edildi. Histopatolojik incelemelerde, akciğerde granülom yapısı saptandı. Bu granülomun merkezinde ise şiddetli nekrozun yanı sıra nötrofiller ve mantar hifalarının varlığı tespit edildi. Akciğer dokusunun PAS boyamasında bu hifaların pozitif reaksiyon verdiği ve boya aldığı kaydedildi. Akciğer ve karaciğer dokularının Ziehl-Neelsen boyamalarında bu dokuların tüberküloz yönünden negatif olduğu tespit edildi. Histopatolojik ve mikrobiyolojik incelemeler sonucunda süt kuzularında oldukça nadir olarak görülen bu vakaya pulmoner aspergillozis tanısı konuldu.

Key Words: *Aspergillus spp., pulmoner aspergillozis, süt kuzusu*

Introduction

Aspergillosis is a systemic, invasive and opportunistic infection caused by fungi belonging to the *Aspergillus* genus in animals and humans. *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus nidulans* and *Aspergillus niger* are among the most common and pathogenic species in this genus (Cafarchia et al., 2012). Aspergillosis causes many pathological changes in humans, especially in the respiratory system. It is also a

common problem in veterinary medicine and poultry are particularly affected by this infection (Şahin et al., 1997; Latif et al., 2015). In addition to poultry, there are some literatures showing that this disease can be seen in dogs, cats, horses and marine mammals (Savelieff et al., 2018). In ruminants, *Aspergillus* species are considered to be the cause of pneumonia, mastitis, gastroenteritis and placentitis (do Carmo et al., 2014).

In this study, it was aimed to evaluate pulmonary aspergillosis infection, which was detected and rarely observed in a 20-day old lamb, by histopathological and microbiological methods.

Case History

The study material was a 20-day-old suckling lamb who had high fever, anorexia, weakness and balance disorder in Susuz District of Kars Province in May 2019 and was brought to the Department of Pathology of the Faculty of Veterinary Medicine of Kafkas University as dead for systemic necropsy. According to the anamnesis obtained from the animal owner, it was recorded that the animal was intensely and indiscriminately supplemented with antibiotics containing amoxicillin, enrofloxacin, oxytetraxacin and vitamin B12 and Selenium-Vitamin E combination. It was found out that Theranecron and Meloxicam were used for papillomatous growths detected in the oral cavity of the animal. Systemic necropsy of the animal was then performed. Rib traces were found in the lung of the animal on macroscopic examination. A yellowish-white colored focus in a diameter of 2x2 mm was detected in an area in the lung. In addition, papillomatous growths were observed on the lips of the animal. One or two small gray areas with a diameter of 1x3 mm were found in the liver. Since the areas in the lung and liver do not have a specific appearance, they were not photographed by us. Numerous large and small calcified white areas were detected in skeletal muscles in the armpit, tail, and gluteal regions. No pathological finding was observed in internal organs such as stomach and intestine.

Various tissue samples (lung, liver, etc.) taken from the animal were fixed with 10% buffered formaldehyde solution. Paraffin blocks prepared after routine tissue follow-up procedures were cut in 5 micron thickness and stained with Hematoxylin-Eosin (H&E), Periodic Acid Shift (PAS) and Ziehl-Neelsen (ZN). Sections were examined under a light microscope and photographed to determine histopathological changes. Intense hyperemia in the lung and a well-circumscribed granuloma structure in a single area were detected (Fig. 1A and Fig. 1B). In the center of this granuloma, intense neutrophil infiltration and fungal hyphae were observed as well as severe necrosis (Fig. 1C and Fig. 1D). Granulomatous lesions were determined in the lung and liver tissue, whereas in the PAS staining (Fig. 1E and Fig. 1F), it was noted that the hyphae gave a positive reaction and stained. In the oral mass, fibrosis and inflammatory infiltration were observed in the dermis. (Fig. 2A). Papillomatous growths and severe necrosis were also detected in the mass (Fig. 2B). Hydropic degeneration and pustule formations

in the papillomatous area were also revealed (Fig. 2C). Severe calcification and fibrosis were observed in skeletal muscles (Fig 3A and Fig. 3B). A single granulomatous area was detected in the subcapsular area of the liver (Fig. 4A). Histocytic cells, giant cells and calcifications were noted in this area (Fig. 4B and Fig. 4C). On the other hand, liver tissue gave negative results in terms of PAS staining. Ziehl-Neelsen staining of the lung and liver tissues revealed that these tissues were negative for tuberculosis. For microbiological analysis of the fungal agents, the tissue samples were plated on Sabouraud Dextrose Agara (SDA) plates and left to incubation for 5 days at 24°C under aerobic conditions. The agent identification was performed according to typical macroscopic and microscopic morphologies and reproductive characteristics of fungi (Swatek et al., 1985; Şahin et al., 1997). In this study, fungal colonies with typical blue-green powdery upper morphology and pale yellow back lower morphology formed by the tissue samples cultured on SDA were defined as *A. fumigatus*. The ethics committee report of this study was obtained from Kafkas University Animal Experimentals Local Ethics Committee (Authorization number: KAU-HADYK-2020/165). Cerrahi hastalıkların yıllara göre dağılımı Tablo 2’de verilmiştir.

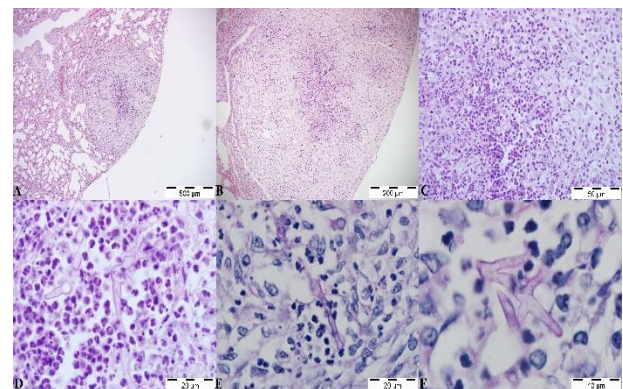


Figure 1. A) Granuloma, lung, H&E, bar = 500 µm. B) Granuloma with a higher magnification, H&E, bar: 200 µm. C) The center of the granuloma, severe necrosis and hyphae, H&E, bar = 50 µm. D) Hyphae and neutrophils, H&E, bar = 20 µm. E) Hyphae, PAS staining, bar = 20 µm. F) Hyphae, PAS staining, bar = 10 µm.

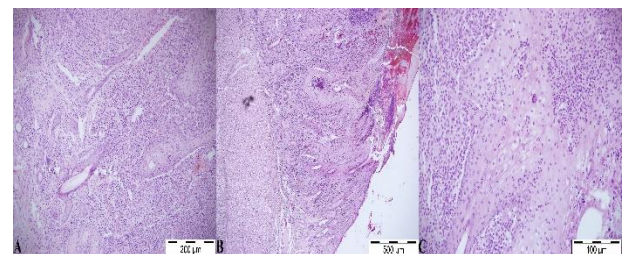


Figure 2. A) Oral mass, fibrosis and inflammatory infiltration in the dermis, H&E, bar = 200 µm. B) Papillomatous growths and severe necrosis, H&E, bar = 500 µm. C) Hydropic degeneration and pustule formations in the papillomatous area, H&E, bar = 100 µm.

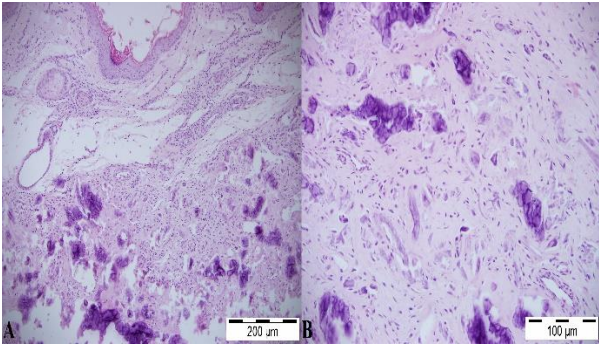


Figure 3. A) Skeletal muscle, severe calcification and fibrosis, H&E, bar = 200 µm. B) Skeletal muscle at a higher magnification, severe calcification and fibrosis, H&E, bar = 100 µm.

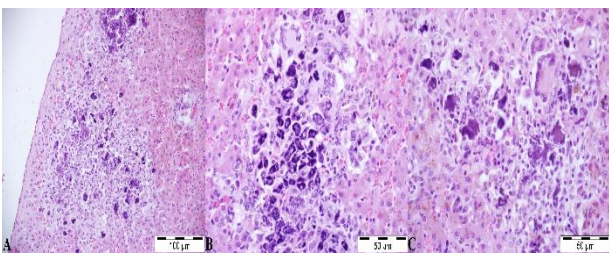


Figure 4. A) Single granulomatous structure in the subcapsular area of the liver, H&E, bar = 100 µm. B) Granulomatous area, histiocytes and calcifications, H&E, bar = 50 µm. C) Granulomatous area, giant cell and pigments, H&E, bar = 50 µm.

Discussion

Aspergillus species are saprophytic filamentous fungi that have the potential to infect many living hosts, including plants, insects, birds and mammals, and live in agricultural land, humid environments and soils (Amchentsev et al., 2008; Seyedmousavi et al., 2015). Although there are more than 300 known species in the *Aspergillus* genus, infections in animals are mainly caused by *A. fumigatus* and rarely by a few other species (Seyedmousavi et al., 2018). (Swatek et al., 1985; Şahin et al., 1997). *Aspergillus* species play a role as opportunistic pathogens in immunocompromised or malformed animals. Suppression of the host's immune system or taking excessive amount of spore are the most important factors in the formation of the disease (Aslan et al., 2015). We think that the age of the lamb in our study is very young and the animal owner used a large number of drugs randomly and without the advice of a veterinarian may have weakened the animal's immune system and these factors may play an important role in the formation of the infection (Özsoy and Hazıroğlu, 2009). Macroscopic and histopathological findings of samples taken from skeletal muscles in the armpit, tail and gluteal regions also support our argument

that the infection is formed as a result of unconscious drug administration. We believe that dystrophic calcification in the muscles occurs as a result of necrosis due to intense and random drug use (Özdemir et al., 2016).

In veterinary sciences, aspergillosis is reported in dogs, horses, cattle and dolphins. Unlike the immunocompromised mammals, poultry show greater sensitivity to *A. fumigatus* (Şahin et al., 1997; Tell et al., 2005). In ruminants, *Aspergillus* species are considered to be the cause of pneumonia, mastitis, gastroenteritis and placentitis (do Carmo et al., 2014). There are few literatures reporting pulmonary aspergillosis in suckling lambs and sheep (Austwick et al., 1960; Gracey and Baxter, 1961; Fragner et al., 1970; Young, 1970; Ohshima et al., 1976; Chihaya et al., 1980; Pérez et al., 1998; Pérez et al., 1999). Fungal infections in sheep are mainly caused by *Aspergillus* spp. or zygomycetes. Systemic mycoses often affect several organs, such as the lungs, mammary glands, or forestomach, and multifocal pyogranulomatous inflammation is observed in these organs (Dagleish et al., 2010). Various researchers have identified a large necrotic area in the middle of these multifocal pyogranulomatous nodules and that this necrotic area is surrounded by neutrophils. They demonstrated the presence of mononuclear cells and a small number of giant cells in the further outer layer of neutrophils (Chihaya et al., 1980; Pérez et al., 1998; Pérez et al., 1999). The macroscopic and microscopic findings of this study were consistent with previous literature data (Chihaya et al., 1980; Pérez et al., 1998; Pérez et al., 1999; Dagleish et al., 2010). Although the general appearance, size, Y-shaped branching structure and septa of the filaments indicate the characteristic morphology of *Aspergillus* in histological sections stained with Hematoxylin-Eosin, special staining techniques such as PAS and Grocott's methenamine silver (GMS) staining are used in the diagnosis of these hyphae (Fragner et al., 1970; Chihaya et al., 1980; Dagleish et al., 2010). In our study, only granulomatous lesions were observed in the lung and liver tissue, whereas in the PAS staining, we observed that the hyphae gave a positive reaction and stained in accordance with the literature data (Fragner et al., 1970; Chihaya et al., 1980; Pérez et al., 1998; Pérez et al., 1999).

In conclusion, there are few studies reporting Aspergillosis infection in sheep and lambs, especially from the lungs (Austwick et al., 1960; Gracey and Baxter, 1961; Fragner et al., 1970; Young, 1970; Ohshima et al., 1976; Chihaya et al.,

1980; Pérez et al., 1998; Pérez et al., 1999). It is quite remarkable that the lamb, which is the material of our study, was 20 days old and it is thought that the findings obtained may contribute to the literature. In line with the findings of the study, it was concluded that the formation of pulmonary aspergillosis infection in a lamb at such a young age may have been caused by the suppression of the animal's immune system by the intensive and indiscriminate drugs the animal owner had taken. It was concluded that suppression of the immune system also plays a major role in the emergence of infection. Therefore, based on these data, the importance of the use of medicines in the conscious and under the supervision of a physician becomes clear.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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