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Olgu Sunumu

A CASE OF DEATH CAUSED BY *BORDETELLA BRONCHISEPTICA* IN A DOG

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Bir Köpekte *Bordetella bronchiseptica*' nın Neden Olduğu Ölüm Olgusu

Özet : Altı aylık, erkek, Danua ırkı bir köpekte, özel bir barınakta kalışı takiben, klinik olarak halsizlik ve öksürük sonrası ölüm şekillenmiştir. Köpeğin nekropsisinde; göğüs boşluğunda kanlı ve bulanık görünümlü sıvı, akciğerlerde yaygın hepatize alanlar ve boz beyaz supuratif odaklar ile birlikte diğer organlarda konjesyon gözlenmiş, bakteriyolojik inceleme sonucunda akciğerlerden saf olarak *Bordetella bronchiseptica* izole edilmiştir.

Anahtar kelimeler: *Bordetella bronchiseptica*, köpek, kennel cough.

Summary: A case of death occurred followed after debility and cough in a six- month- old, male Dane that developed following stay in a private household. In the necropsy of the dog, hemorrhagic and cloudy fluid in thorax, broad hepatized areas and white-gray suppurative foci in lungs and congestion in other organs were observed and after the bacteriological examinations, *Bordetella bronchiseptica* were isolated as a pure culture from the lung.

Key words: *Bordetalla bronchiseptica*, dog, kennel cough

I n t r o d u c t I o n

Bordetella bronchiseptica is an important respiratory pathogen of many species, including dogs, cats, pigs and laboratory animals such as rabbits. In dogs, the agent may cause infectious tracheobronchitis (ITB)/ Kennel cough as a primary pathogen or may also be involved as a secondary agent following a viral respiratory tract infection (**8, 11**). The incubation period range from one to 8 days and clinical signs persist for 1-2 weeks, but the organism can be shed for 2-3 months following clinical recovery (**4**). One of the clinical sign of uncomplicated ITB is a hacking or honking cough. Nasal

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discharge occurs less frequently. In the history of the coughing dog, recent contact with a single or group of dogs, through the clinical signs would help for the diagnosis (**8, 9**). Beside, alterations observed in pathological examinations, such as tracheabronchitis and bronchopneumonia which are changing from serous to mucopurulent, verify the clinical diagnosis of *B. bronchiseptica* infection.(**6**). Since most of the cases are self limiting, antibiotic therapy is not required in every case; however this should be decided on an individual basis. For prevention of the disease, especially in group housing environments, high attention should be given to hygiene and sanitation (**8, 9**).

In this report, a case of death because of *B. bronchiseptica* in a six- month- old, male dog was described.

M a t e r i a l a n d M e t h o d s

The dog: A male, six-month-old, Dane which had been in a kennel 15 days before referred to a Small Animal Clinic because of debility. In the clinic, lactated ringer's and dextrose solution, Rocephin® (1 g), diuretics and caffeine were administrated. But despite antibiotic and supplemented treatments, the clinical condition of the dog did not improve and the dog died after two days.

Postmortem examination: Necropsy was performed after death and the organs were investigated macroscopically. For histopathology evaluation, tissue samples from all visceral organs were fixed in 10 % formalin-saline solution and routinely processed. The samples were embedded in paraffin wax and cut at about 5-6 µm thickness and finally stained with Hematoxylin & Eosin.

Bacteriological examination: Lung, liver, spleen, and kidney samples inoculated onto sheep blood agar, MacConkey agar plates. Inoculations onto blood agar plates were duplicated for each sample. Half of them, along with the MacConkey agar plates, were incubated aerobically, while the others were incubated microaerobically at 37 ºC for 24 hours. Gram staining was performed from the cultures and routine bacteriological methods were used for the identification (**10**).

The antibiotic susceptibility pattern of the isolate was investigated by use of Kirby-Bauer disc diffusion procedure. For this purpose, antibiotic discs comprising amikacin, ampicillin/sulbactam, amoxicillin/clavulanic acid, cefixime, ceftriaxone, chloramphenicol, ciprofloxacin, enrofloxacin, erythromycin, gentamicin, kanamycin, oxytetracycline, penicillin G and sulphamethoxazole/trimethoprim were used (**10**).

Results

Postmortem examination of the dog

Gross Pathology: Opaque, reddish fluid with an amount of 500-600 cc within thorax was observed and petechial hemorrhage was determined on the pleura. The heart had a hypertrophic appearance. Dilatation in the right ventriculus; gelatinous appearance and thickening of the cardiac valves; intense foamy fluid in the lumen of trachea and severe hyperemia on the mucosal surface; diffuse congestion in the lobuli of the lungs; wide hepatised areas and white-gray suppurative focal lesions especially in apical lobus were observed. There was diffuse congestion in the liver, kidney and spleen.

Histopathology:

Heart: Thickening in the spongious layer of bicuspidal and tricuspidal valvuli due to the increase of fibroblastic tissue, degeneration and myxomatous transformation in the fibrous layer (Figure 1), oedema and infiltration of mononuclear inflammatory cells in the endocardium and epicardium of ventriculi were observed.

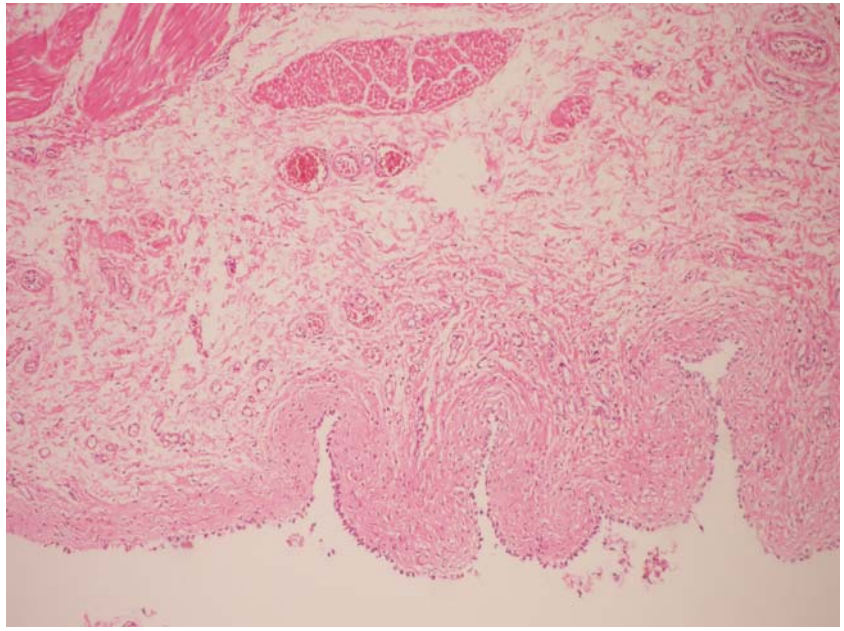

Figure 1: Heart. Thickening in the cardiac valves due to the increase of fibroblastic tissue, degeneration and myxomatous transformation. H.E.,10x

Şekil 1: Kalp. Kapakçıklarda fibroblastik doku artışına bağlı kalınlaşma, dejenerasyon ve miksomatöz değişim H.E.,10x

Lung: Mucopurulent bronchopneumonia characterized with wide necrotic and hemorrhagic areas, hemolysed erythrocytes, fibrin and abundant neutrophil leucocyte in the alveolar tissue (figure-2); severe degeneration, excessive desquamation in the bronch and bronchiole luminal epithelial and neutrophil leucocyte infiltration were observed (figure-3) Inflammatory oedema and pseudomembrane formation within the pleura were determined (figure-4).

There was intensive hemorrhage and congestion in the other visceral organs.

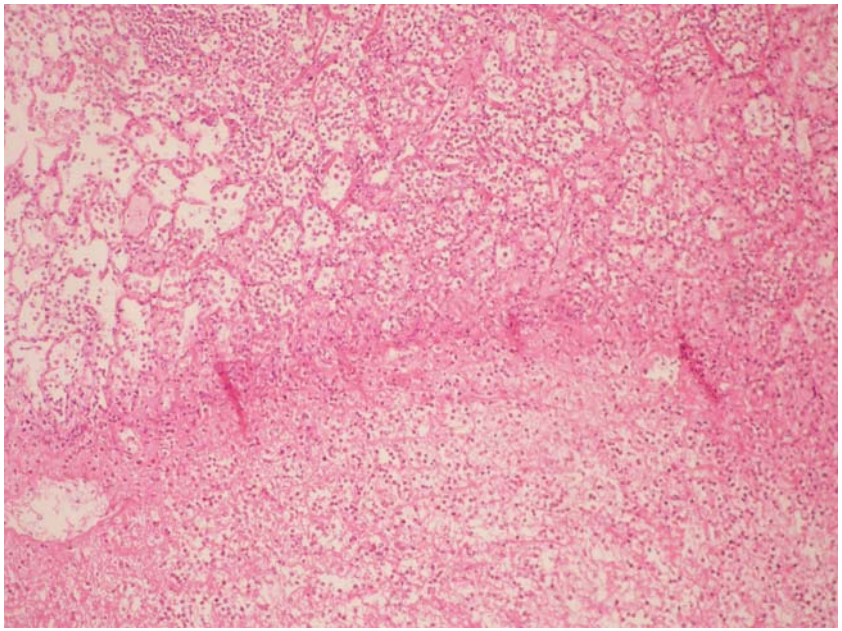

Figure 2: Lung. Wide necrotic areas and abundant neutrophil leukocyte infiltration in the alveolar tissue. H.E.,10x

Şekil 2: Akciğer. Alveolar dokuda geniş nekrotik alanlar ve bol nötrofil lökosit infiltrasyonu. H.E.,10x

Bacteriological examination

Non-hemolytic, small and smooth colonies were observed as pure cultures on the blood agar plates under both aerobic and microaerobic conditions after 24 hours of incubations, only from the lung of the dog. Pure, lactose negative pale, slightly tan colonies were observed on MacConkey agar plates after 24 hours of incubations. As a

result of gram staining from the colonies, Gram negative coccobacilli were seen. The isolate was non-fermentative, oxidase and catalase positive and identified as *B. bronchiseptica* according to biochemical properties (Table 1).

Antibiotic susceptibility test result of the isolate is shown in Table-2

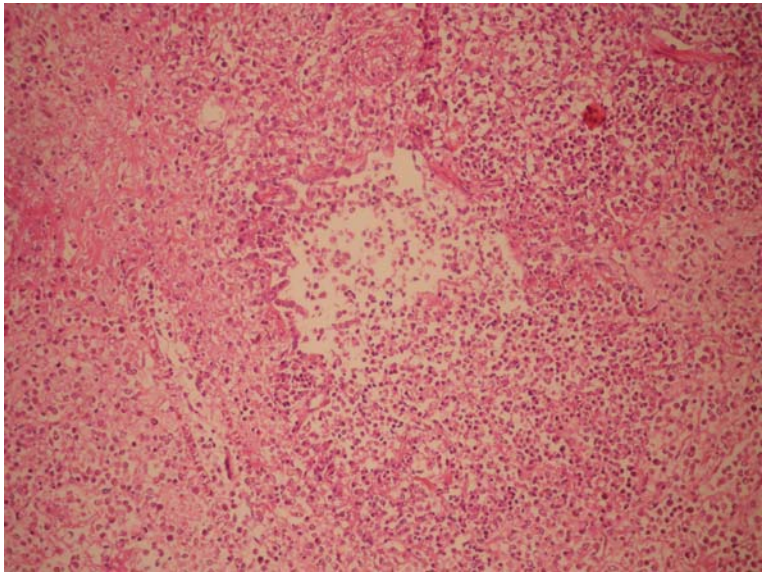

Figure 3: Lung. Severe degeneration, desquamation in the bronchiole luminal epithelial and neutrophil leukocyte infiltration. H.E.,10x

Şekil 3: Akciğer. Bronşiol lumen epitellerinde şiddetli dejenerasyon, deskuamasyon ve N. lökosit infiltrasyonu. H.E., 10x

Discussion

B. bronchiseptica has been recognized as an important respiratory pathogen of various animals species such as dogs, cats, pigs and rabbits since early 1900s (**5, 7, 8, 11**). Dawson et al (**3**) reported an outbreak of coughing in two dogs in a private household, closely followed by similar signs in two in-contact cats. They indicated that both strains were identical on Pulsed Field Gel Electrophoresis (PFGE) examination and suggested that it can be transmitted between dogs and cats. *B. bronchiseptica* may survive in the environmental surfaces, especially in group housing environments. And in the clinical diagnosis of the disease one of the most important evidence is a history of exposure to other dogs in a kennel, hospital or etc. In this case the symptoms started within 2 week period following a stay in a private kennel. Although we could not have

an opportunity to check if the disease exists there, it is highly possible that the kennel would be the source of the infection.

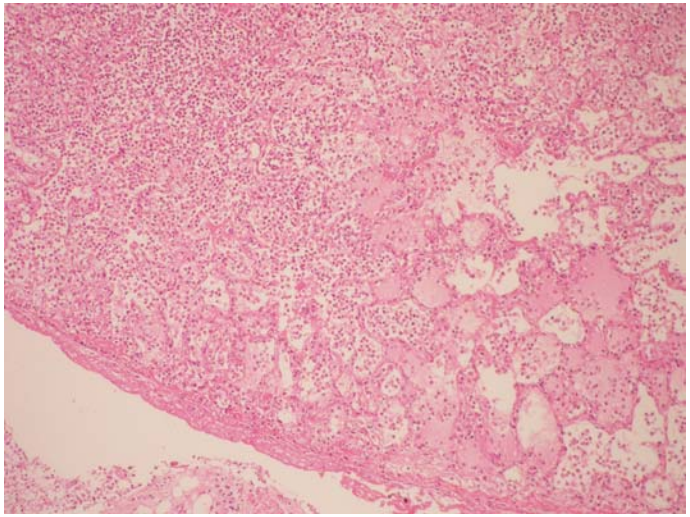

Figure 4: Lung. Inflammatory oedema and pseudomembrane formation on the pleura H.E.,10x
Şekil 4: Akciğer. Plörada yangısel ödem ve psöydomembran oluşumu. H.E.,10x

Angus et al. (**1**) determined amikacin, gentamicin, amoxycillin clavulonic acid and ticarcillin clavulonic acid as the most effective antimicrobial agents. Speakman et al (**11**) reported tetracycline, doxycycline, enrofloxacin and amoxycillin clavulonic acid as the most effective antibiotics. In this case the isolate was sensitive to amoxycillin clavulonic acid, chloramphenicol, ciprofloxacin, enrofloxacin, gentamicin, kanamycin, oxytetracycline. These results suggest that for the treatment of the disease as same as in other bacterial diseases, antimicrobial sensitivity testing should not be missed.

Recently, *B. bronchiseptica* is being isolated increasingly from especially immunusupressed patients (**3, 5, 7, 11**). Gueirard et al (**5**) reported repetitious *B. bronchiseptica* infection from a 79-year-old woman with bronchopneumonia which was related to contact with infected rabbits that were living near the patient. Bauwens et al (**2**) described a patient with *B. bronchiseptica* pneumonia and bacteremia that developed following bone marrow transplantation and acquired through contact with an ill dog. According to these human cases, the potential risk of transmission between dogs and cats and their owner should always be remembered.

Table 1: Biochemical properties of the isolate
Tablo 1: İzolatın biyokimyasal özellikleri

Gram staining		Gram negative	Adonitol	-
Oxidase		+	Arabinose	-
Catalase		+	Dulcitol	-
Motility		+	Fructose	-
O-F test reaction	Oxidative	-	Galactose	-
	Fermantative	-	Glucose	-
	Unreactive	+	Inositol	-
Growth on MacConcey Agar		+	Inulin	-
Haemolysis		-	Xylose	-
Triple Sugar Iron Agar	Slant	Acid	Lactose	-
	Butt	Alkaline	Maltose	-
	H_2S	-	Mannitole	-
	Gas	+	Mannose	-
Methyl red		-	Mellibiose	-
Voges-Proskauer		-	Rafinose	-
Indol		-	Rhamnose	-
Nitrate		+	Ribose	-
Citrate		+	Salicin	-
Urease		+ (rapid)	Sellobiose	-
Lysine		-	Sorbitole	-
Arginin		-	Sorbose	-
Ornithin		-	Sucrose	-
Gelatinase		-	Trehalose	-
Phenylalanine		-	Eskulin	-
Growth on Smith-Baskerville Medium		+*	Malonate	-

+ = positive reaction, - = negative reaction; *The colonies on the Smith-Baskerville Medium after 24 hours inoculation are blue colonies with a lighter blue reaction in the medium around them. After 48 hours incubation the colonies are blue/blue with green centre and the surrounding medium is blue.

Table 2: Antibiotic susceptibilities of the isolate
Tablo 2: İzolatın antibiyotiklere duyarlılık sonuçları

Antimicrobial agent	**S**	**I**	**R**	**Antimicrobial agent**	**S**	**I**	***R***
Amikacin (30µg)		I		Erythromycin (15µg)			*R*
Amoxicillin/Clavulonic Acid (30µg)	S			Gentamicin (10µg)	S		
Cefixime (5µg)			R	Kanamycin (30µg)	S		
Ceftriaxone (30µg)			R	Oxytetracycline (30µg)	S		
Chloramphenicol (30µg)	S			Penicillin (10µg)			*R*
Ciprofloxacin (5µg)	S			Sulbactam/Ampicillin (10µg)			R
Enrofloxacin (5µg)	S			Sulphamethoxazole/Trimethoprim (25µg)			R

S: Susceptible; I: Intermediate susceptible; R: Resistant

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