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Case Report

A necrobacillosis case determined in a sheep herd

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ABSTRACT

In this study, cases of necrobasillozis detected in a sheep herd in Kars province are described.A total of 12 dead animals were noted in 2-4 month-old sheep in a herd containing 210 animals. Three of dead animals were brought to Kafkas University Faculty of Veterinary Medicine Department of Pathology for necropsying. Systemic necropsy was performed and tissue samples were collected for histopathological and microbiological investigations. On gross examination, yellow-white multifocal necrotic areas sizing up to 3 cm in liver of one animal, and up to 1 cm in lung of another animal were recognized. No gross lesions were detected in the third animal. In microscopic examination of these two animals in which lesions were detected, coagulation necrosis with central calcification and peripherally located crumbs of leukocyte nucleus were noted. In the animal with lung lesions, alveolar capillary hyperemia and mononuclear cells infiltration including neutrophil leukocytes were also detected. In microbiological analysis, samples were inoculated onto specific agars, incubated at aerobic, microaerobic and anaerobic conditions for 48 hours and finally colonies grown on anaerobic environment in Eugon agar and blood agar were evaluated. In inoculations made from the 2 lungs and 1 liver samples that were collected from different animals, colonies that produce large hemolytic areas were viewed. In gram staining, fusiform and gram (-) bacteria were named as *Fusobacterium necrophorum*. The results of the study indicate that necrobasillozis might be an important cause of death in lambs in Kars province. It was also concluded that death due to necrobasillozis might be seen in lambs with no apparent gross lesions.

INTRODUCTION

Fusobacterium necrophorum is a Gram negative, non-spore forming, obligate anaerob pleomorphic microorganism in human and animal flora of the digestive tract (13, 15). There are two subtypes of agent called as *F. necrophorum* subsp. *necrophorum* (biovar A) and *F. necrophorum* subsp. *funduliforme* (biovar B) which are opportunistic pathogens of various animal species. *F. necrophorum* may be the primary disease agent in ruminants or as an important component of the mixed infections in those they form together with other pathogenic bacteria (1). Toxins such as leucotoxin, endotoxin, hemolysin, hemagglutinin and adhesin are thought to be closely related to the virulence of *F. necrophorum* (16, 21). Due to its high polysaccharide content and leukotoxin production ability, *F. necrophorum* subsp. *necrophorum* subspecies is more pathogenic and commonly isolated from necrotic cases (18, 21). *F. necrophorum* causes rumenitis, liver abscess syndrome, interdigital necrobacillosis, calf diphtheria (stomatitis, laryngitis and pharyngitis) and abortion in sheep (6, 22). All these diseases are generally called as necrobacillosis (13, 20). Though anaerobic culture methods are used in the diagnosis of disease, these methods are inadequate. Therefore, the true incidence of *F. necrophorum* infections cannot be fully established (17). Thus, macroscopic lesions and histopathological findings may contribute to the diagnosis of *F. necrophorum*.

The aim of this study was to determine the necrobacillosis cases by histopathological and microbiological methods in a sheep herd in Kars province, Turkey.

MATERIALS and METHODS

The material of this study was consisted of 12 lambs of 2-4 months age, which died in about one week with respiratory distress, observed in a herd consists of 210 sheep in April 2016 in Selim district, Kars. Unfortunately, only 3 of the dead animals could be reached without putrefaction and they were brought to the Department of Pathology, Veterinary Faculty, Kafkas University in order to make laboratory diagnoses. Systemic necropsies of the animals were done and histopathological and microbiological studies were performed on infected tissues, liver and lungs.

In macroscopic examination, multifocal white necrotic areas of 3 cm diameters in liver of one lamb and 1 cm diameter in lung of another lamb were observed (Figure 1a, 1b). No other findings were found in the internal organs of the remaining lamb.

Tissue samples were fixed with 10% buffered formaldehyde solution and following the routine tissue follow-up procedures and paraffin blocked. 5 microns thickness sections were

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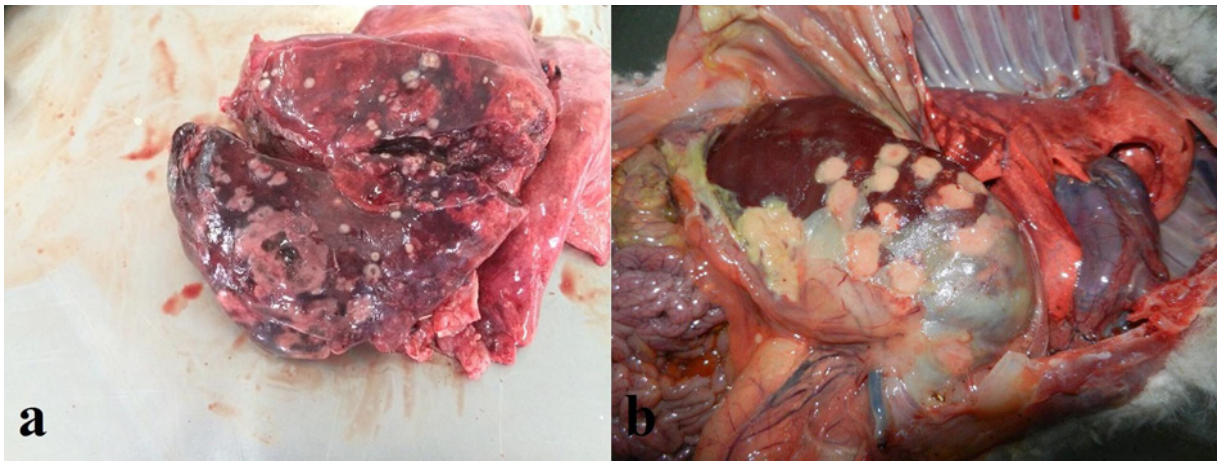

Figure 1. a) Multifocal necrosis areas in lung b) Multifocal necrosis areas in liver

cut and stained with Hematoxylin-Eosin (H & E). Inoculums prepared from tissue samples were inoculated on Brain-Heart-Infusion (BHI) (Sigma, DE) agar plates supplemented with 10% defibrinated sheep blood, 0.5% yeast extract, 0.01% magnesium sulfate, vancomycin (5µg / ml) and neomycin (100 µg / ml). The agar plates were placed in anaerobic jar using AnaeroGaspack kits (Becton and Dickinson, USA) and incubated at 37 ° C for 48-72 hours. Following the incubation, suspected colonies in terms of *F. necrophorum* were subjected to identification tests such as Gram staining (microscopic morphology), lipolytic activity on egg yolk agar and biochemical tests (8).

of Gram staining morphology (long or short Gram negative fusiform bacilli), lipolytic activity on egg yolk agar and biochemical activities (indole positive, whereas catalase, methyl red and voges-proskauer negative). However, *F. necrophorum* could not be subtyped.

DISCUSSION

Necrobacillosis caused by *F. necrophorum* is a very serious disease which is commonly seen in animals such as cattle, deer and antelopes (6, 19). Subtype *F. necrophorum* subsp. *necrophorum* is responsible for the most of cases and pathological lesions.

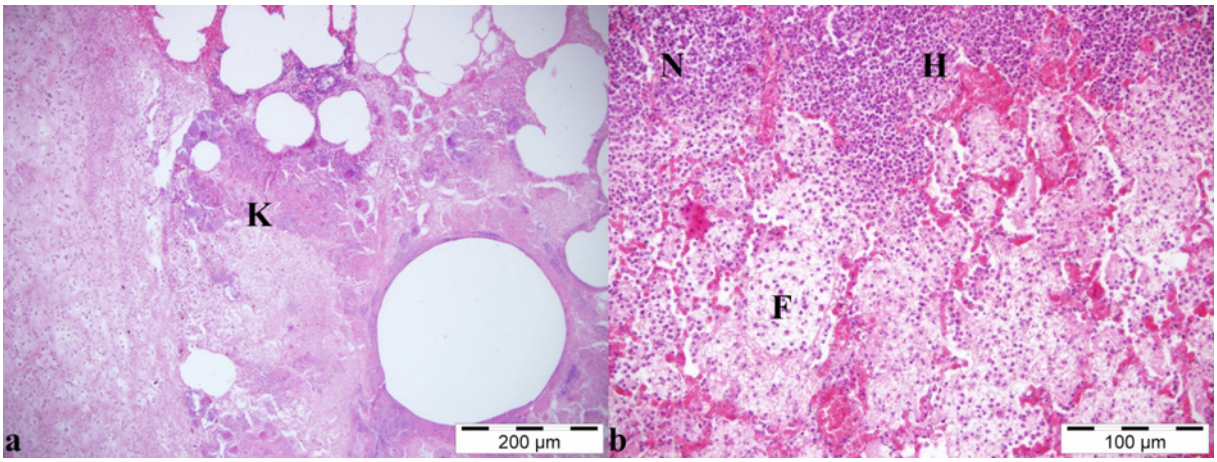

Figure 2. a) Coagulation necrosis (K) in lung, Bar: 200 µm, HE b) neutrophil infiltration (N), hyperemia (H) and fibrin (F) in alveolar lumen, Bar:100 µm, HE

Histopathological examinations of both animals with macroscopic lesions revealed coagulation necrosis areas in the middle of the lesions and demolished leukocytes around these areas. In addition, hyperemia in alveolar capillaries and mononuclear cells infiltration including neutrophil leukocytes in alveolar lumen were observed in lamb with lung lesions (Figure 2 a-b, 3 a-b).

In the microbiological analysis, the smooth, grayish and butyric acid scented β-hemolytic colonies were evaluated in terms of *F. necrophorum* after the culture of lung samples taken from two lambs and liver sample taken from one lamb. *F. necrophorum* was identified by considering the characteristics

F. necrophorum subsp. *funduliforme* subtype is known as the flora agent of intestinal tract (2). Cultural, biochemical and phenotypic features are insufficient in the exact differentiation of these two species. Therefore, different molecular methods such as 16S rRNA, restriction fragment length polymorphisms (RFLPs) and ribotyping should be used (12). In this study, *F. necrophorum* identification was carried out with the phenotypic methods from tissue samples taken from 3 lambs that died with the suspicion of necrobacillosis. The present case resembles to those previously reported (4, 7, 9, 15). The case was supported by pathological findings, as well. However, these methods were inadequate for differentiation of the subtype of the agent.

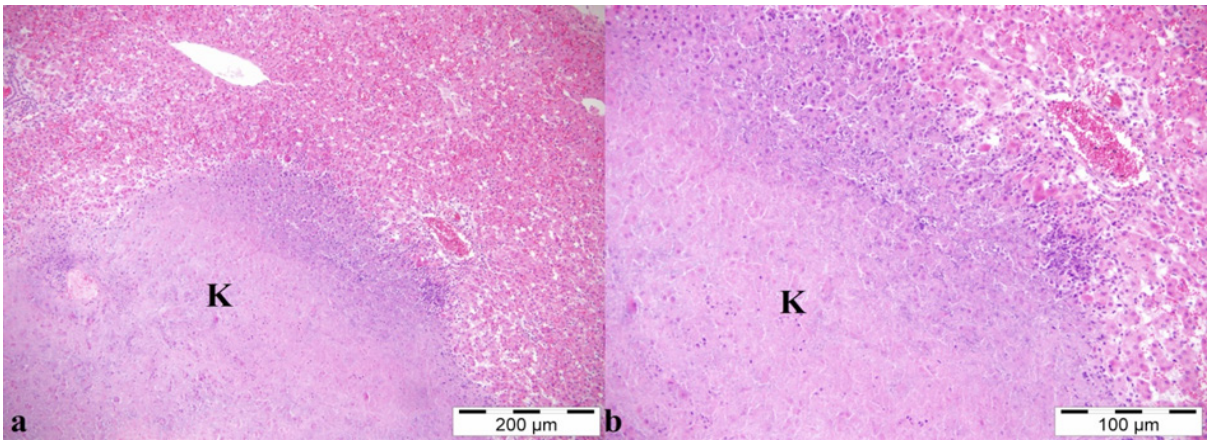

Figure 3. a) Coagulation necrosis(K) in liver, Bar: 200 µm, HE b) Higher maginification, coagulation necrosis (K) in liver, Bar: 100 µm, HE

Fusobacterium necrophorum, which is one of the most important bacterial agent encountered in liver, can takes place naturally in the mouth and intestinal tract in ruminants (16, 17). Necrobacillosis can be formed by omphalophlebitis in lambs. The lesions in liver are multifocal and exhibit typical characteristics of *F. necrophorum* infection (10). The characteristic lesions in the liver are miliar, yellow colored, slightly raised, round and dry coagulation necrosis areas surrounded by a hyperemic band (11). There are large amounts of filamentous Fusobacterium agents in the vicinity of the necrotic mass between the degraded leukocyte and the core crumbs (5). Severe hyperemia, hemorrhage and thrombosis in the vessels are found in the outer parts (3, 10, 15). Similar to the liver lesions, necrosis areas of different sizes are observed in the lungs (14).

In this study, variable necrotic foci with gray color, which were detected macroscopically in liver (5, 15) and lung samples (1, 16) taken from the lambs, are in parallel with the literature data. In addition, histopathological examinations of both animals revealed that coagulation necrosis areas were found in the middle of the lesions and leukocytes were destroyed around these areas. In addition, hyperemia in alveolar capillaries and mononuclear cells infiltration including neutrophil leukocytes in alveolar lumen were observed in lamb with lung lesions.

Fusobacterium necrophorum is an opportunistic pathogen that causes deaths in lambs and leads to significant economic losses. There are few studies in which the *F. necrophorum* was identified from lambs. In the present report, we describe the histopathological lesions and microbiologic results of necrobacillosis in lambs. It was concluded that necrobacillosis should be considered in the neonatal lamb deaths. It is hoped that this data will have a literature contribution in the elucidation of these cases.

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CONFLICT of INTEREST STATEMENT

The authors declare no conflicts of interest with respect to the publication of this manuscript.

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