Kafkas Univ Vet Fak Derg
24 (2): 315-318, 2018
DOI: 10.9775/kvfd.2017.18879

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://submit.vetdergikafkas.org

Case Report

Concomitant Mammary Tuberculosis and Malignant Mixed Tumor in a Dog [1]

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[1] *This case presented as a poster presentation at the 50 th European Veterinary Conference, 19-21 April 2017, Den Haag - Netherland*

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Article Code: KVFD-2017-18879 Received: 14.10.2017 Accepted: 21.01.2018 Published Online: 22.01.2018

How to Cite This Article

Kutlu T, Yücel Tenekeci G, Kaya İB, Müştak HK, Kutsal O: Concomitant mammary tuberculosis and malignant mixed tumor in a dog. *Kafkas Univ Vet Fak Derg,* 24 (2): 315-318, 2018. DOI: 10.9775/kvfd.2017.18879

Abstract

Tuberculosis and tumors are two major health problems in both humans and animals; and there are still many question marks in the association between these two important diseases. In this case, malignant mixed tumor and productive tuberculosis were observed simultaneously in 8 years old, terrier, and female dog. After mastectomy of the left mammary chain; removed tissue was brought for pathological examination. For the histopathology examination, specimens were fixed in 10% neutral buffered formalin. Tissues were processed routinely and stained with hematoxylin-eosin and Ziehl-Neelsen for acid-fast bacilli detection. Microscopically, atypical neoplastic cells and caseation necrosis located in the center of tubercle were seen. After these findings, *Mycobacterium* spp. was investigated in the same sample and was confirmed by PCR amplification. Concomitant tuberculosis and neoplasm, previously reported in only a goat, is a rare occurrence in domestic animals.

Keywords: *Dog, Histopathology, Malignant mixed tumor, Mycobacterium spp.*

Bir Köpekte Memede Beraber Seyreden Tüberküloz ve Malign Mikst Tümör

Öz

Tüberküloz ve tümörler hem insanlarda hem de hayvanlarda gözlenen önemli birer sağlık problemi olup bu iki hastalık arasındaki ilişki hakkında halen çok soru işaretleri mevcuttur. Bu olguda 8 yaşlı, dişi, terrier bir köpekte malign miks tümör ve prodüktif tüberkülozün birlikte seyrettiği gözlendi. Klinikte operasyonla alınan sol meme zinciri patolojik yönden incelenmesi için getirildi. Histopatolojik inceleme için doku %10'luk tamponlu formalin solüsyonunda tespit edildi. Rutin doku takibinin ardından hazırlanan parafin bloklardan kesitler alınarak hematoksilen eozin ve asid fast tüberküloz basili tespiti için Ziehl- Neelsen boyamaları yapıldı. Mikroskobik incelemede atipik neoplazik bez epitel hücreleri ile merkezinde kazeifikasyon nekrozu bulunan tüberküllere rastlandı. Ayrıca *Mycobacterium* spp. PCR amplifikasyonu ile teyit edildi. Tüberküloz ve tümörün bir arada bulunması daha önce sadece bir keçide bildirilmiş olup evcil hayvanlarda nadir gözlenen bir olgudur.

Anahtar sözcükler: *Histopatoloji, Köpek, Malign mikst tümör, Mycobacterium spp.*

INTRODUCTION

Tuberculosis is one of the major zoonotic diseases. *Mycobacterium tuberculosis* and *Mycobacterium bovis* are important pathogenic agents, which are able to infect many animals and humans [1-3]. Dog to dog transmission is rare and also most cases of tuberculosis are transmitted from human reservoirs to dogs [4].

Tuberculosis lesions in carnivores are different from the other species. Typical tubercles are not always observed; and when observed, caseous necrosis of the tubercles are not macroscopically significant. Microscopically, typical granulomas are composed of mononuclear cell infiltration and epithelioid histiocytes surrounded by fibrous tissue. Also, necrosis is present in the center of the granulomas. Giant cells are rare or not seen [5].

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Mammary tumors are neoplasms that are commonly seen in domestic animals such as dogs and cats. The mammary malignant mixed tumor is the most frequent neoplasm in female dogs. This tumor is composed of two cell components: epithelial and connective tissue components, both having malignant character [6,7].

Co-existence of tuberculosis and neoplasm has been found to be important for many years. Previously, concomitant tuberculosis and different types of neoplasms, such as meningioma, renal carcinoma and lymphoma were reported in humans [8-10]. In animals, concomitant mammary carcinoma and tuberculosis was reported only in a goat [11].

The purpose of the case is to describe concomitant mammary tuberculosis and malignant mixed tumor for the first time in a dog.

CASE HISTORY

An 8-year-old terrier, female dog was brought to a private clinic with tumor suspicion in mammary glands and surgical operation was performed. Left mammary chain was removed and sent to Ankara University, Faculty of Veterinary Medicine, Department of Pathology for pathological examinations.

Macroscopically, multifocal areas, smaller than 1 cm in diameter, with yellowish/white caseous appearance were seen on mammary glands. For the histopathological examination, specimens were fixed in 10% neutral-buffered formaldehyde. The tissue samples subjected to routine tissue processing, were embedded in paraffin and cut at a thickness of 4-6 µm. First, all sections were stained with the routine haematoxylin-eosin. In addition to routine staining, Ziehl- Neelsen staining was done for detection of acid-fast bacilli.

With haematoxylin-eosin staining, neoplastic mammary gland cells that had differences in size and shape, variations in the cytoplasm/nucleus ratio, pale cytoplasm with unobvious border with mitotic figures were observed *(Fig. 1)*. Metaplastic changes in the mesenchymal tissue accompanied these cells. Also, a few areas of hyalinization and necrosis were recognized *(Fig. 1)*. In the same areas, chronic granulomatous inflammation resembling tubercles was seen. At the center of the tubercles caseous necrosis was present, surrounded by lymphocytes and epithelioid cells infiltration and a fibrous tissue capsule *(Fig. 2)*. With Ziehl- Neelsen staining, acid-fast bacilli were freely scattered in the granulomas.

After these findings, frozen tissue sample suspected to be *Mycobacterium* spp. was sent to Microbiology Department of Ankara University, Faculty of Veterinary Medicine, for the investigation of *Mycobacterium* spp. The confirmation of the histopathological examination was performed by Mycobacterium genus specific PCR *(Fig. 3)*. DNA was extracted from the tissue sample with a commercial kit (Genomic DNA Purification, Catalog No: K0512, Thermo Fisher Scientific, U.S.A.) following the manufacturer's recommended protocol. PCR was performed using specific primers (Forward- B16F 5'-GGG ACGAAGTCGTAACAAGG-3', Reverse- B16R 5'-TGATGCTC GCAACCACTATC-3'), which were designed and tested in-silico for this study to amplify a 270 bp product. The PCR reaction was performed containing 0.2 µM of each primer, 0.2 mM dNTPs (10 mM dNTP mix; (Thermo Fisher Scientific, USA), 3 mM of $MgCl_2$ (Thermo Fisher Scientific, USA), 2.5 µL PCR reaction buffer, 2U of Taq DNA polymerase (Thermo Fisher Scientific; EP0402), and nuclease-free water to a final volume of 25 µL. In the reaction, 1 µL of DNA was used as template. The amplification was performed as follows: strand separation at 95°C for

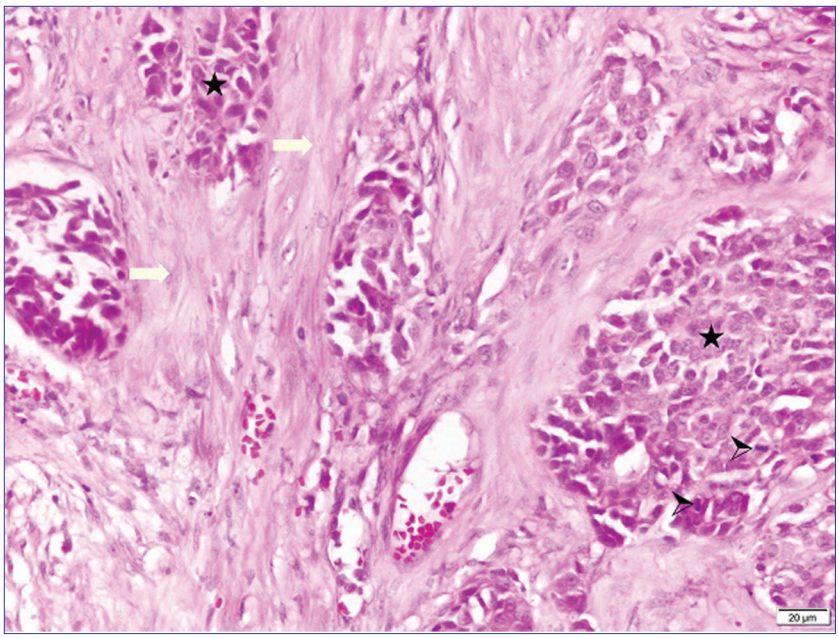

Fig 1. Anaplastic mammary gland cells *(stars)*, mitotic figures *(arrow heads)*, hyalinization of fibrous connective tissue *(white arrows)*, HxE, bar: 20 µm

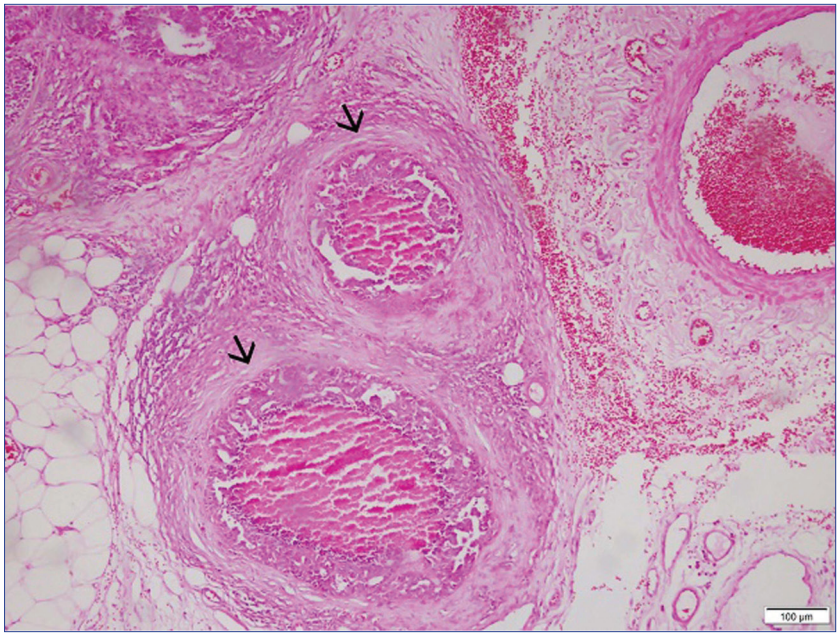

Fig 2. Microscopic structures of tubercle *(arrows):* caseous necrosis at its centre, mononuclear cell infiltrations and fibrous capsule at periphery, HxE, bar: 100 µm

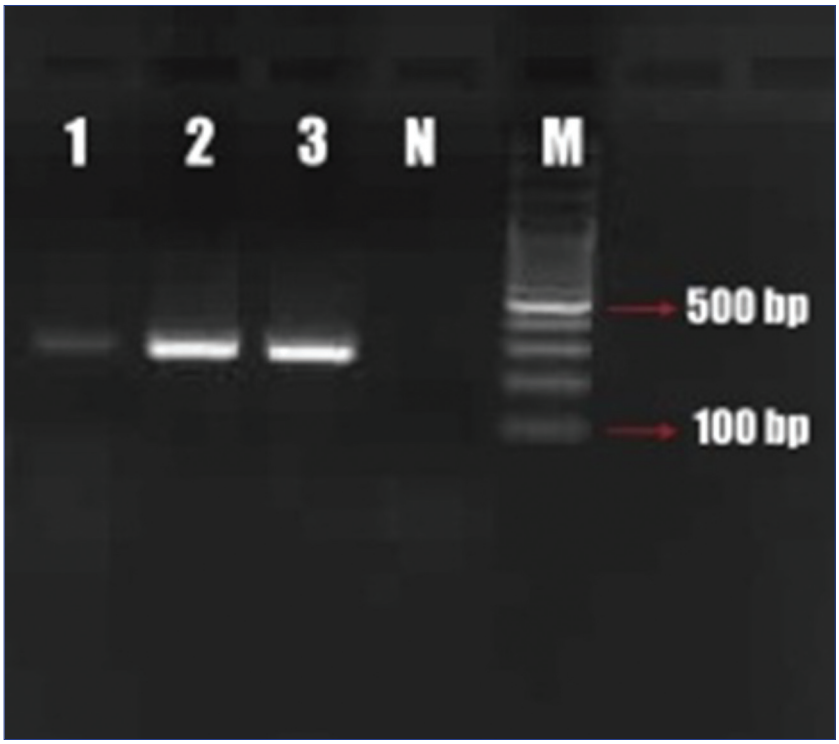

Fig 3. Result of DNA Gel Electrophoresis. 1: sample (270 bp); 2: *Mycobacterium tuberculosis* (positive control); 3: *Mycobacterium bovis* (positive control); N: negative control; M: 100 bp plus DNA Ladder (Thermo Fisher Scientific, USA)

7 min, followed by 40 cycles of 95°C for 45 sec, 60°C for 45 sec, and 72°C for 45 sec. Finally, there was a 7 min at 72°C for further strand extension. Ten microliters of the amplified PCR product was analyzed by electrophoresis on 1.5% agarose gel (Promega Corporation, USA) with 4 µL of SafeView Classic (Applied Biological Materials, Canada) in Gel Electrophoresis Apparatus with 90v for 45 min. *Mycobacterium tuberculosis* and *Mycobacterium bovis* strains which were selected from the collection of Microbiology Department were used as a positive control in all reactions.

DISCUSSION

Inflammation of the mammary gland is known to be rare and nonspecific in dogs [12]. Conversely, in this case, a specific agent such as mycobacterium has been diagnosed in mammary gland. Additionally, malignant mixed tumor was also detected histopathologically in the same mammary gland. In this direction, appearance of tumor was similar to other literature in terms of age, breed and gender susceptibility [6]. The appearance of tuberculosis and malignant mixed tumor together in the same tissue is

an unusual condition. So, tuberculosis should also be also considered if there is a tumor in a dog by veterinarians.

The mechanism of concomitant tuberculosis and neoplasm has been unclear and keep its uncertainty [13]. In humans, observing tumors and tuberculosis at the same time and the same organ causes difficulties in diagnosis and treatment [14]. It was confirmed in experimental studies that prolonged chronic infection, scar tissue formation and irritation lead to the carcinogenesis in time [15]. Also, a study performed in mice reported that chronic tuberculosis infection is enough to cause multi-step transformation of cells like dysplasia, metaplasia and finally carcinomas [16]. Lungs with tuberculosis infection in humans, compared with those without tuberculosis infection were found to be 11 times more sensitive to lung cancer [17].

On the other hand, it is a well-known fact that due to the local and systematic effects (causing malnutrition and immunodeficiency) of the neoplastic disease, susceptibility to tuberculosis infection increases [18,19].

Although there are various opinions; currently the relationship has remained controversial. So, more research is needed to enlighten the association between tumor and tuberculosis.

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