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Research Article

Evaluation of Pulmonary Infection Risk in Dogs with Pulmonary Contusion [1]

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Abstract

There is a dilemma about usage and selection of the antibiotics following pulmonary contusion. Thus, this study aimed to evaluate pulmonary infection risk in dogs with pulmonary contusion. In total, eleven dogs with pulmonary contusion included in the study. Diagnosis of the pulmonary contusion and its degrees were determined based on the clinical and radiological findings. Under general anesthesia, bronchoscopy and bronchoalveolar lavage (BAL) was applied on 0, 24 and 48th h and BAL samples were collected. Cytological examination, culture and antibiotic susceptibility analysis of the BAL samples were performed. Clinically, mild, moderate and severe degree pulmonary contusions were diagnosed, but radiological results pointed out severe degree of pulmonary contusion in all dogs. Bronchoscopy showed the hemoptysis in all dogs except one. Cytologically, all preparations had the inflammatory results at 0th hour and these were increased at 24 and 48th hours. Additionally, evidence of infection was determined in the cytological preparations of four dogs (case 1, 2, 5 and 8) taken at 0, 24 and 48th hours. These cytological results were compatible with microbiological results of case 1, 2, 5 and 8. *Pasteurella multocida* in case 1 and 2, and *Escherichia coli* in case 5 and 8 was cultured microorganism from the BAL samples, separately. While *P. multocida* was resistant to gentamicin, erythromycin and oxytetracycline; *E. coli* was resistant only sulfamethoxazole/trimethoprim. In conclusion, it may be stated that pulmonary contusion triggers inflammation process, and if there is a pathogenic opportunistic flora in the lower respiratory tracts, the pulmonary infections might accompany with inflammation process. Thus, if it is able, antibiotic usage must be planned considering to the results of BAL culture and antibiogram tests.

Keywords: *Bronchoalveolar lavage, Bronchoscopy, Cytology, Dog, Microbiology, Pulmonary contusion*

Pulmoner Kontüzyonlu Köpeklerde Pulmoner Enfeksiyon Riskinin Değerlendirilmesi

Özet

Pulmoner kontüzyon sonrası antibiyotik kullanımı ve seçimi hakkında bir çelişki vardır. Bu nedenle bu çalışma pulmoner kontüzyonlu köpeklerde pulmoner enfeksiyon riskinin değerlendirmesini amaçladı. Çalışmaya pulmoner kontüzyonlu 11 köpek dahil edildi. Pulmoner kontüzyon tanısı ve derecelendirilmesi klinik ve radyolojik bulgular temelinde belirlendi. Genel anestezi altında, 0, 24 ve 48. saatlerde köpeklerde bronkoskopi ve bronkoalveolar lavaj (BAL) uygulandı ve BAL numunesi alındı. BAL numunelerin sitolojik muayenesi, kültür ve antibiyotik duyarlılık analizleri yapıldı. Klinik olarak hafif, orta ve şiddetli derece pulmoner kontüzyon tanıları konuldu, ancak radyolojik bulgular tüm köpeklerde şiddetli derecede pulmoner kontüzyonu belirtti. Bronkoskopi, biri hariç tüm köpeklerde hemoptiziyi gösterdi. Sitolojik olarak, tüm preparatlar 0. saatte enflamasyon bulgularına sahipti ve bu bulgular 24 ve 48. saatlerde artıyordu. Ek olarak, enfeksiyon varlığı, dört köpeğin (olgu 1, 2, 5 ve 8) 0, 24 ve 48. saatlerde alınan sitoloji preparatlarında belirlendi. Bu sitolojik bulgular olgu 1, 2, 5 ve 8'in mikrobiyolojik bulguları ile uyumluydu. Sırasıyla, *Pasteurella multocida* olgu 1 ve 2'de, *Escherichia coli* olgu 5 ve 8'de BAL numunelerinden kültüre edilen mikroorganizmalardı. *P. multocida* gentamisin, eritromisin ve oksitetrasiklin'e dirençli iken, *E. coli* sadece sulfametoksazol/trimetoprim'e dirençliydi. Sonuç olarak, pulmoner kontüzyononun yangısal süreci tetikleyebildiği ve eğer alt solunum yollarında patojenik fırsatçı bir flora var ise akciğerdeki yangısal sürece enfeksiyonun da eşlik edebileceği söylenebilir. Bu yüzden, antibiyotik kullanımı olanaklar elveriyor ise BAL kültür ve antibiyogram sonuçları göz önüne alınarak kurgulanmalıdır.

Anahtar sözcükler: *Bronkoalveolar lavaj, Bronkoskopi, Köpek, Mikrobiyoloji, Pulmoner kontüzyon, Sitoloji*

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INTRODUCTION

Thoracic trauma results in pulmonary contusion, pneumothorax, hemothorax, rib fractures, intercostal muscle ruptures, diaphragmatic hernia and external thoracic wall injuries in dogs [1-5]. Pulmonary and myocardial contusions are severe traumatic injuries that they may occur if the blunt trauma affects the thorax [3,6].

Pulmonary contusion is the most common traumatic lesion in the dogs, accounting for approximately 50% of all thoracic injuries [6-9]. It is informed as an anatomic and pathologic lesion of the lung occurring after a compression-decompression injury of the thoracic wall [4,7]. Pulmonary contusion classifies as mild, moderate and severe according to pulmonary injury and associated findings (pneumothorax, rib fractures etc.) [5-7,9-11]. Pulmonary contusion has 20-50% mortality rate in dogs, and its progression leads to unresponsive dyspnea and died; thus, patients had blunt thoracic trauma should be examined carefully, and history, physical and radiological examinations, bronchoscopic findings and microbial analysis of BAL samples should be assessed in conjunction with the cytological results to clarify the exact diagnosis and estimate the prognosis of the dogs [7,12-15]. Additionally, cytological examination and bacterial cultures of intraluminal secretions may be performed before antibiotic usage to assist the diagnosis and evaluate the possible establishing lower respiratory tract disease [16,17].

Identification of the normal reference range of cell counts is impeded by a lack of standardization due to BAL fluid handling techniques and high variability of the clinically normal dogs [13]. Increased numbers of the neutrophils and phagocytosis of the organisms or macrophages, which are containing intracellular bacteria, is the evidence of the bacterial infections [13,15,16]. This condition can together with the acute inflammation, because acute inflammatory process often associated with bacterial infections [13,15]. Pulmonary contusion is a risk factor for pulmonary infection in dogs, which begins the cellular inflammatory process and activate the normal bacterial flora [13,18]; thus, BAL fluid should be analyzed in terms of enzymatic, cytological, microbiologic and histopathologic changings to determinate the exact diagnosis in dogs [17].

Antibiotic usage is recommended by surgeons to prevent the possible secondary infections to be encountered following pulmonary contusion; however, there is still a dilemma about usage and selection of the antibiotics in pulmonary contusion cases [9,18]. If bronchopneumonia develops following pulmonary contusion, the antibiotic usage is indicated [7,17]. On the other hand, there is no reported data on secondary pulmonary infections following pulmonary contusion in dogs, which is more common complication in humans [7]. Thus, to demonstrate the pulmonary infection risk, this study aimed to investigate cytological and microbiological analysis results of the BAL fluids in dogs with pulmonary contusion.

MATERIAL and METHODS

This study plan was approved by Local Ethic Committee of Uludag University (Decision no: 2013-01/05).

Material of the study consisted of 11 owned dogs (different breed, sex and age) presented in different times to Uludag University, Faculty of Veterinary Medicine, Department of Surgery Clinics with suddenly onset thoracic trauma. These dogs were included in the study by taking to permissions of the owners after diagnoses of the pulmonary contusion.

Initial Approaches and Diagnosis

Based on the history, physical examination, laboratory analysis and radiological results, pulmonary contusion was diagnosed in the dogs. Severity of these results helped the grading of the pulmonary contusion both clinically and radiologically; thus, vital parameters, general condition, thoracic auscultation, respiratory tracts examinations, breathing model and radiological findings were attentively evaluated for the study plan.

According to severity of the traumatic results and associated injuries, emergency medical protocols such as diuretics (furosemide), fluid therapy (lactate ringer and mannitol), and hemostatic (tranexamic acid) were applied to the dogs. When the dogs were stable, clinical, laboratory (routine hematology and blood gas analysis) and radiological examinations were completed to reach the exact diagnosis of pulmonary contusion and its degree.

For blood gas analysis, a specific 2 mL injector (containing 100 µL lithium heparin) was inserted to femoral artery with sterile technique, and about 2 mL arterial blood was aspirated into the injector. Body temperature of the case was recorded, and the arterial gases were instantly sent to laboratory to achieve an exact blood gas analysis results. Blood gas kits (Irma® blood analysis system kits, USA) and an analyzer (Truepoint®, IRMA, USA) were used to determinate the pH, pO_2 and pCO_2 values.

The effected hemithorax and lung lobe(s) from the trauma were identified by radiological examinations. Close monitorisation (SPO_2, pulsation and respiration controls: frequency and tidal volume) and ECG examinations were performed in the dogs. In ECG examinations, cardiac arrhythmias, amplitude of ECG waves and duration of intervals were measured on lead II (50 mm/s; 10 mm (mV; Esoate®, Italy). Furthermore, intensive care (instant oxygenation with mask or after endotracheal intubation) was taken to manipulate the complications, and thoracocentesis, tube thoracostomy and surgery were also carried out, if required.

Anesthesia

The dogs were anesthetized for tracheobronchoscopic examinations. After catheterization of the vena cephalica parva, the dogs were premedicated and induced with injectable anesthetic protocols (10 mg/kg ketamine HCl + 0.5 mg/kg diazepam combination, iv), and then they were intubated with a proper size of sterile endotracheal tube according to body weight and tracheal size compliance. The maintenance of the anesthesia was provided by iv administration of the same combination.

BAL Procedure

The same surgeon was performed all tracheobronchoscopy procedures as described previously [12]. A fiber optic flexible endoscope (5.2 mm diameter, 85 cm long) (Karl Storz®, Germany), a recorder (Tele Pack Vet X, Karl Storz®, Germany) and their endoscopic equipment were used during this procedure. A mouth gag was placed to fix the chin and an assistant fixed the head of the dog to prevent the bronchoscope from the trauma for any reason. The endoscope was inserted into the endotracheal tube and trachea, right and left bronchus and bronchioles were examined for the possible pathological findings. These endoscopic findings were recorded and bronchoalveolar lavage (BAL) procedure was performed to obtain specimens from lower respiratory tracts. For flushing to bronchial three, 0.5 mL of sterile saline (0.09% NaCl) per kg bodyweight was instilled through bronchoscope port, and then the fluid was aspirated slowly with gentle pressures through suction canal of the bronchoscope. The obtained fluid samples were collected into a sterile tube connecting to the suction system. The samples were taken to the laboratory for cytological examination, microbiological culture and antibiotic susceptibility. As performed in 0th h, BAL procedures were repeated at 24 and 48th h in dogs under general anesthesia.

Cytology

BAL fluids were centrifuged 1.000xg for 5 min, and the preparation was made from the precipitate of the fluids. This precipitate laid on the lam by a pipet and slides were performed. These preparations were stained by Diff-quick stain. All slides were evaluated in a blindly, ten randomly selected areas at higher magnification (x400 magnification) were evaluated and number of epithelial cells, macrophages, neutrophils and other cellular components (eosinophils and mast cells) were recorded and averaged in each time period. According to the microscopic findings, evidence of the inflammation and infection at 0, 24 and 48th h were evaluated.

Microbiology

BAL fluid samples taken 0, 24 and 48th h were processed within 2 h as soon as obtained from dogs. The samples were cultured for bacteria and yeasts with 3 pair on 5% defibrinated sheep blood (one of them was incubated in aerobically, one of them micro-aerobically and the other was incubated anaerobically), MacConcey (MCA, Oxoid) agar and Sabouraud dextrose (SAD, Oxoid) agar (aerobically incubated at 37°C and 28°C), respectively. Pleuropneumonia-like organism base agar (PPLO, Oxoid) was used for *Mycoplasma* isolation with incubation at 35°C in 5% CO_2. After incubation for 48-72 h, plates were examined for growth. Standard biochemical methods and commercial miniaturized identification systems (BBL Crystal Panel™) were used for identification of pure cultures [19]. Furthermore, the long time period was awaited for growing of *Mycoplasma* and mycotic microorganism to achieve the definitive microbiological results, as well.

Bacterial susceptibility testing was performed according to standards established by the Clinical Laboratory Standards Institute (CLSI) using Kirby-Bauer disc diffusion procedure [20]. Fourteen antibiotics were used for *P. multocida* isolates; azithromycin (AZM, 15 µg, Oxoid CT0906B), chloramphenicol (C, 30 µg, Oxoid CT0013B), doxycycline (DO, 30 µg, Oxoid CT0018B), enrofloxacine (ENR, 5 µg, Oxoid CT0639B), erythromycin (E, 15 µg, Oxoid CT0020B), gentamicin (CN, 10 µg, Oxoid CT0024B), ofloxacin (OFX, 5 µg, Oxoid CT0446B), penicillin G (P, 10units, Oxoid CT0043B), streptomycin (S, 25 µg, Oxoid CT0048B), sulfamethoxazole/trimethoprim 19:1 (SXT, 25 µg, Oxoid CT0052B), kanamycin (K, 30 µg, Oxoid, CT0026B), amoxicillin clavulanic acid (AMC, 30 µg, Oxoid CT0223B), ampicillin (AMP, 10 µg, Oxoid CT0003B), oxytetracycline (OT, 30 µg, Oxoid CT0041B). Twelve antibiotics were used for *E. coli* isolates; sulphamethoxazole/trimethoprim 19:1 (SXT, 25 µg, Oxoid CT0052B), ceftriaxone (CRO, 30 µg, Oxoid CT0417B), gentamicin (CN, 10 µg, Oxoid CT0024B), ampicillin (AMP, 10 µg, Oxoid CT0003B), ceftazidime (CAZ, 30 µg, Oxoid CT0412B), tobramycin (TOB, 10 µg, Oxoid CT0056B), amoxicillin clavulanic acid (AMC, 30 µg, Oxoid CT0223B), amikacin (AK, 30 µg, Oxoid CT0107B), ciprofloxacin (CIP, 5 µg, Oxoid CT0425B), cloksacilin (OB, 5 µg, Oxoid CT0016B), cefoperazone (CFP, 75 µg, Oxoid CT0249B), cefotaxime (CTX, 30 µg, CT0166B). The reference bacterial strains *E. coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were used as quality control strains following the recommendations of CLSI.

Considering to antibiotic susceptibility results of the BAL samples, a selective antibiotic would be administered to all dogs both prophylactically and as a treatment protocol as described previously [21].

Statistical Analysis

Average and standard deviations of blood gas analysis results (pH, pO_2 and pCO_2) were estimated in a statistical program (SPSS 23.0, IBM®, USA).

Considering to microbiological results into consideration, Fisher's exact test (SPSS 23.0, IBM®, USA) was applied to number of the cases, which had positive or negative culture results ($P<0.05$).

RESULTS

Clinical, Laboratory and Radiological Results

The causes of the thoracic trauma in dogs were traffic accident (n=7), fighting (n=3) and falling dawn (n=1). These dogs had different clinical appearance. According to severity of the clinical examination (changings in the vital parameters, abnormal lung sounds during auscultation, evidence of the hemoptysis, cardio-respiratory system abnormalities and etc.) and laboratory analysis results, pulmonary contusion was determined as mild in 3 dogs, moderate in 5 dogs and severe in 3 dogs *(Table 1)*. Because the cases were acutely presented to our clinics, there was no markedly abnormal changings in hematological parameters; however, there was minimal changes in the blood gas analysis results performed at 0th h (pH: 7.237±0.08; pO_2: 89.01±45.07 mmHg; pCO_2: 51.51±16.14 mmHg). The other associated thoracic pathologies were flail chest in case 7, penetrated wounds in case 3 and 7, costal fracture in case 7 and skin lacerations in case 7 and 11. All dogs had respiratory system problems (increased respiration rate, dyspnea, abnormal respiratory sounds, hemoptysis etc.), which were related to degree of pulmonary condition. In addition, thoracic region of the dogs were pointed out the thoracic trauma because all dogs had moderate respiratory dyspnea.

ECG examinations of the cases were showed some abnormalities: small complex QRS's (n=3), sinus tachycardia (n=6), electrical alternans (n=3), sinus arrhythmia (n=2), atrial premature complexes (n=2) increased amplitude of R wave (n=1) and P pulmonale (n=2). Specifically, considering to severity of the thoracic trauma, ECG abnormalities were small complex, sinus tachycardia and electrical alternans in case 3 and 5; sinus arrhythmia, P pulmonale and atrial premature complex in case 7.

Radiological findings of the dogs were changing due to influence of the trauma, but all dogs had third degree pulmonary contusion findings as described previously [6] *(Table 1)*. These dogs had increased radiopacity in a hemithorax (case 1, 4, 6, 7 and 8) or both hemithoraxes (case 2, 3, 5, 9, 10 and 11). The increased fluid density in the lung because of the hemorrhage and atelectasis of the lung lobes secondary to pneumothorax *(Fig. 1)* and air-bronchogram pattern appearance due to more severe lung contusion indicated the pulmonary contusion in the cases. The other radio-abnormalities were also pneumothorax in case 2, 3, 5, 7 and 10, pneumomediastineum in case 3, and flail chest and fractured ribs in case 7 that observed secondarily to the pulmonary contusion.

After diagnosis of the cases, as well as the medical therapy, some surgical manipulations were carried out as treatment regimens under general anesthesia. Thoracocentesis was applied in case 2, and pneumothorax was treated with tube thoracostomy in case 3, 5 and 7. Surgery was performed in case 7 to reconstruct the thoracic wall injury. However, in order to perform the study plan, antibiotic regimens were not applied to the dogs during the first 48 h. After the last sample was taken by BAL at 48th h, ampicillin sulbactam (20 mg/kg, bid, for 5 days) was started to the cases considering to previously reported

Table 1. *Signalments, clinical and radiological degree of the pulmonary contusion and broncoscopic findings of the dogs*

Case no	Signalments	Degree of the Clinical Findings	Degree of the Radiological Findings	Bronchoscopic Findings
1	Mixed-breed, ♂, 1 year-old, 5.4 kg	moderate	3	Hemoptysis, left bronchial hemorrhage, cyanotic left bronchial mucosa
2	Mixed-breed, ♂, 8 month-old, 13 kg	mild	3	Bilateral cyanotic bronchial mucosa
3	Anatolian shepherd, ♂, 10 month-old, 45 kg	severe	3	Hemoptysis, bilateral bronchial hemorrhage and cyanotic bronchial mucosa
4	Anatolian shepherd, ♂, 2 month-old, 9.8 kg	moderate	3	Hemoptysis, bilateral bronchial hemorrhage and cyanotic bronchial mucosa
5	Anatolian shepherd, ♀, 4 year-old, 48 kg	moderate	3	Hemoptysis, right bronchial hemorrhage, bilateral cyanotic bronchial mucosa
6	Staffordshire Bull Terrier, ♀, 3 month-old, 9.4 kg	mild	3	Hemoptysis, right bronchial hemorrhage, bilateral minimal cyanotic bronchial mucosa
7	Mixed-breed, ♀, 5 year-old, 5.5 kg	severe	3	Hemoptysis, right bronchial hemorrhage and cyanotic bronchial mucosa
8	Anatolian shepherd, ♂, 3 year-old, 55 kg	moderate	3	Hemoptysis, bilateral bronchial hemorrhage and minimal cyanotic bronchial mucosa
9	Mixed-breed, ♂, 5 month-old, 5.5 kg	moderate	3	Hemoptysis, left bronchial hemorrhage and minimal cyanotic bronchial mucosa
10	Pitbull Terrier, ♂, 5 month-old, 7 kg	mild	3	Hemoptysis, right bronchial hemorrhage and minimal cyanotic bronchial mucosa
11	White Terrier, ♂, 7 year-old, 12 kg	severe	3	Hemoptysis, left bronchial hemorrhage and minimal cyanotic bronchial mucosa

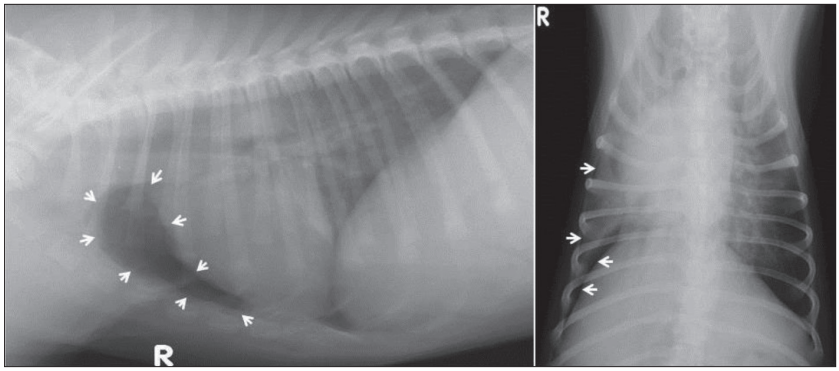

Fig 1. Radiograms of case 10 taken at 0th h, the ventrodorsal radiograph points out increased fluid density in the lung due to 3rd degree pulmonary contusion. There is minimal evidence of the pneumothorax, atelectatic caudal lung lobe border *(right arrows)* and free air accumulation *(left arrows)*. The right lateral radiograph shows the free air accumulation and air-bronchogram pattern view due to more severe lung contusion

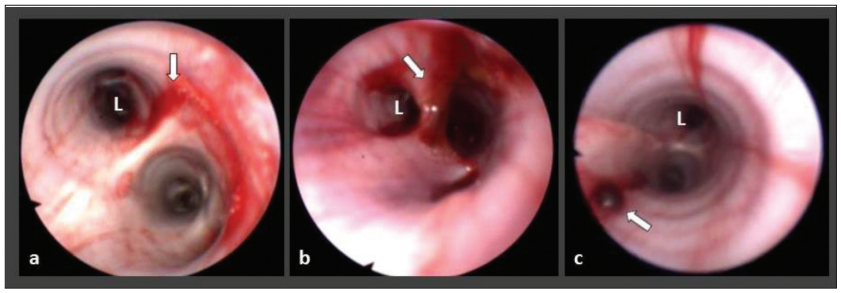

Fig 2. Bronchoscopic views of the case 1, hemorrhage *(arrow)* in the left (L) bronchus at 0th h (a), fibrin formation *(arrow)* at 24th h (b), and mucoid-hemorrhagic sputum *(arrow)* upcoming from the left (L) bronchus to the tracheal lumen at 48th h (c)

data [20], which had found compatible with the antibiotic susceptibility results afterwords.

Bronchoscopic Results

At presentation; during bronchoscopic evaluations, mucosal surface of the trachea had bloody appearance in all dogs except case 2, which was pointed out the hemoptysis. There was unilateral *(Fig. 2a)* or bilateral bronchial hemorrhage and also cyanosis in the bronchial mucosa of the cases *(Table 1)*.

At 24th h; there was a bloody appearance in the tracheal lumen and on the effected bronchial mucosa. Fibrin formations on the mucosal surface *(Fig. 2b)* and foamy hemorrhagic fluid accumulations were markedly observed.

At 48th h; broncoscopic evaluations of the cases revealed minimal hemorrhagic spots on the mucosa, minimal fibrin formation, muco-purulent and mucoid-hemorrhagic sputum in the lumen of trachea and effected bronchus *(Fig. 2c)*.

Cytological Results

Taking the cytological findings into consideration, inflammation was determined in all dogs; however, case 1, 2, 5 and 8 had both inflammatory and infective findings in the preparations. Comparative evaluations of the preparations at 0, 24 and 48th h in dogs implied that;

- *Inflammation*

0th hour: All preparations of the cases had intense erythrocyte, neutrophil (80-85%), bronchial epithelial cells, macrophage (10%), muco-protein fibers and mast cells (0.5-1%).

24th hour: There were more severe inflammatory findings. Neutrophil counts (85-90%) had increased and many degenerated respiratory epithelial cells were noticed in all cases.

48th hour: Degenerated respiratory epithelial cells, increased number of macrophages (80%) and lower neutrophil count (30-40%) were evident at that time in all cases *(Fig. 3)*.

Infection

0th hour: Existence of various shaped bacteria was found in four cases (case 1, 2, 5 and 8).

24th and 48th hour: The intensity of bacterial cells was increased *(Fig. 4)* and phagocytized bacteria by macrophages were evident.

Microbiological Results

P. multocida was cultured from the BAL samples of case

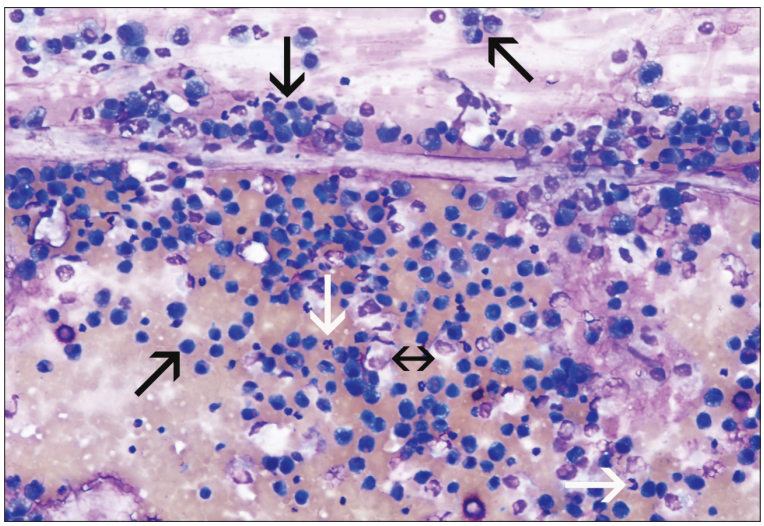

Fig 3. Severe inflammatory results of case 3 at 48th h, cytological examination view of the BAL samples demonstrates respiratory epithelial cells (double-headed arrow), neutrophil leukocytes *(white arrow)* and alveolar macrophages *(black arrows)* (200X magnification)

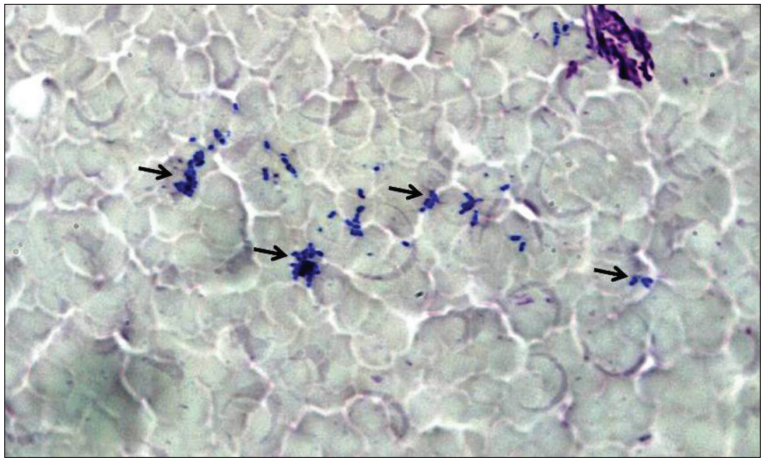

Fig 4. The encountered very intense bacterial cells *(arrows)* in the cytological preparation of the case 1 (1000X magnification)

1 and 2, and *E. coli* was the other cultured microorganism from the BAL samples of case 5 and 8. There was no *Mycoplasma* and mycotic microorganisms in the cultures. Microbiological results were obtained 0th h BAL sample of case 1, 5 and 8, but it was cultured at 24th h in case 2. According to bacterial susceptibility testing, *P. multocida* isolates were resistant to gentamicin, erythromycin, oxytetracycline and *E. coli* isolates were resistant only to sulfamethoxazole/trimethoprim 19:1.

Considering to these antibiotic susceptibility results, which were time-consuming procedure, the previously planned antibiotic regimen of ampicillin sulbactam (20 mg/kg, im., bid for 5 days) were repeated to all dogs.

Statistical Analysis Results

Statistical analysis results pointed out that there was no significant difference in terms of cultured microorganisms between the numbers of the cases (P= 0.2774).

DISCUSSION

Pulmonary contusion results in structural and functional changes in the lung tissues, which may lead to respiratory failure. In the management of the pulmonary contusion, antibiotic therapy is not indicated unless bronchopneumonia develops. If bacterial pneumonia develops at the early stage, bacterial culture of the tracheal fluid is recommended [7,18]. This study was planned to evaluate the pulmonary infection risks of the dogs with pulmonary contusion, and our results showed to some supportive findings of the infections following pulmonary contusion in the studied dogs. In addition to clinical and radiological examinations, bronchoscopic examinations of the respiratory tracts were made to determinate the traumatic results of the respiratory tracts and to take BAL samples for cytological and microbiological analysis at 0, 24 and 48th h. Cytological evaluations targeted to presence of the inflammation and infection as well as microbiological culture and antibiotic susceptibility analysis to answer the possible questions about to pulmonary trauma in dogs. Because inflammatory process begins in the lung parenchyma following trauma, the organ might be labile to opportunistic bacterial infections due to immunocompromised status of the parenchyma [22,23]. Thus, this study was planned to answer the questionable issues about antibiotic usage in dogs with pulmonary contusion.

Physical examination of the patients with mild to moderate pulmonary contusion exhibit some superficial respiratory system abnormalities and findings (tachypnea, dyspnea, orthopnea, hypoxia and hemoptysis), and auscultation of the thorax include abnormal respiratory sounds such as increased bronchial and broncho-vesicular sounds [5,7-9]. If the pulmonary contusion degree is mild, only tachypnea may be observed in pulmonary contusion cases. Severe degree pulmonary contusion includes cyanosis, dyspnea and shock. Hemoptysis is a rare clinical appearance, but if it is clinically detected, the prognosis of the case should be evaluated [9]. In this study, we clinically evaluated changings of the vital parameters, abnormal lung sounds and evidence of hemoptysis as well as cardio-respiratory system abnormalities, while pulmonary contusion was graded in the dogs. These clinical findings of the pulmonary contusion were different in dogs due to severity of the trauma, and we clinically diagnosed mild, moderate and severe degree of pulmonary contusion in the dogs. Hematoma and edema in the lung parenchyma following contusion increases the airway pressure and may responsible to irregular gas exchange in the alveoli [9]. Clinical deterioration is the appearance of the cellular damage in the patient that it may be seen at the first 24-48th h after trauma [24]. Progressive lung dysfunction and hypoxia may occur at the early stage of the pulmonary contusion due to hypoventilation, gas-diffusion abnormality, intra-pulmonary shunt and discordance of ventilation-perfusion [7,25]. In presented cases, hematological results were not congruent with the pulmonary contusion, but blood gas analysis results had minimal parametric difference. The time between the trauma and formation of the clinical findings is about 1-28 days [26]. It was clearly seen in this study that there was a meaningful difference between the clinical and radiological grading of the pulmonary contusion in dogs. Although all degrees of the pulmonary contusion were clinically observed, radiological results of the dogs pointed out that all dogs had 3rd degree pulmonary contusion. Radiological findings of the pulmonary contusion are uncertain in the first 4-6 h [27]. The interstitial and severe alveolar model pulmonary findings and air-bronchogram pattern radiopacities are usually observed in the radiographs taken initially [2,6,7,9].

Irregular, complex alveolar-interstitial model spots are the evidence of the pulmonary contusion [6,7,27]. After 24-36 h from the trauma, the lesions seen on the radiographs may be matching to the patients' healthy status [6-8]. In the cases with pulmonary disease, asymmetrical radiological lesions and diffuse pattern radio-abnormalities may be seen in dogs [22]. Radiologically, diagnosis of the dogs was third degree pulmonary contusion that the dogs had unilateral or bilateral increased fluid density in the ventrodorsal radiographs because of the hemorrhage and atelectasis of the lung lobes and air-bronchogram pattern appearance due to more severe pulmonary contusion and associated thoracic pathologies. Treatment of the pulmonary contusion changes according to degree of the pulmonary injury. In the mild degree, there is no advised treatment protocol; thus, restriction of the cases in a box is recommended until the clinical symptoms terminate. The cases with moderate degree should be treated with diuretics and colloids. If there is a severe respiratory problem, oxygenation by mask or mechanical ventilation following intubation, and treatment protocols should be planned to manage the vital parameters. Fluid therapy is required to improve the tissue perfusion and optimize the cardiac output. Corticosteroid usage has a confliction, because it may exaggerate to pulmonary disturbance. Bronchodilatators and analgesics may be given to regulate the respiration [9]. In this study, to complete the study plan, the dogs were medically treated following to emergency medical protocols. For this purpose, furosemide, lactate ringer and mannitol, and tranexamic acid were applied to the dogs. Moreover, thoracocentesis, tube thoracostomy and surgery were performed, if required.

Bronchoscopy is a valuable procedure in evaluation of canine respiratory disease [12,14,28]. It is usually used in referral hospitals to characterize respiratory disease processes and provides valuable visual information and documentation of the diseases and theirs characterize [23]. Bronchoscopy is also more sensitive for detecting airway pathology than diagnostic imaging techniques [14,23]. Thus, bronchoscopic evaluation was considered in dogs with pulmonary contusion. It permitted to visual assessment of the traumatized lower respiratory tracts and facilitated collection of deep respiratory samples by BAL for cytological and microbiological examination as described previously [14,15,28]. At 0th h, in bronchoscopic evaluations, bloody appearance (hemoptysis) in both tracheal and bronchial lumens and cyanosis in the bronchial mucosa was determined in the dogs. At 24 and 48th h, the results were not severe and there were only fibrin formation, mucopurulent and mucoid-hemorrhagic sputum in the trachea and effected bronchus.

BAL is a minimally invasive technique that is widely used in veterinary medicine [12-15], because it is the most effective procedure to take samples from respiratory tracts of immunocompromised patients and patients with opportunistic bacterial pneumonia, pulmonary hemorrhage or neoplasm [22,23,29]. By flushing to saline from the airways, samples are collected from bronchi, bronchioles and alveolar spaces for laboratory analysis [12-15,22]. Considering to simplicity and effectivity of the technique, BAL was applied by bronchoscopy at 0, 24 and 48th h under general anesthesia and the samples were investigated in pathology and microbiology laboratories, separately.

Diagnosis of the bacterial pneumonia is made on the basis of microbial culture results of BAL samples, and treatment should be planned by BAL cytology, microbial culture and susceptibility results [12,15-17,22,29]. Inflammation identifies according to total nucleated cell counts in BAL samples [12,13]. Inflammatory process is classified as neutrophilic, eosinophilic, lymphocytic, macrophagic, mixed or suppurative (>12% neutrophil) [12,16,17,22]. In the presented study, inflammation was evaluated by cytological analysis of BAL fluids at 0th h, and its progression was controlled at 24 and 48th h. The obtained results showed that there were intense erythrocyte, neutrophil, bronchial epithelial cells, macrophage, muco-protein fibers and mast cells at 0th h. It was considered that this cell intensity was due to hemopthysis (intrabronchial bleeding). However, the inflammation was more severe (neutrophilic, neutrophil count: 80-85%) and suppurative as described previously [12,13,16,22] at 24 and 48th h. There were also many degenerated respiratory epithelial cells in the cytological preparations. These conditions was interpreted that the inflammation was continuing and there is an immune response by the immune defense system. The presence of the bacteria in the cytological preparations is definitive diagnosis for the infectious disease in the respiratory tracts or parenchyma [12,13,15]. The presence or absence of the hemorrhage, etiologic agents, and other causes should compare for diagnosis in both suppurative and inflammatory diseases, and exact diagnosis is possible, only if infectious organisms are presented in cytology [13,15,22]. The cytological diagnosis of the infection in case 1, 2, 5 and 8 were made by determination of the existence of various shaped bacteria in the preparations. There were also phagocytized bacteria in the macrophages. It was deduced that these suppurative condition was together with the inflammatory process for case 1, 2, 5 and 8.

In general, Gram - and anaerobic bacteria is isolated from the lower respiratory tracts of the animals [17,18,29]. *Mycoplasma* is cultured in the normal pharyngeal flora of healthy dogs, and it can be isolated tracheo-bronchial lavages due to contamination. In a retro-spective study, *Pseudomonas* sp. is the more common encountered bacteria. *Staphylococcus, Streptococcus, Flavobacterium, Pseudomonas, Enterococcus, Bacillus, Bordettella bronchiseptica* and *Actinomyces* have been isolated in dogs with chronic bronchitis and dogs with lower respiratory tract infections [15,16]. In another study, the

culture results of the BAL fluids in diseased dogs include *E. coli, Streptococcus, Klebsiella, Mycoplasma* and *Enterobacter sp.*[15,17]. In the current study, *P. multocida* in case 1 and 2, and *E. coli* in case 5 and 8 were the cultured organisms from the BAL samples. Microbiological results were obtained 0th h samples in case 1, 5 and 8, but 24th h in case 2. According to these microbiological results, it has been concluded that pathogenic and opportunistic microorganisms, together with the inflammatory process, may be active with the pulmonary contusion due to immune system disturbance; hence, microorganisms could be cultured following to the pulmonary trauma in the bronchial lumen.

According to the patients' general status and suspected underlying disease, prophylactic antibiotic may be given with appropriate dosages [18,21]. Antibiotics should be considered on basis of the suspected pathologies and applied after culture and antibiotic susceptibility results [15,18,21,29,30]. In presented study, because pulmonary infection risk was evaluated, the antibiotic was not applied to dogs due to study plan at 0, 24 and 48th h. However, considering to antibiotic susceptibility results of a previous study [21], a selective antibiotic (ampicillin sulbactam) was applied to all dogs both prophylactically and as treatment protocol.

As a conclusion, thoracic trauma may result in pulmonary contusion, and clinical degree of the pulmonary contusion may not be compatible with the radiological degrees. Regardless of the pulmonary contusion degree, it should be noticed that pulmonary contusion triggers the inflammation process. If there is a pathogenic opportunistic flora in the lower respiratory tracts, the pulmonary infections may start together with the inflammation process according to our study results. Hence, antibiotic usage must be planned considering to culture and antibiogram test results of BAL fluids.

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