Benha university

Faculty of Veterinary Medicine

Department of Animal Medicine(Internal Medicine)

Clinical and Diagnostic studies on Respiratory System Affections in dogs and cats

Thesis Submitted by

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قسم طب الحيوان (الامراض الباطنة)

دراسات إكلينيكية وتشخيصية للإصابات التنفسية في الكلاب والقطط

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"Clinical and Diagnostic Studies on Respiratory Affections in Dogs and Cats"

For the Master Degree of ***Veterinary Medicine*** **(Internal Medicine) has been approved on 19/5/2022 by the examining committee:**

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﴿ قالوا سبحانك لا علم لنا الا ما علمتنا

إنك انت العليم الحكيم ﴾

صدق الله العظيم

الآيه(32) سوره البقره

Dedication

This work is dedicated to:

My lovely family, My lovely husband and my lovely son Yahia Algendy….

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DECLARATION

I declare that this thesis has been complied by myself and is the result of my own work. It has not been submitted for any other degree and all sources of information have been properly acknowledged.

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List of Abbreviations

A/G	Albumin globulin ratio
APPs	Acute phase proteins
APR	Acute phase response
BAL	Bronchoalveolar lavage
BP	Bacterial pneumonia
Ca	Calcium
CAP	Community acquired pneumonia
CBC	Complete blood count
CL	Chloride
CIRD	Canine infectious respiratory disease
CRP	C-reactive protein
CT	Computed tomography
ELISA	Enzyme linked immunosorbent assay
FCV	Feline calici virus
FHV	Feline herps virus
FURD	Feline upper respiratory disease
Hb	Hemoglobin
HP	Haptoglobin
IgG	Immune globulin G
IL6	Interleukin 6
K	Potassium
Mg	Magnesium
Na	Sodium
P	Phosphorous
PCV	Packed cell volume
RBCs	Red blood cells
RTD	Respiratory tract disease
SAA	Serum amyloid A
SBP	Secondary bacterial pneumonia

T FAST	Thoracic focused assessment with sonography for trauma
TNF	Tumor necrosis factor
URTD	Upper respiratory tract disease
WBCs	White blood cells

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Animal Medicine Department

Abstract:

Title: Clinical and Diagnostic studies on Respiratory System Affections in dogs and cats

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Abstract

Respiratory diseases in dogs and cats are one of the most common complicated affections affecting animal health with high morbidity and mortality. The first study aimed to evaluate the clinical, hematological, acute phase proteins (APPs) and radiographic changes during different naturally respiratory affections in dogs and cats. Aimed to isolate and identify the most common associated bacterial agents in pneumonic cases. The affected cases showed inflammatory leukogram, changes in the level of APPs and different radiographic patterns associated with different respiratory affections, The most common isolated pathogens were Klibsiella, E.coli, Pseudomonas and other types. This study revealed that clinical, hematological, biochemical, radiographic examination and bacterial isolation are essential for accurate diagnosis of different respiratory affections in dogs and cats. The second study investigated the role of APPs, Il6 and ultrasonography in the early diagnosis of induced bacterial pneumonia in dogs. The pneumonic dogs showed significant increase in the levels of APPs, Il6 and serum proteins. Ultrasonographic changes in the lung tissues appeared on the 3rd day post induction. This study revealed that ultrasonography, acute phase proteins and Il6 could be used as valuable tools for early prediction and diagnosis of pneumonia in dogs.

Key words: Acute phase proteins, Bacterial pneumonia, Dogs and cats, Radiography, respiratory affections, Ultrasonography

1-Respiratory affections in dogs and cats:-

1.1.Causes of respiratory affections in dogs and cats

Many of canine diseases have medicolegal importance as zoonotic view, so dogs play an important role in the epidemiology and control of these diseases. Respiratory and cardiac disorders are amongst common diseases of dog**.** The respiratory system is a complex, extensive airway that extend from external nares to alveoli. It is responsible for the transportation of oxygen from the atmosphere to all living cells of body and get out of carbon dioxide from cells back to the atmosphere**(Ali .,2011).** There for, respiratory diseases are classified as life threatening.

Respiratory diseases in dogs and cats involved upper respiratory affections(kennel cough in dogs and feline upper respiratory affections in cats) and Lower respiratory affections such as pneumonia that may be caused by viral, bacterial, fungal infection, other causes and predisposing factors. The most common cause of pneumonia is bacterial cause. Bacterial pneumonia (BP) in dogs has been known for decades. Reports of dogs serving as experimental models in attempts to shed light on human bacterial infections were reported in the 1910s and beyond **(Dale et al., 1974a).** Naturally occurring BP in dogs was later described in connection with contagious respiratory diseases **(Rosendal .,1978)**and thereafter, clinical and microbiological findings have been reported in retrospective studies **(Epstein et al., 2010; Proulx et al., 2014;)**. However, several aspects of BP require further studies. BP in small animals defined as inflammation of lung parenchyma that caused by bacterial infection. it is very dangerous disease due to complex pathogen interactions, variable clinical signs and long duration of antibiotic therapy **(Dear.,2020)**.

Based on the etiology, clinical appearance, and patient history, canine bacterial pneumonia is classified as either community-acquired pneumonia(CAP) or secondary bacterial pneumonia (SBP). CAP is caused by known contagious pathogens such as Bordetella bronchiseptica and Streptococcus equi subspecies zooepidemicus, and it is commonly seen in dogs with a history of acute onset clinical signs following exposure to reservoirs of infectious agents such as shelters, boarding facilities, and dog parks **(Priestnall et al ., 2010).**

CAP which known also by canine infectious respiratory diseases(CIRD) or kennel cough considered one of the most complicated canine disease due to appearance of new pathogen and the continuous circulation of common etiological agents in dog populations which play an important role in humans' daily lives**(Hao et al.,2019).** Also known as any contagious, acute-onset respiratory infection in dog, affecting the upper respiratory tract, it caused by multiple pathogen including several viruses and bacteria such as Canine Parainfluenza virus, canine adeno virus2, canine distemper virus, canine Herpes, Reovirus, Corona virus, Canine Influenza irus, Bordetella bronchiseptica, Mycoplasma spp., Staphylococcus spp., and other bacteria **(Ford.,2013).** CAP in dogs commonly begin with viral colonization and infection of the upper respiratory tract(canine respiratory coronavirus, herpesvirus, pneumovirus, and parainfluenza virus, among others). Often, such diseases are acute and self-limiting, but in a subset of dogs inflammation associated with these organisms immobilizes the host's immune defenses and predisposes infection with other respiratory pathogens mainly bacterial pathogen**(Radhakrishna et al .,2007).**

Canine infectious respiratory disease is similar to syndromes in cattle (bovine respiratory disease complex) and pigs (porcine respiratory disease complex), it is considered as a complex infection . Clinical signs including coughing, nasal discharge, and dyspnea are rarely caused by a single pathogen and more often caused by multiple agents that act synergistically to cause disease **(Priestnall et al.,2014).** The clinical signs can persist for several weeks, often resulting in severe disease, such as bronchopneumonia, and may lead to death or result in euthanasia. Worldwide, CIRD is a major cause of morbidity in kenneled dog populations, It is most likely that a single pathogen alters the protective defense mechanisms of the respiratory tract, that allowing other pathogens to infect the respiratory tissues**(Mitchell et al.,2017).** Dogs with viral or bacterial co-infections suffer from moderate to severe clinical signs more than dogs with single infection **(Decaro et al.,2016).**

Secondary bacterial pneumonia occurs as a sequela to a predisposing anatomic or physiological condition, such as megaesophagus, laryngeal paralysis, or ciliary dyskinesia**(Dhein et al .,1990).** Aspiration pneumonia ,canine infectious pneumonia ,inhalation of foreign body ,nosocomial infection ,and immune dysfunction result in occurrence of bacterial pneumonia in dogs and cats. Common bacteria that isolated from lower respiratory tract of dogs and cats

include Staphylococcus, Streptococcus, E. coli, Pseudomonas, Bordetella, Pasteurella, Klebsiella, Bacteroides and Clostridium**(Dear.,2014).** The role of these bacteria as a primary pathogen is less clear **(ford.,2013).**

Aspiration pneumonitis mean aseptic inflammation of air ways and pulmonary tissue, the severity of inflammation depend up on the nature of aspirated materials **(Schlze and Rahilly .,2012).** These changes cause immunosuppression and provide suitable media for bacterial colonization result in secondary bacterial infection **(Bahr et al.,2014).**

Mucociliary clearance is one of the most important defense mechanisms of the air way, any defect such as primary ciliary dyskinesia result in inhalation of any foreign material carrying bacteria causing bacterial pneumonia **(Merveille et al.,2014).** Congenital or acquired immune deficiency result in conversion of normally inhabitant non- pathogenic bacteria into pathogenic **(Lobetti .,2000 ; Kanemoto .,2015).**

Infectious feline upper respiratory tract disease (URTD) is a common and serious cause of morbidity and mortality in kittens, particularly those kept in overcrowded or stressful environments. Lethargy, inappetence, sneezing, conjunctival hyperemia, serous to mucopurulent nasal and ocular discharges, hypersalivation, and, in some cases, respiratory distress caused by bronchopneumonia and death are among the clinical indicators of disease **(Sykes ., 2014).** URTD in cats is particularly common in stressed and congested environments **(Nguyen et al .,2019).**

Feline Herpesvirus-1(FHV) and Feline Calicivirus are the two most common causes of feline upper respiratory tract illness(RTD). Other pathogens that can induce RTD in cats include Bordetella bronchiseptica and Chlamydia psittaci, albeit C psittaci is mostly a conjunctival pathogen. Feline herpesvirus and feline cytomegalovirus(FCV) had long been thought to be equally important in feline RTD, but new evidence reveals that FCV may be more prevalent **(Binns et al .,2000).**

1.2. Diagnosis of respiratory diseases in Dogs and Cats:

Diagnosis of respiratory diseases in canine and feline depend on case history, clinical findings, laboratory diagnosis and diagnostic imaging.

1.2.1. Clinical findings:

Bacterial pneumonia characterized by wide range of clinical signs include a productive cough, nasal discharge, hyperpnoea or dyspnea,and lung sound abnormalities such as crackles, increased bronchovesicular sounds, or wheezes. Signs of systemic disease are present and may include fever, anorexia, depression, weight loss, and dehydration **(Dear.,2014).**

Dogs and cats that affected by CIRD suffer from different clinical signs as it is a complex infection caused by viruses and/or bacteria that act synergistically with each other to cause disease. These signs include paroxysmal cough that can be stimulated by exercise and finger manipulation of trachea in case of tracheitis, honking sound can be heard during cough in case of laryngitis, serous, mucoid or mucopurulent nasal and conjunctival discharge and sneezing. Fever, lethargy and inappetance may develop but are not common **(Ford.,2013).**

1.2.2. Haematological changes:

In animals with respiratory affection, a complete blood count is a valuable diagnostic test. An inflammatory leukogram is linked to this condition, neutrophilia, with or without left shift, and varying signs of toxic alterations**(Dear ., 2020).**

1.2.3. Acute phase protein and pro-inflammatory cytokines:

An effective innate immune response, consisting of local cytokine generation, neutrophil emigration, and extravasated plasma components, is required for successful host defense against any pathogens in the lungs **(Mizgerd.,2008).** This local response is accompanied by a systemic acute phase response (APR), which is characterized by changes in the circulating levels of acute-phase proteins (APPs) **(Gabay and Kushner.,1999).** The intention of this inflammatory reaction is to stop the infection from spreading and kill germs. The production of both pro-inflammatory and anti-inflammatory cytokines, which increase phagocytosis, macrophage activation, cell-mediated immunity, and up-regulate APP synthesis, is part of the host response to many illnesses and bacterial infections **(Idoate et al. 2015).**

Anti-inflammatory cytokines such as tumor necrosis factor (TNF-) and interleukin (IL-1, IL-6, and IL-8) are generated during this phase are pro-inflammatory**(Cray et al.,2009)**. T-cells generate pro-inflammatory cytokines, which act as chemical messengers in response to the host

immunological response. TNF-alpha is a pro-inflammatory cytokine that plays a key role in host immunological responses, including the production of IL-6. Increased TNF-alpha and Il-6 levels indicate that the animal has been exposed to various stresses and is experiencing immunological suppression **(Ditchkoff et al.,2001).**

The release of cytokines varies depending on the type of pathogens (bacteria, viruses) **(Gånheim et al.,2007).** It has been shown that released cytokines are responsible for the release of acute phase proteins in the hepatic cells, and that cytokine levels and acute phase protein levels have a positive association **(Gånheim et al.,2003).** Basic mediators of APPs generated by the liver include pro-inflammatory cytokines including IL-6 and TNF. While IL-6 is more effective in cases of hepatic APR, TNF- is effective in cases of extrahepatic APR. These cytokines are mostly produced by macrophages, although they may also be produced by other cells in response to internal or external stimuli **(Murata et al.,2004).**

APPs are generated in the hepatic cells during infection and circulate in large amounts in the blood. Many functionalities of APPs may be useful to the infected host **(Gabay and Kushner.,1999).** The primary function of APPs is to protect the host from various pathogenic reactions while also assisting in the restoration of homeostasis at various stages of inflammation. Some APPs have anti-protease activity, whereas others (Haptoglobin and Serum amyloid A) have scavenging functions and are defined by their capacity to impact the host immunological response **(Tothova et al., 2014).**

The concentration of APPs in the blood changes depending on the severity of the condition. They also stated that the stimulus of inflammation causes a significant change in APPs serum concentrations, which remain high as long as the infection is present in the body (**Dinler et al., 2017).** APPs can be utilised in diagnosis, prognosis, and monitoring response to treatment, as well as in general health screening, as quantitative biomarkers of disease. These biomarkers are relatively sensitive indicators of inflammation, although they lack specificity, and the APP response varies greatly between species**(Eckersall and Bell .,2010).** In bacterial infections, APR is characterised by an accelerated turnover of APPs, but in viral infections, it is less pronounced or absent. Based on their serum increase and decrease during APR, the APPs are categorized as positive or negative(**Kilicarslan et al. 2013).**

C-reactive protein (CRP) is a prominent APP in a variety of species, including dogs, and its serum concentration can rapidly rise from 100 mg/L as a result of infectious disorders such as babesiosis, leishmaniosis, leptospirosis, parvovirus infection, and E. coli endotoxaemia **(Cerón et al.,2005).** CRP has been used as a diagnostic, prognostic, and follow-up marker in patients with CAP for decades. Current human guidelines advocate testing serum CRP in patients suspected of having CAP, in addition to other diagnostic techniques**(Lim et al., 2009; Woodhead et al., 2011).**

According to human guidelines, CAP is considered very likely when CRP is > 100 mg/l in a patient with compatible clinical presentation and unlikely when CRP is<20 mg/l and symptoms have lasted more than 24 hours **(Woodhead et al., 2011).** Serum CRP measurement can be applied in humans to distinguish bacterial pneumonia from non-bacterial infections **(Flood et al., 2008; Bafadhel et al., 2011).**

The utility of serum CRP measurement as a follow-up biomarker in patients with CAP has been extensively studied. CRP levels were found to be useful in assessing treatment response and identifying patients who had a poor response to therapy when measured consecutively throughout the first week of CAP **(Coelho et al., 2007; Bruns et al., 2008).** Failure to see a decrease in serum CRP by day three or four after starting therapy was linked to a worse result **(Coelho et al., 2007; Moreno et al., 2010).**

Patients with an inadequate antibiotic treatment regimen, infectious complications, and a risk of worsening may benefit from daily CRP readings **(Coelho et al., 2012).** The normalisation of CRP has been proposed as a potential goal for antimicrobial treatment since serum CRP lowers promptly following beginning of effective therapy **(Ehl et al.1997).**

Serum CRP is significantly increased in dogs with BP relative to healthy dogs and dogs with other respiratory diseases, and therefore, serum CRP can be used as an additional diagnostic biomarker in BP **(Viitanen ., 2017).** In dogs, serum amyloid A (SAA) is a prominent positive APP. Although SAA is mostly produced in the liver, it has also been shown to be produced locally in inflamed tissue **(Kjelgaard-Hansen et al., 2007).** Two hours after an experimentally induced inflammation, SAA concentrations were found to be significantly higher **(Higgins et al., 2003).**

Similar to CRP, SAA may be elevated after stress or strenous exercise in healthy dogs **(Casella et al., 2013 and Fazio et al., 2014).** CRP and SAA measurement in dogs with systemic inflammation were found to be sensitive indicators of inflammation .However, SAA showed a wider range of concentrations and was therefore considered inferior to CRP **(Christensen et al. 2014).**

Despite substantial human medicine research on CRP, SAA has gotten far less attention in individuals with CAP. Only a few studies have looked at the value of SAA measurement, and it is not currently used in clinical practice. In humans, CRP and SAA have a strong correlation, and their diagnostic value in individuals with systemic bacterial infections is similar **(Huttunen et al., 2003).** SAA is regarded a more sensitive marker of modest inflammatory activity typical of viral and non-invasive bacterial infections since it is triggered by subtle inflammatory stimuli in humans **(Lannergard et al., 2003)**.

In dogs, Hp is a moderate positive APP **(Conner et al., 1988).** Although canine Hp is similar to human Hp, canines only have one subtype of Hp, whereas humans have three **(Ceron et al., 2005).** Hp lowers inflammation by binding to toxic and pro-inflammatory free haemoglobin in plasma. Hp inhibits granulocyte chemotaxis, phagocytosis, and bactericidal activity, as well as mast cell and T-cell proliferation, among other immunomodulatory actions **(Murata et al., 2004).**

The glycosylation pattern of Hp differs between disorders, and more research is needed to fully understand the clinical utility of these modifications in distinguishing and monitoring various diseased processes**(Andersson and Sevelius, 2001).** Elevated Hp concentrations were first observed 24 hours after an experimentally or medically caused injury in dogs, and maximal concentrations were detected 3-4 days after the commencement of inflammation **(Dabrowski et al., 2007).** Hp levels have been found to be elevated in a variety of viral and non-infectious disorders in dogs, including nasal infections**(Sheahan et al., 2010).**

In dogs with BP, serum CRP, SAA, and Hp values were considerably higher upon presentation. CRP and SAA levels dropped rapidly after starting therapy and mirrored the recovery process, suggesting that they could be used as treatment response markers in dogs with BP **(Viitanen .,2017).**

1.2.4. Ultrasonographic examination:

Thoracic ultrasonography has been used for diagnosis of pulmonary ,pleural and heart lesions in veterinary medicine **(Babkine and Blond .,2009)** Ultrasonography in unsedated animals is not has any harmful effect that allowing it possible to do serial examinations to identify disease sequence and treatment response. Based on their nature, the ability of ultrasonography to identify fluid from soft tissue and differentiate between soft tissues making it better than x-ray technique in the examination of soft tissues**(Nyland and Mattoon, 2002).**

The presence of aerated lung tissue obstructs the evaluation of intrathoracic structures in a normal animal. However, a suitable acoustic window can be provided by a collapsed or consolidated lung, thoracic masses, or pleural effusion. To confirm the presence of a lesion, identify a good acoustic window, thoracic radiography or thoracic CT should be performed before thoracic ultrasonography **(Tidwell .,1998).**

The use of thoracic ultrasound is useful in the detection of many thoracic diseases and has the ability to detect pleural effusions, atelectasis, pneumothorax, and pneumonia with sub pleural involvement of the lung **(Koenig et al .,2011).** However, the use of ultrasound as an indication of underlying parenchymal pulmonary disease, when the organ is still filled with air, is a relatively new application **(Picano and Pellikka .,2016).**

Despite detecting other lung pathology, the thoracic FAST(Thoracic Focused Assessment with Sonography for Trauma [TFAST] protocol is the only standardized lung ultrasound technique currently use in small animals **(Lisciandro and Gregory.,2011)**.

Only a single lung view (one view bilaterally two total acoustic windows) called the chest tube site, is examined by the Thoracic FAST protocol. So A more inclusive lung ultrasound survey has been developed and named Vet BLUE. This lung survey provide four bilaterally applied lung views (eight total acoustic windows)**(Boysen and Søren .,2013).**

The chest tube site view has been defined as the highest point on the thoracic wall over lung directly dorsal from the xiphoid approximating the eighth and ninth intercostal spaces and was the starting point for the Vet BLUE examination **(Lisciandro et al .,2008).**

Standard orientation of normal lung ultrasound consisted of the observation of the "gator sign(alligator)"represented by two rib heads(the gator's eyes)with an interposed intercostal space (gator's bridge of nose)similar to a partially submerged alligator (gator) peering over the water at the sonographer **(Boysen and Søren .,2013).** The pulmonary–pleural line represented by the proximal hyper echoic (bright white) line along the intercostal space **(Lisciandro et al .,2008).**

Dry lung(glide sign) is ultrasonographically represented by the to-and-fro motion along the pulmonary–pleural line with air reverberation artifact called A-lines.9. A-lines are parallel lines extending from the pulmonary–pleural line on equal distance**(Lichtenstein and Meziere et al .,2008).** Wet lung is represented by ultrasound lung rockets also called B-lines **(Volpicelli et al., 2012).** Ultrasound lung rockets are hyper echoic laser-like streaks that extend from the pulmonary–pleural interface through the far field and vibrate in synchronization with inspiration and expiration**(Lichtenstein and Karakitsos et al., 2012).** In human beings, the frequency of ultrasonography lung rockets is determined by the level of edema in the lungs **(Soldati et al ., 2011).**

1.2.5. Radiographic examination:

The investigation of suspected thoracic diseases relies heavily on diagnostic imaging, particularly radiography. Thoracic radiographs are used to verify suspected disease, identify the extent and location of the lesion, detect additional complicating abnormalities, plot the course of the disease, and select further alternative imaging procedures. However, thoracic radiographs have limitations **(Saunders and Keith .,2004).**

2-Therefore ,this study was designed to achieve the following aims:-

1- Recording the clinical, hematological, biochemical, and radiographic changes in dogs and cats with natural respiratory affections.
2- Isolation and identification of the possible causative bacterial agents associated with certain respiratory affection in dogs and cats.
3- Recording of clinical, hematological, biochemical, ultrasonographic and histopathological changes in case of experimentally induced bacterial pneumonia in dogs.
4- Addressing the possible use of serum CRP and other acute phase proteins measurement in the early diagnosis of bacterial pneumonia in dogs.

Clinical, Hematological, Acute phase proteins and Radiographic changes in different respiratory affections in dogs and cats

1. Abstract:

Respiratory affections are important clinical problems recorded in dogs and cats affecting their health condition. This study was carried out on a total of 84 animals including 32 dogs and 52 cats of both sexes and different breeds suffering from different respiratory affections including pneumonia, aspiration pneumonia, Feline upper respiratory diseases(FURD) and Canine infectious respiratory disease(CIRD). Clinical, hematological, biochemical and radiographic changes during these affections were evaluated. In addition the most common incriminated bacteria was isolated and identified. The affected cases showed variable respiratory signs including dyspnea, nasal and ocular discharge, sneezing, cough, abnormal respiratory sound and abnormal lung sound. Hematological changes showed inflammatory leukogram represented by increasing in WBCs and neutrophil count Serum analysis showed marked increase in CRP, SAA and HP levels, with hyperproteinemia and hypoalbuminemia compared to reference value. The most common bacteria isolated from pneumonic cases were Klebsiella, E.coli, Staph., Pseudomonas, Pasteurella, Proteus and Serratia. Radiographic examinations revealed abnormal radiographic patterns associated with the different affections. The present study concluded that clinical, hematological, biochemical combined with chest radiographic findings are essential for precise diagnosis of different respiratory affections in dogs and cats and the early diagnosis facilitate the prescription of relevant therapy and follow up procedures.

Key Words: Acute phase proteins, Dogs and Cats, Radiography, Respiratory affections, Serum proteins.

2. Introduction:

Respiratory diseases in dogs and cats currently receive a great deal of attention, particularly the diagnosis and treatment. Pneumonia is one of the most common systemic diseases in dogs and cats, may be caused by primary bacterial infection such as Bordetella bronchiseptica, Mycoplasma spp., Streptococcus spp. and Yersinia or secondary to other affections such as viral infection , aspiration or inhalation of any foreign materials, also may be resulted from immunodeficiency syndrome. Pneumonia manifested clinically by cough, nasal discharge, dyspnea, fever and abnormal lung sound**(Kogan et al ., 2008).** Most common organisms isolated from dogs and cats with lower respiratory disease include E. coli, Pasteurella spp., klebsiella, Streptococcus spp., Bordetella bronchiseptica, Enterococcus spp., Mycoplasma spp., Staphylococcus spp., and Pseudomonas spp.**(Rheinwald et al ., 2015).**

Canine infectious respiratory disease (CIRD) or kennel cough is a syndrome occurs in dogs especially young puppies, manifested clinically by acute onset of cough that may be accompanied by other signs such as sneezing, nasal discharge, conjunctivitis and ocular discharge according to causative agent. Bacterial infection may be implicated as a primary pathogen or secondary to viral infection **(Mochizuki et al ., 2008).** FURD is a syndrome occurs in cats, manifested clinically by sneezing, nasal discharge, fever, salivation, conjunctivitis, ocular discharge, oral ulceration and epistaxis according to the cause of infection. One or more pathogens including viral pathogen such as herps virus, calici virus and canine distemper virus or bacterial pathogen such as Bordetella bronchiseptica, Chlamydia spp. and mycoplasma spp. or mixed infection are occurred**(Quimby and Lappin ., 2009).**

Diagnosis of respiratory affections in canine and feline depend on case history, clinical findings, laboratory diagnosis and diagnostic imaging. Respiratory sampling is very useful in accurate detection of causative agent in case of respiratory infection to achieve accurate diagnosis, good treatment and prognosis **(Finke., 2013).** The investigation of suspected thoracic diseases relies heavily on diagnostic imaging, particularly radiography. Thoracic radiographs are used to verify suspected disease, identify the extent and location of the lesion, detect additional complicating abnormalities, plot the course of the disease, and select further alternative imaging procedures **(Saunders and Keith ., 2004).**The acute phase proteins (APPs) are blood proteins that can be used to assess the innate immune system's systemic response to infection and inflammation. The major APPs that respond to inflammatory stimuli are CRP and SAA in dogs, SAA in cats while the moderate APPs are HP and α1 acid glycoprotein (AGP) in dogs and cats **(Eckersall and Bell., 2010).**

This study was aimed to assess the clinical features, hematological, acute phase proteins and radiographic changes in case of different respiratory affections in dogs and cats, in addition to isolation and identification of the most common bacterial agents that may be included in case of pneumonia.

3. Materials and methods:

3.1.Animals:

This study was applied on total of 84 animals including 32 dogs and 52 cats belonged to pet animal veterinary clinic of faculty of veterinary medicine, Benha University and private pet animal clinics located in Cairo governorate, Egypt during the period from December2020 to February2022. These cases suffered from different respiratory affections. The diagnostic evaluation included history, physical examination(84/84), hematology(25/84), serum biochemistry(25/84), thoracic radiographs (38/84) , bacterial isolation and identification (24/84). Dogs and cats suspected to be suffering from respiratory disease were visually examined for the presence of nasal or ocular discharge, dyspnea, cough, depression and inappetance. Using the clinical respiratory scores **(Maboni et al ., 2019)**summarized in **Table(2-1)**, the affected animals were classified into score1or score2.

Table (2-1): Clinical respiratory scores in dogs and cats.

Clinical score	Clinical signs
0 (asymptomatic)	No history of respiratory disease
1 (mild)	Cough or sneeze or nasal discharge
2 (moderate/severe)	Cough or sneeze or nasal discharge, in addition to one of the following signs: Fever or lethargy/depression or inappetance or pneumonia

3.2.Ethical approval:

The study was done after the approval of the Ethics committee of Benha University with the approval number (BUFVM 15-03-22). All samples were collected after owner consents.

3.3.Samples:

Two sets of blood samples were collected from cephalic vein. The first blood sample was collected on a labeled test tube with anticoagulant (potassium salt of EDTA) for determination of hematological parameters. The second blood sample was collected without anticoagulant, clotted at room temperature for 20 min, centrifuged at 3,000 rpm for 10 min, and then the clear non-

hemolyzed serum samples were separated and stored at −20°C until subsequent biochemical analysis.

3.4.Hematological examination:

Hematological parameters were determined by hematological analyzer(Model No.93-91098-00-GF) as previously described by **Feldman et al., (2000).** Results were compared with normal reference values according to **Latimer.,(2011).**

3.5.Bacteriological examination:

Respiratory samples were collected from cases suffered from pneumonia (12dogs and 12cats) for bacterial culture. Sampling methods included were tracheal wash (2/24), sputum samples (8/24) and nasal swabs from nasal discharge (14/24). The respiratory samples swabs were inoculated separately in nutrient broth for activation of the microorganism at 37 ^{o}C for 24 hours. A sterile lop full from the broth with activated microorganism was directly sub-cultured on blood agar, paired barker media, Eosin methylene blue media and MacConkey's agar. Plate readings occurred at 24 hours. and 48 hours. The isolates recovered were sub-cultured and further identified using colony morphology, Gram stain and biochemical tests**(Alton et al.,1996)**. The identification of suspected bacteria colonies was achieved by observation of colonial morphology under microscopy and the use of some biochemical tests: hemolysis, motility, indole formation, glucose, lactose, methyl red, voges proskauer and citrate utilization. Assay for biochemical properties of the bacteria isolates was conducted according to **MacFaddin .,(2000).**

3.6. Serum proteins analysis:

Serum total proteins were determined spectrophotometrically according to the method described by **Pagana and Pagana (2017).** Serum albumin was determined calorimetrically by using the dye-binding technique with bromocresol green according to the method described by **Fischbach and Dunning (2009).** Serum globulin was determined by the differences between total protein and albumin according to **Chernecky and Berger (2008)** and A/G ratio was calculated by dividing the albumin value over globulin value according to **Fischbach and Dunning (2009).** Results were compared with normal reference values according to **Latimer.,(2011).** Serum CRP was analyzed using ELISA Kit (Eucardio laboratory, Inc.,

Encinitas, and CA.,USA), previously validated for use in dogs according to **Ibraimi et al., (2013).** SAA were measured with a commercially available ELISA kit according to method described by **Alsemgeest et al. (1995).** Serum Hp concentrations were determined by ELISA kit according to method described by **Idoate et al. (2015).** Results were compared with normal reference values according to **Ceron .,(2005).**

3.7.Radiographic examination:

Lateral and ventrodorsal views of the chest area are standard, both right and left lateral views are required to gain maximum information, radiographic evaluation of the lung fields is enhanced by exposing the radiograph at maximum inspiration if possible .According to **Kealy et al ., (2010).**

4. Results:

4.1.Clinical findings:

Pneumonic cases were admitted with a history of vaccination, anorexia, dullness, cough, nasal discharge and some cases with previous illness **Fig. (2-1)**. Cases of aspiration pneumonia were admitted with a history of accidental fluid inspiration may be water or drugs, vomiting, dyspnea, nasal discharge, coughing with digestive disorders and death within few days in some cases. Cases of FURD were admitted with a history of housing with other infected unvaccinated cats with signs of anorexia, inappetance, salivation, nasal and conjunctival discharge, sneezing and epistaxis, these affected cases were not vaccinated **Fig.(2-2) & Fig.(2-3)**. All cases of kennel cough were admitted with a history of housing with other infected dogs, hacking paroxysmal cough, serous nasal discharge, dullness, inappetence and ocular discharge. All data are represented in **Table (2-2)**

Table(2-2):Result of clinical examination of different respiratory affections in dogs and cats.

Disease	Clinical parameters			Clinical score		Other signs
	Temp. C°	Pulse rate pulse/min	Respiratory rate RC/min	Score 1	Score 2	
Pneumonia	39.3± .67 in dogs 40.2±.5 in cats	129±20 in dogs 135±10 in cats	57±16 in dogs 62±20 in cats	–	12 dogs 12 cats	Congested or pale or cyanosed mucous membrane, lung crackles and wheezes, tachycardia and abdominal respiration.
Aspiration pneumonia	39.1 ± .5 in dogs 39.9± .4 in cats	120 ±5 in dogs 130±7 in cats	48±5 in dogs 54±7 in cats	_	3 dogs 5 cats	Dyspnea, abnormal lung sound (moist rales or crackles), megaesophagous in some cases, abdominal respiration and mouth breathing
CIRD	39.2 ±.14	100±7	40 ±5	12 dogs	5 dogs	Harsh sound of trachea, recovery occur within 3-7 days.
FURD	40.3±.4	130±10	58±10	10 cats	25 cats	Ulceration in oral cavity, petechial hemorrhage in gum and hard palate, corneal ulcer, abnormal respiration, severe conjunctivitis and lacrimation.

Table(2-3): Showing the different breeds included in the study, their age average and the percentage of each affection.

Disease	Affected animals	
	Dogs	**Cats**
Pneumonia (28.6 %)	(12dogs) 3German shepherd, 3Golden Retriever, 4 Rottweiler, 2 Yorkshire	(12 cats) 4 Baladi, 5 Persian, 3 Siami
	Age : 9m - 4 y	Age :1.5-3y.
Aspiration pneumonia (9.5 %)	(3 dogs) 1 Mixed breed, 1 Griffon, 1 Golden	(5 cats) 3 Persian, 2 Himalayan
	Age:9m - 3 y.	Age: 1-3 y.
CIRD (20.2 %)	(17 dogs) 6 German shepherd, 4 Golden Retriever, 1 Labrador Retriever, 3 Rottweiler, 1 Begal, 1 Griffon, 1 Cocker spaniel	
	Age :10m – 2y.	
FURD (41.6 %)		(35 cats) 11 Baladi cats, 5 Siami, 19 Persian
		Age :2m -1.5 y.

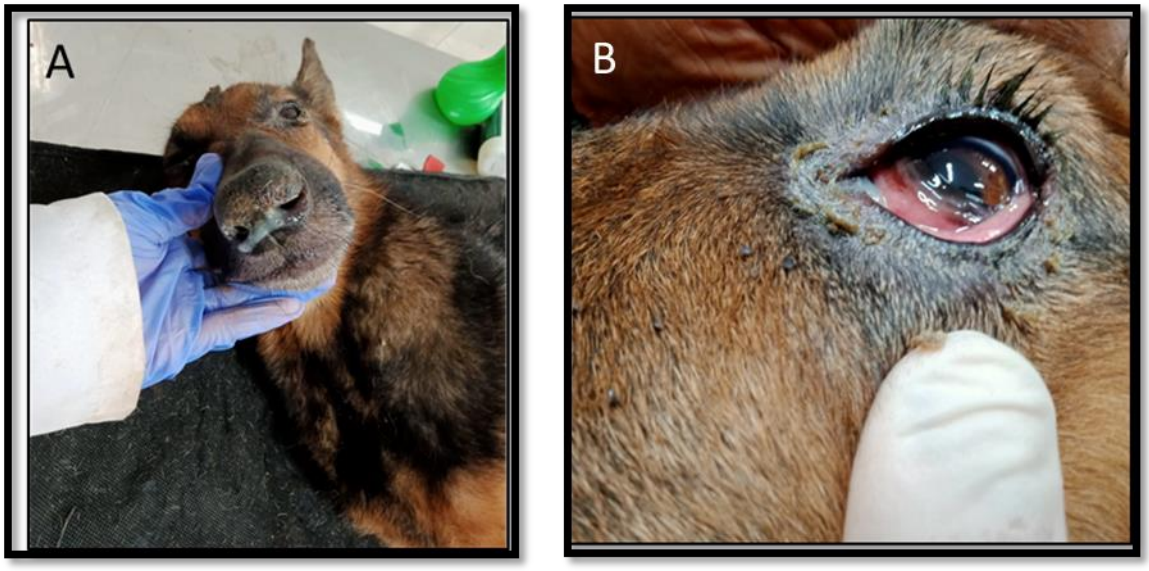
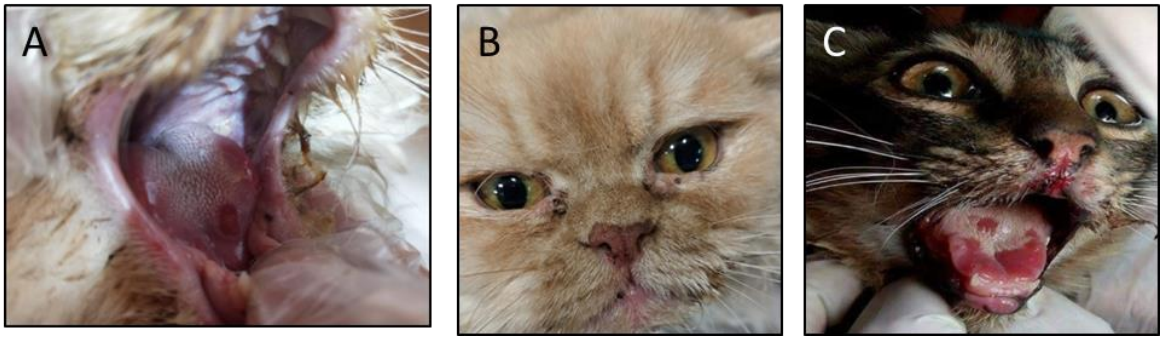
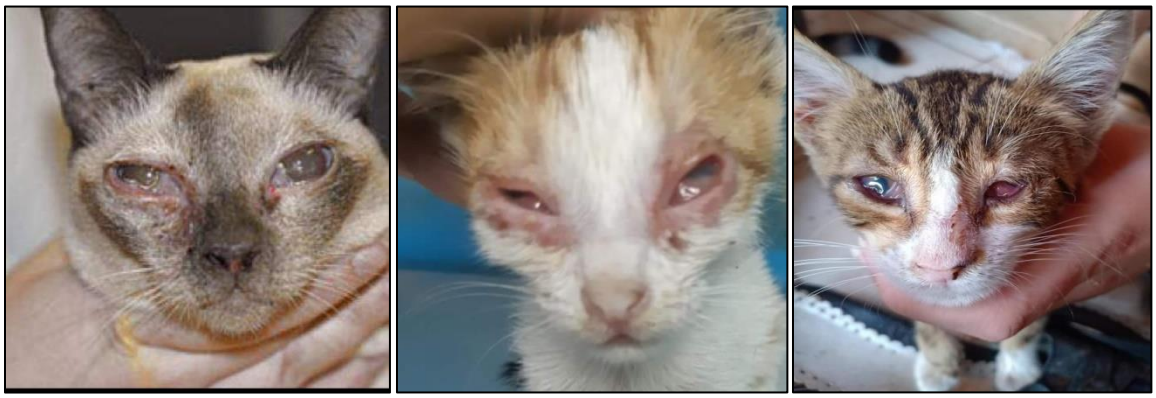


Figure (2-1) : German Shepherd dog suffering from pneumonia showing bilateral mucopurulent nasal discharge (A), congested mucous membrane and ocular discharge (B).



Figure(2-2): Persian and baladi cats suffering from upper respiratory disease showing oral ulceration(A,C), ulceration at nasal region(C), salivation, ocular and nasal discharge(B).



Figure(2-3): Siami and baladi cats suffering from upper respiratory disease showing conjunctivitis, ocular and nasal discharge.

4.2. Hematological findings:

The results of CBC analysis are represented in **Table(2-4).** The mean value of RBCs count, Hb content and PCV% were within normal reference value in cases affected with aspiration pneumonia, CIRD and FURI but lower than reference value in case of dogs and cats affected with pneumonia. The mean value of WBCs count and neutrophils were higher than normal reference value in case of pneumonia, aspiration pneumonia, CIRD and FURI. The mean value of lymphocytes was lower than normal reference value in case of pneumonia, FURI and CIRD but within normal level in case of aspiration pneumonia. The mean value of eosinophils was lower than normal reference value in all affections. The mean value of monocytes was higher than the normal reference value in all affections except FURD was within the normal value.

Table (2-4): Hematological changes in dogs and cats suffering from different respiratory affections.

Hematological parameters	Pneumonia (n=7dogs)	Aspiration Pneumonia (n=3dogs)	FURD (n=6cats)	CIRD (n=9dogs)	Reference value	
					Dogs	Cats
RBCs (10^6 /µl)	4.3±.92	7.4±.25	7.07±1.21	5.9±1.40	**5.7-8.5**	**6.9-10.1**
Hb (g/dl)	9.8±.71	14.6±.51	12.5±1.25	13.6±1.13	**14.1-20.1**	**10.9-15.7**
PCV%	32.6±3.1	47.8±3.18	40.9±8.34	36.2±8.35	**41-58**	**31-48**

WBCs (10^3/mm^3)	26.1±3.30	22.8±2.63	20.2±5.23	19.4±5.42	**5.7-14.2**	**5.1-16.2**
Neutrophils %	87.8±4.62	85.6±2.92	81.2±9.26	83.2±7.35	**43-80**	**25-77**
Lymphocytes %	5.9 ±1.94	27.3±1.43	8.1±1.72	8,3±1.21	**14-45**	**14-61**
Eosinophils %	.47±.30	.52±1.21	.6 ±.42	.4±.34	**1-18**	**2-23**
Monocytes%	12.4±2.91	10.6±1.90	4.9 ± 2.60	10.4±.61	**2-9**	**1-5**

4.3.Bacterial isolation and identification:

The result of bacteriological isolation revealed that the most common isolated pathogen in pneumonic cases were Klebsiella, E.coli, Staph., Pseudomonas, Pasteurella, Proteus and Serratia.

4.4.Changes in serum proteins:

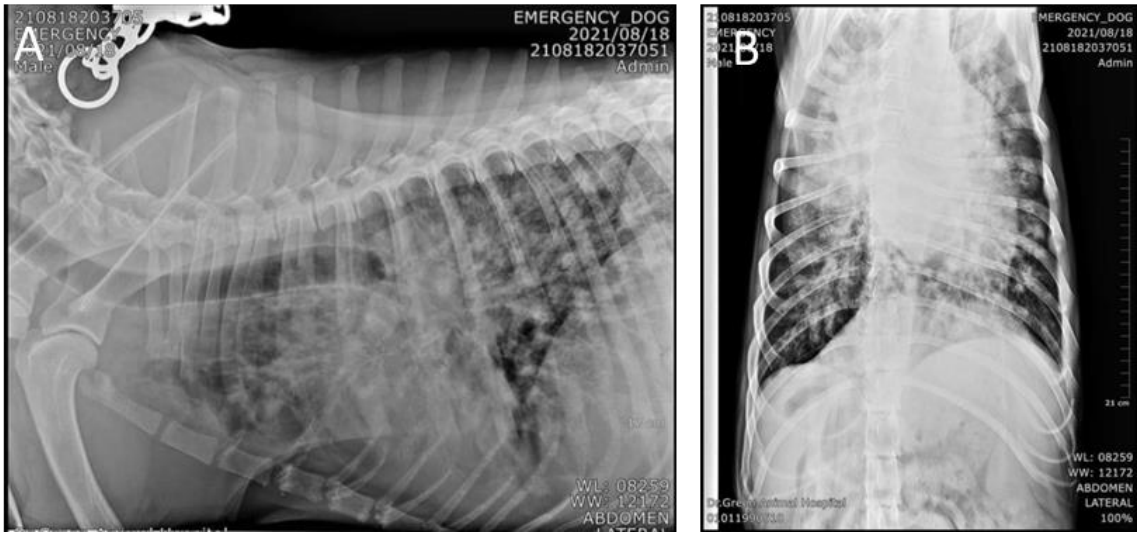
The mean value of SAA and HP were higher than normal reference value in case of pneumonia, aspiration pneumonia and FURD but within the normal level in case of CIRD. The mean value of CRP was higher than normal reference value in all cases, especially in pneumonic cases was 6 times more than reference value. Pneumonic cases showed increase in total protein and globulin, decrease in globulin and A/G ratio. Cases of aspiration pneumonia showed increase in total protein and decrease in albumin but globulin and A/G ratio were within the normal level. Cases of FURD and CIRD showed a slight increase in total protein and globulin while showing decrease in albumin and A/G ratio compared to normal reference value. All data are represented in **Table (2-5)**

Table(2-5): Changes in acute phase proteins and serum proteins concentrations in the different affected cases .

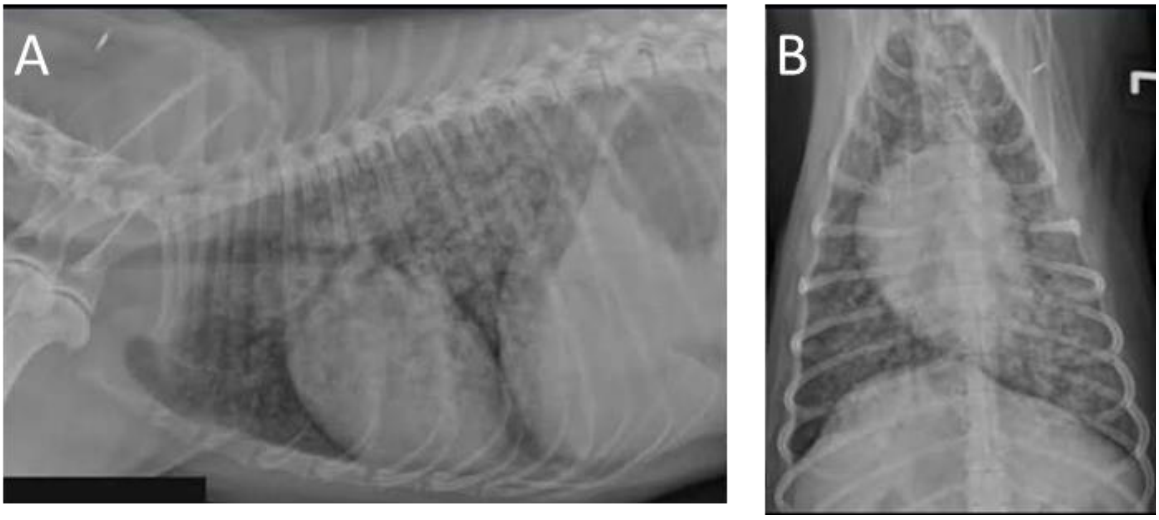
	Pneumonia	**Aspiration pneumonia**	**FURD**	**CIRD**	**Reference value**	
	(n=7dogs)	**(n-3dogs)**	**(n=6cats)**	**(n=9dogs)**	**dogs**	**cats**
CRP (mg/l)	30.00 ±7.62	17.93±2.82	7.35±1.07	12.29±.34	< 5	0-.03
HP (mg/dl)	76.24 ±17.38	83.90±9.64	54.86±10.30	11.67±.33	0 – 30	3.84 – 40
SAA (mg/l)	3.74±1.34	4.31±.72	4.78 ±1.25	1.97±.09	0 - 2.19	-
Total protein (g/dl)	9.52±.92	8.76±.26	8.46±.94	7.55±.47	5.5 – 7.2	6.6 - 8.4
Albumin (g/dl)	2.01±.49	2.42±.09	2.31±.19	3.00±.02	3.2 - 4.1	3.2 – 4.3
Globulin (g/dl)	6.13±.59	3.32±.19	4.92±.95	4.29±.46	1.9 – 3.7	2.9 – 4.7
A/G ratio	.52 ±.06	.72±.10	.73±.19	.72±.08	.9 – 1.9	.8 – 1.5

4.5 Radiographic findings:

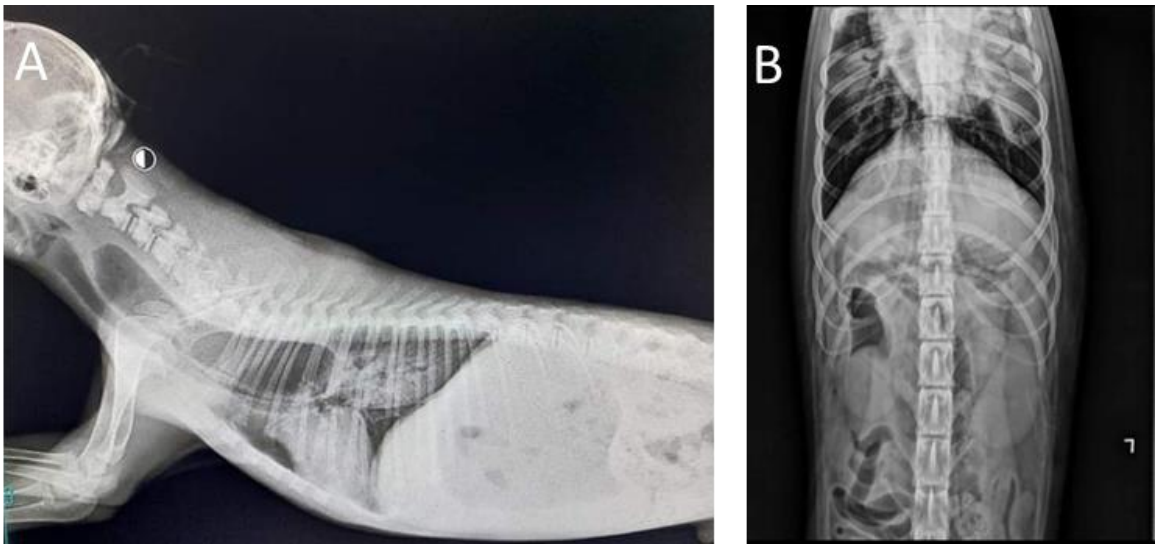
The radiographic views of cases suffering from pneumonia and aspiration pneumonia revealed a predominantly interstitial pattern and alveolar infiltrate **Fig.(2-4)& Fig.(2-5)**, some cases showing lung consolidation. Some cases of aspiration pneumonia were caused by megaesophagous as demonstrated by x-ray examination **Fig.(2-6)**. The radiographic views of some cases suffering from CIRD revealed pneumonia represented by bronchial pattern mainly, but some cases showed mixed pattern **Fig.(2-7).**



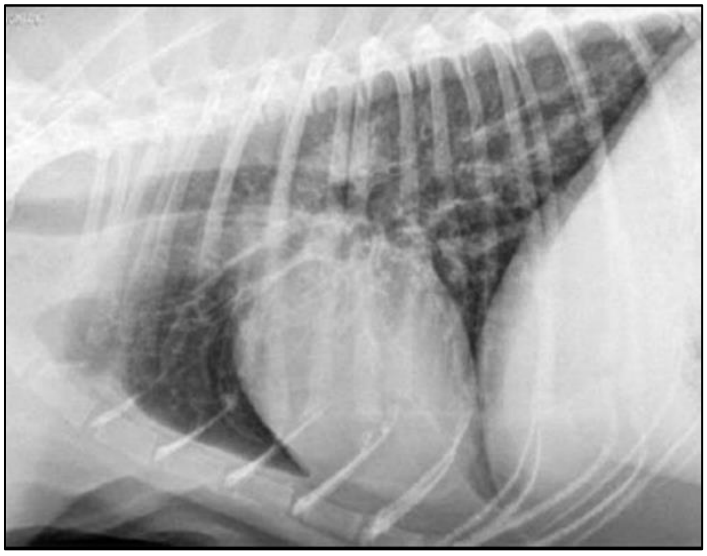
Figure(2-4): Lateral(A)and ventrodorsal view(B) of chest radiography in Golden retriever dog showing interstitial pattern to patchy alveolar pattern with mild degree of pleural effusion in a dog lung suffering from pneumonia.



Figure(2-5): Lateral(A) and ventrodorsal view(B) of chest radiography in German shepherd dog suffering from pneumonia showing diffuse alveolar and interstitial patterns.



Figure(2-6): lateral and ventrodorsal view of chest radiography in young puppy showing megaesophagous, consolidation in the cranial , caudal(A) and left middle lung lobes(B) in a dog suffering from aspiration pneumonia.



Figure(2-7): lateral view of chest radiography showing bronchial and alveolar pattern in the cranial and caudal lung lobes in Rottweiler dog suffering from CIRD.

5. Discussion:

The dogs and cats affected by pneumonia showed signs of cough, nasal discharge, anorexia, lethargy, fever in some cases but other cases were with a normal temperature, also showed tachypnea, tachycardia, dyspnea, abdominal respiration and abnormal lung sound, so all cases of pneumonia took score 2 according to respiratory scoring system, the same result was previously recorded by **Radhakrishnan et al.,(2007); Dear .,(2014)**. Cases of aspiration pneumonia showed normal body temperature, history of vomiting or digestive troubles, dyspnea, nasal discharge and abnormal lung sound such as harsh sound or crackles or wheezes, most cases die within few days due to complication by secondary bacterial infection the same result was previously recorded by **Tart et al .,(2010).** All cases of aspiration pneumonia took score 2 due to presence of nasal discharge in addition to pneumonia. Ten cats suffered from FURD showed signs of sneezing or cough or nasal discharge only so, these cases took score 1. Other 25 cats showed signs of fever, anorexia, sneezing, nasal discharge, ocular discharge, salivation, oral ulceration and epistaxis in some cases, these cases took score 2, the same results was previously recorded by **Hurley and Sykes ., (2003) ; Lappin et al .,(2017**). Dogs affected by CIRD(n=12) showed signs of acute onset of cough, sneezing, serous ocular and nasal discharge, normal body temperature and normal lung sound, these cases took score 1, recovery occur within 3 to 7 days. Some cases(n=5) showed signs of fever, mucopurulent nasal discharge and abnormal laryngeal and tracheal sound, these cases took score2, the same result was previously recorded by **Reagan and Sykes.,(2020)**. Occurrence of dyspnea in some cases may be attributed to hypoxia that resulted from sever inflammation in the bronchioles and alveoli, which interfere with gas exchange and respiration. Nasal discharge was observed may be due to inflammatory changes in the nasal mucous membrane. Heart rate and respiratory rate were increased in some cases to compensate hypoxia. Abnormal lung sounds such as crackles or wheezes may be due to the accumulation of exudate produced by inflammatory cells and goblet cells as a result of pneumonia **(Ramadan ., 2019).** Most of CIRD and FURD cases if not treated rapidly it may be complicated by secondary bacterial infection result in occurrence of pneumonia.

The mean value of RBCs count, Hb content and PCV% were within normal reference value in cases affected with aspiration pneumonia, CIRD and FURI but lower than reference value in case of dogs and cats affected with pneumonia. The mean value of WBCs count and

neutrophils were higher than normal reference value in all affections. The mean value of lymphocytes was lower than normal reference value in case of pneumonia, FURI and CIRD but within normal level in case of aspiration pneumonia. The mean value of eosinophils was lower than normal reference value in all affections. The mean value of monocytes was higher than the normal reference value in all affections except FURD was within the normal value , the same results were previously described by **Hurley and Sykes.,(2003); Kogan et al .,(2008); Reagan and Sykes., (2020) and Berliner., (2021).** The decrease in RBCs, Hb content and PCV% was attributed to, respiratory affections especially pneumonia. The mononuclear phagocytic system under the circumstances of inflammatory conditions becomes hyperplastic, trapping free iron and hence increases iron storage in phagocytic cell, reducing iron transfer to growing erythroid cells in the bone marrow, resulting in a decrease in Hb production and the development of microcytic hypochromic RBCs **(Ismael et al., 2017).** The increase of WBCs, mainly neutrophils and monocytes is a frequent finding in many diseases because of acute inflammatory response due to presence of bacterial infection **Kogan et al ., (2008).** Lymphopenia could be attributed to stress response and endogenous release of corticosteroids that may play a secondary role in redistribution of recirculating lymphocytes leading to their sequestration in the lymphoid tissues rather than entering efferent lymph and blood to participate in the developing inflammation **(Ramadan et al ., 2019).**

The bacteriological examination of the cultured swab collected from the pneumonic cases revealed that, the most common pathogens isolated in case of pneumonia were Klebsiella, E.coli, Pasteurella, Pseudomonas, Staphylococcus spp., Proteus and Serratia. The same result was previously recorded by **Rheinwald et al.,(2015)and Viitanen.,(2017).**

The major positive APPs, such as CRP and SAA in dogs, have low physiological levels, but rise rapidly within hours after inflammatory stimulus and normalize quickly when inflammatory stimulus ceases **(Eckersall and Bell, 2010).** Due to these properties, the major positive APPs have received the most attention as inflammatory biomarkers than the intermediate positive APPs, such as Hp. **(Ceron et al., 2005).** The mean value of SAA and HP were higher than normal reference value in case of pneumonia and FURD but within the normal level in case of CIRD. The mean value of CRP was higher than normal reference value in all cases especially pneumonic cases. The same results were previously described by **Cerón et al .,**

(2005); Eckersall and Bell (2010); Viitanen., (2017). SAA is the major APPs in cats, significantly increase in the early stage of pneumonia and upper respiratory infection compared to other APPs. SAA has been clinically applied as a biomarker for occurring the disease in cats as it is the most rapidly responsive AAP in case of inflammatory and infectious conditions. **(Yuki et al ., 2020).** HP is the moderate APP in dogs and cats was moderately increased in case of infection and inflammation **Eckersall and Bell (2010).**

Pneumonic cases showed marked increase in total protein and globulin, decrease in globulin and A/G ratio compared to normal reference value. The same results were previously recorded by **Cerón et al ., (2005); Kogan et al ., (2008)**. Cases of FURD and CIRD showed a slight increase in total protein and globulin while showing decrease in albumin and A/G ratio. The same results were previously described by **Trumel et al ., (2019); Hong et al ., (2021).** The changes in protein profile during acute phase response were caused by increasing in synthesis of positive acute phase proteins, complement proteins, and immunoglobulins, so hyperproteinemia is usually associated with infection and inflammation. Albumin is a negative acute phase protein that decreased during inflammation because about 30–40% of hepatic protein anabolic capacity is utilized for the creation of positive acute phase proteins during the acute phase response, resulting in a reduction in other proteins**(Ramadan et al.,2019).** Hypoalbuminemia could be suggestive of lung inflammation and vasculitis that resulted in leakage of albumin into the alveolar space**(Kogan et al., 2008).**

The radiographic views of cases suffering from pneumonia and aspiration pneumonia revealed a predominantly interstitial pattern and alveolar infiltrate, some cases showing lung consolidation, the same results was previously recorded by **Dear ., (2014); Levy et al., (2019).**The radiographic views of dogs chest suffering from kennel cough revealed a predominantly bronchial pattern, but some cases showed mixed pattern, the same result was previously recorded by **Chand et al.,(2015); Vindenes et al .,(2015).** Lung patterns are simply the radiographic appearance of disease in the lung. Common pattern include bronchial pattern, interstitial pattern and alveolar pattern. Bronchial pattern represented by diffuse thickening of the air way lines and rings throughout the pulmonary tissue, it was observed in case of bronchitis, feline asthma, kennel cough and mycoplasma pneumonia. An un structured interstitial pattern represented by increasing the soft tissue opacity that partially obscure the blood vessels margin,

it was observed in case of pulmonary edema and pneumonia. A structured interstitial pattern represented by ovoid or rounded shaped nodules of soft tissue distributed all over the lung tissue, it was observed in case of fungal pneumonia. Alveolar pattern is more severe than interstitial pattern, it represented by an area of increased soft tissue opacity in the lung tissues that completely obscure pulmonary blood vessels, it was observed in the same cases as interstitial pattern **(Thrall .,2013); (Thrall and Robertson .,2015).**

6. Conclusion:

The present study concluded that clinical, hematological, biochemical combined with chest radiographic findings are essential for precise diagnosis of different respiratory affections in dogs and cats and the early diagnosis facilitate the prescription of relevant therapy and follow up procedures .

Clinical, Hemato-biochemical, Ultrasonographic and Histopathological Changes in dogs with Induced Bacterial Pneumonia

1. Abstract:

Pneumonia in small animals is a multifactorial complex disease. It is considered one of the most common systemic diseases in dogs causing high mortality. This study was aimed to evaluate the role of acute phase proteins, Il6 and ultrasonography in the early diagnosis of bacterial pneumonia in dogs. For that purpose ten healthy baladi dogs were infected by intra-tracheal instillation of 2ml of Klebsiella pneumoniae broth. Clinical, hematological, biochemical and ultrasonographic examinations of the pneumonic dogs were performed on zero day before induction and on the 1^{st}, 3^{rd},5^{th} and 7^{th} day post induction. The result of the study showed a significant increase in C-reactive protein(CRP), Haptoglobin(HP), Serum amyloid A (SAA) and interleukin 6 (IL6) within 24 hours post induction. There was a significant increase in total protein and globulin and a significant decrease in albumin and A/G ratio. The infected lungs showed ultrasonographic changes on the 3^{rd} day post induction. This study showed that CRP is a diagnostic biomarker in case of bacterial pneumonia in dogs as it increased 6 folds within 24 hours post infection more than its value on zero day. Ultrasonographic examination was helpful in the detection of the early changes in pulmonary tissues in case of pneumonia.

Key Words: Acute phase protein, Bacterial pneumonia, Dogs, IL6, ultrasonography.

2. Introduction:

Bacterial pneumonia (BP) is still one of the most prevalent clinical diagnosis in dogs with acute or chronic respiratory diseases. According to new research, viral respiratory disorders and the development of bacterial pneumonia in dogs have a complicated association. Much has been learned about the complicated interplay between host and environmental factors that contributes to this complex of diseases in the last decade **(Dear ., 2014)**. BP may be resulted from aspiration pneumonia, canine infectious pneumonia, inhalation of foreign body, nosocomial infection, and immune dysfunction. Common bacteria that isolated from lower respiratory tract of dogs and cats include Staphylococcus, Streptococcus, E. coli, Pseudomonas, Bordetella, Pasteurella, Klebsiella, Bacteroides and Clostridium **(Viitanen.,2015).** The role of these bacteria as a primary pathogen is less clear **(Ford.,2013).** Wide range of clinical signs are included in case of BP such as a productive cough, nasal discharge, hyperpnoea or dyspnea and lung sound abnormalities such as crackles, increased bronchovesicular sounds, or wheezes, additionally

signs of systemic disease are present that include fever, anorexia, depression, weight loss, and dehydration **(Ali.,2011).**

The use of thoracic ultrasound as an indication of underlying parenchymal pulmonary disease when the organ is still filled with air, is a relatively new application**(Picano and Pellikka.,2016).** It is useful in the detection of many thoracic diseases and has the ability to detect pleural effusions, atelectasis, pneumothorax, and pneumonia with sub pleural involvement of the lung **(Koenig et al .,2011).**

The acute phase response is an unspecific systemic reaction of the organism that occurs after infection or inflammation. This reaction includes changes in the concentrations of some plasma proteins called acute phase proteins(APPs) **(Pomorska-Mól et al., 2013)**. APPs are sensitive markers of inflammation, and especially serum CRP is currently an important diagnostic and follow-up marker in humans with community-acquired pneumonia **(Lim et al., 2009)**. CRP is produced mainly by the liver in response to the cytokine such as interleukin 6, which is released from monocytes and macrophages at site of inflammation. CRP has been shown to be elevated in dogs with BP**(Christensen et al., 2014)**.

This study was aimed to assess the clinical features, hematological, biochemical and histopathological changes during bacterial pneumonia. In addition, this study investigated the applicability of APPs as diagnostic biomarker and the role of chest ultrasonography in the early diagnosis of bacterial pneumonia.

3. Materials and methods:

3.1. Animals:

10 healthy baladi dogs were used in this study, with body weight ranged from 10 to 15 Kg and their age ranged from1-2years. They were healthy based on clinical, hematological, biochemical, radiographic and ultrasonographic examination. They were housed in separate boxes with free access of food and water. After two weeks of adaptation, bacterial pneumonia was induced as previously described **(Bookstein et al ., 1983)**, with some modifications. Briefly, each dog was anesthetized with intravenous propofol (6-8 mg/kg b.wt) **(Short and Bufalar., 1999)**,then dogs were instilled intra-tracheally using endotracheal tube in upright position by

2ml of klebsiella broth, that was isolated from germen shepherd dog affected by bacterial pneumonia. The klebsiella broth was prepared through collection of a fresh sputum sample from the affected dog then inoculated in nutrient broth for activation of the microorganism at 37 ^{o}C for 24 hours. A sterile lop full from the broth with activated microorganism was directly sub-cultured on blood agar, paired barker media and MacConkey's agar. Plate readings occurred at 24 hrs. and 48 hrs. The isolates recovered were sub-cultured and further identified using colony morphology, Gram stain, biochemical tests and identification confirmed by PCR **(Alton et al ., 1996)**. Clinical examination was performed daily for 7day, hematological and biochemical Examination were performed on zero day, 1st , 3rd and 5th day of induction, ultrasonographic examinations were performed for all dogs on zero day before induction and on the 1st, 3rd, 5th and 7thday post induction. Histopathological examination was performed at the end of experiment.

3.2. Ethical approval:

All examinations were done after the approval of the Ethics committee of Benha University with the approval number (BUFVM 07-02-22).

3.3. Samples:

Two sets of blood samples were collected from each dog from the cephalic vein. The first blood sample was collected on a labeled test tube with anticoagulant (potassium salt of EDTA) for determination of hematological parameters. The second blood sample was collected without anticoagulant, clotted at room temperature for 20 min, centrifuged at 3,000 rpm for 10 min, and then the clear non-hemolyzed serum samples were separated and stored at −20°C until subsequent biochemical analysis.

3.4. Hematological analysis:

Total erythrocytic count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), total leukocytic count (WBCs), and differential leukocytic counts were determined by a hematological analyzer as previously described by **Feldman et al. (2000).**

3.5. Biochemical analysis:

3.5.1 Serum proteins

Serum total proteins were determined spectrophotometrically according to the method described by **Pagana and Pagana (2017).** Serum albumin was determined calorimetrically by using the dye-binding technique with bromocresol green according to the method described by **Fischbach and Dunning (2009).** Serum globulin was determined by the differences between total protein and albumin according to **Chernecky and Berger (2008)** and A/G ratio was calculated by dividing the albumin value over globulin value according to **Fischbach and Dunning (2009).** Serum IgG was measured by nephelometry according to **Whicher et al ., (1982).**

3.5.2 Acute phase proteins and IL6

Serum CRP was analyzed using ELISA Kit (Eucardio laboratory, Inc., Encinitas, and CA.,USA), previously validated for use in dogs according to **Ibraimi et al., (2013).** SAA were measured with a commercially available ELISA kit according to method described by **Alsemgeest et al. (1995).** Serum Hp concentrations were determined by ELISA kit according to method described by **Idoate et al. (2015).** IL-6 levels were determined from undiluted serum samples using commercially available ELISA Kits according to method described by **Kabu and Sayin(2016).** Serum cortisol concentration was determined using ELISA Kit(Eucardio laboratory, Inc., Encinitas , and CA.,USA).

3.5.3 Serum minerals and electrolytes

Serum chloride(Cl), sodium(Na) and potassium(K) levels was determined using spectrophotometer according to the method described by **Dacie and Lewis (1991).** Serum calcium and phosphorus were determined by spectrophotometer according to the method described by **Cheesbrough, (1991).** Serum magnesium (Mg) levels were determined by using atomic absorption spectrophotometer by as described by **Devlin (1997).**

3.6. Ultrasonographic examination:

Ultrasonographic examinations were performed without sedation or anesthesia while animal in standing position or lateral recumbency through using an intercostal approach, which includes placing the transducer on the chest wall between adjacent ribs and examining the thoracic tissues according to **Penninck and d'Anjou et al., (2015)**. Hair in the scanning area should be shaved, and isopropyl alcohol should be applied to improve image quality. The thorax of each dog was examined in a systemic manner using chison E3portable ultrasound with 5-8 MHZ micro convex transducer.

3.7. Histopathological examination:

Dogs were euthanized by injection of a high dose of anesthetic drug according to **Reilly.,(2001)**, lungs were removed and examined by visual examination. Autopsy samples were taken from the lung of infected dogs and fixed in 10% neutral buffered formalin for 24 hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl)were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin &eosin stain for routine examination through the light electric microscope according to **Banchroft et al (1996)**.

3.8. Statistical analysis:

All the statistical procedures were performed using SPSS V.25.0. The data was analyzed using the general linear model procedures (One way ANOVA with repeated measures) to compare the mean difference among the different time points followed by the tukey's multiple comparison post-hoc test to compare all pairs of means. The graphic presentation of the results was performed using graphpad prism V.9**.** Data of minerals and electrolytes were analyzed using the paired sample T-test to compare the two mean values of the before and after induction. A statistically significant difference between the before and after mean values was be considered at *p-value* < 0.05.

4. Results:

4.1. Clinical findings:

The pneumonic dogs showed wide range of clinical signs throughout the days of experiment including signs of systemic diseases(fever, anorexia, dullness, depression and congested mucous membrane). In addition, respiratory manifestations approved as dyspnea, painful cough, rapid breathing, nasal and conjunctival discharge**(Fig. 3-1 &3-2)**, tachycardia, painful palpation of chest during lung examination and abnormal lung sounds appeared from the 3rd day post induction in form of fine crackles, then heard as harsh exaggerated sound on the 4th day, respiratory wheezes were auscultated on the 5th and 6th day, while on the 7th day lung sound become weak and the heart sound become more obvious. The pneumonic dogs showed a significant increase (P<0.05) in respiratory rate **(Fig.3- 5)** appeared on the 2nd day post infection. Whereas the significant increase (P<0.05) in body temperature**(Fig.3- 4)**and pulse rate **(Fig.3- 3)** appeared on the 4th day and 5th day post infection respectively**(Table 3-1) .**

Table (3-1): physical examination of dogs on zero day and on the 1st, 2nd, 3rd,4th, 5th and 7th day post intra-tracheal instillation of bacterial broth.

	0 day	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	*P-value*
Pulse rate (beat\min)	104.75^{b} ± 2.29	112.3^{ab} ± 1.03	118.75^{ab} ± 4.27	127.27^{ab} ± 2.5	134^{ab} ± 3.5	137.25^{a} ± 3.4	136.25^{a} ± 2.4	136.25^{a} ± 1.7	**0.001****
Temp. ^{0}C	38.8^{b} ± 0.23	39.2^{ab} ± 0.21	39.5^{ab} ± 0.15	39.8^{ab} ± 0.14	40.4^{a} ± 0.16	40.6^{a} ± 0.06	40.4^{a} ± 0.12	40.4^{a} ± 0.13	**0.001****
Respiratory rate (breath\min)	45.5^{d} ± 3.4	56^{cd} ± 5.9	64.3^{bc} ± 1.3	76.3^{ab} ± 2.17	83^{a} ± 1.8	84.8^{a} ± 1.5	85.8^{a} ± 1.3	83.5^{a} ± 0.87	**0.000*****

Results are presented as (mean ± S.E.). The mean values of different superscript in the same raw are significantly different at (P-value < 0.05).

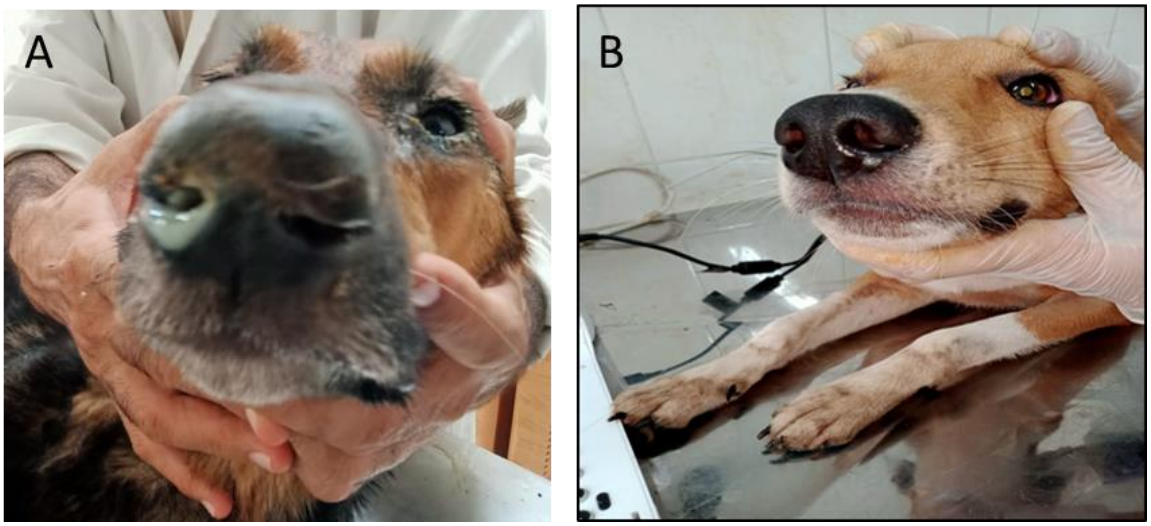
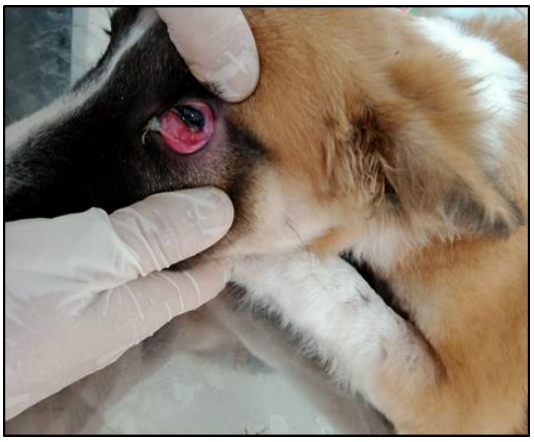


Figure (3-1): Pneumonic dogs showing mucopurulent nasal discharge(A) and signs of dyspnea (B).



Figure(3-2): Pneumonic dog showing congested mucous membrane.

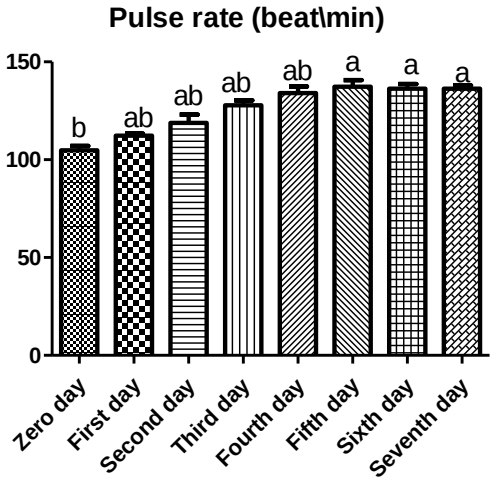


Figure (3-3) : changes in pulse rate of dogs at zero day before induction and during 1^{st},2^{nd} 3^{rd} ,4^{th} , 5^{th} and 7^{th}

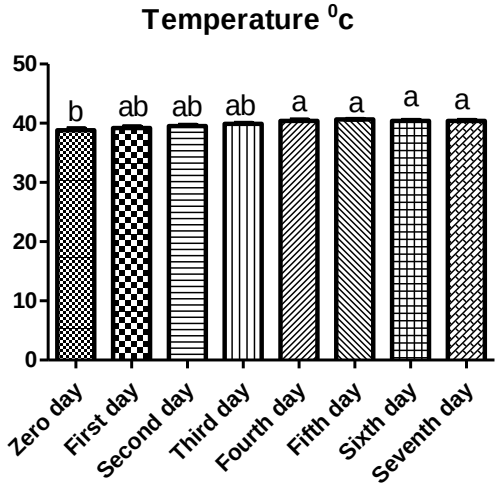
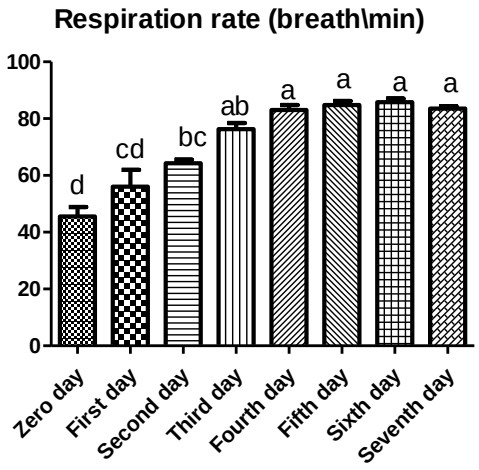


Figure (3-4): Changes in body temp. of dogs at zero day before induction and during 1st ,2nd,3rd ,4th , 5th and 7th day after induction.



Figure(3-5): changes in respiratory rate of dogs at zero day before induction and during 1st ,2nd,3rd ,4th , 5th and 7th day after induction.

4.2. Hematological findings:

The results of CBC analysis are represented in **Table(3-2)**. The pneumonic dogs showed a significant increase (P<0.05) in WBCs count on the 5th day post intra-tracheal instillation, **(Fig.3- 6)**. Neutrophils % was slightly increased on the 1st and 3rd day then significantly increased (P<0.05) on the 5th day post intra-tracheal instillation**(Fig.3-7).** RBCs count was slightly decreased on the 1st and 3rd day then significantly decreased (P<0.05) on the 5th day post intra-tracheal instillation**(Fig.3-8)**. There was a significant decrease in Hb content and PCV% (P<0.05) from the 1st day post intra-tracheal instillation **(Fig.3-9 &Fig.3-10)**.There was a significant decrease in lymphocytes (P<0.05) on the 1st day then decrease gradually until significantly decrease on the 5th day post intra-tracheal instillation **(Fig.3-11)**. There was no significant change(P>0.05) in monocytes % and eosinophils % **(Fig.3-12&Fig.3-13)**.

Table (3-2) : Hematological parameters in dogs on zero day and on the 1st ,3rd and 5th day post intra-tracheal instillation of bacterial broth.

	Zero day	First day	Third day	Fifth day	*P-value*
RBCS (million\mm^3)	**5.91^a ± 0.23**	**5.63^{ab} ± 0.18**	**5.64^{ab} ± 0.28**	**5.12^b ± 0.12**	**0.02***
Hb (g\dl)	**11.58^a ± 0.43**	**10.45^b ± 0.48**	**9.98^b ± 0.53**	**9.65^b± 0.48**	**0.001****
PCV (%)	**37.83^a ± 1.55**	**33.65^b ± 1.13**	**31.63^b ± 1.98**	**31.9^b ± 1.57**	**0.003****
WBCS (Thousand\mm^3)	**10.45^b ± 2.01**	**14.55^b ± 2.28**	**16^b ± 2.46**	**23.18^a ± 1.31**	**0.001****
Neutrophils (%)	**61.1^b ± 1.95**	**68.38^{ab} ±3.25**	**70.38^{ab} ± 4.28**	**75.9^a ± 3.5**	**0.03***
Lymphocytes(%)	**29.55^a ± 2.08**	**22.65^b ± 2.54**	**20.78^{bc} ± 4.48**	**15.18^c ± 3.81**	**0.000*****
Eosinophils (%)	**0.58^a ± 0.13**	**0.5^a ± 0.11**	**0.53^a ± 0.21**	**0.63^a± 0.33**	**0.9^{NS}**
Monocytes (%)	**6.08^a ± 1.26**	**5.88^a ± 1.01**	**4.9^a ± 0.74**	**4.95^a ± 0.95**	**0.54^{NS}**

Results are presented as (mean ± S.E.). The mean values of different superscript in the same raw are significantly different at (*P-value* < 0.05).

NS: non-significant, *: Significant at $P < 0.05$, **: Significant at $P < 0.01$, ***: Significant at $P < 0.001$

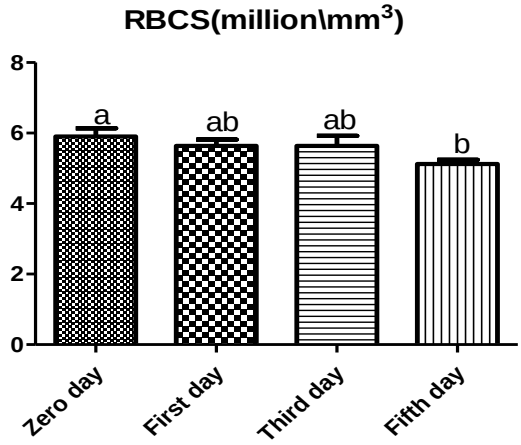


Figure (3-6): Changes in RBCs count of infected dogs at zero day before induction and during 1st ,3rd and 5th day after induction.

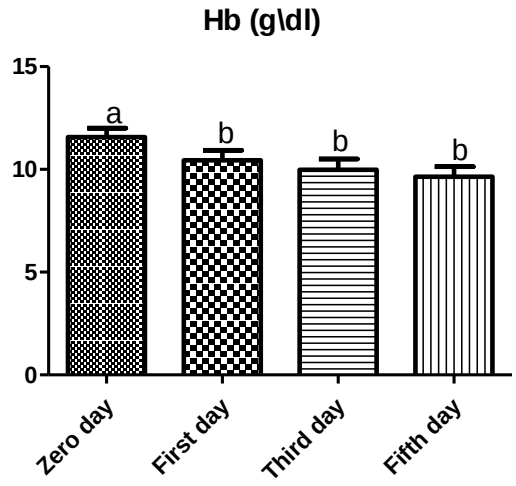
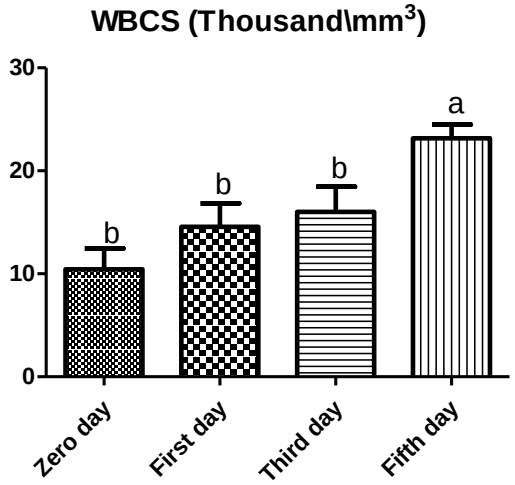
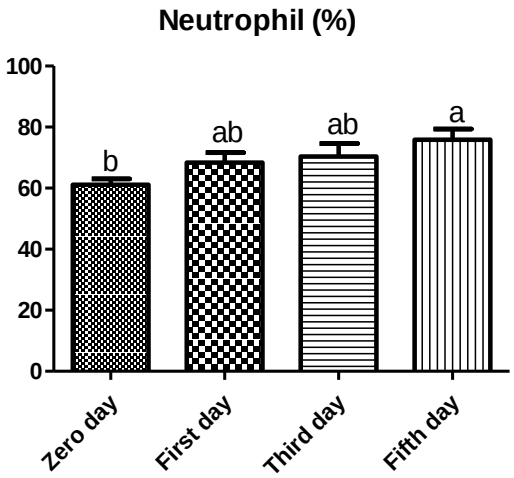


Figure (3-7): Changes in Hb value of infected dogs at zero day before induction and during 1st , 3rd and 5th day after induction.



Figure(3-6): Changes in WBCs count of infected dogs at zero day before induction and during 1st,3rd and 5th day after induction.



Figure(3-7): Changes in Neutrophils% of infected dogs at zero day before induction and during 1st,3rd and 5th day after induction.

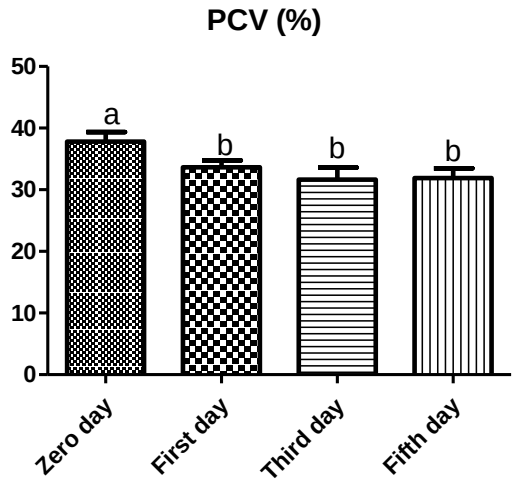
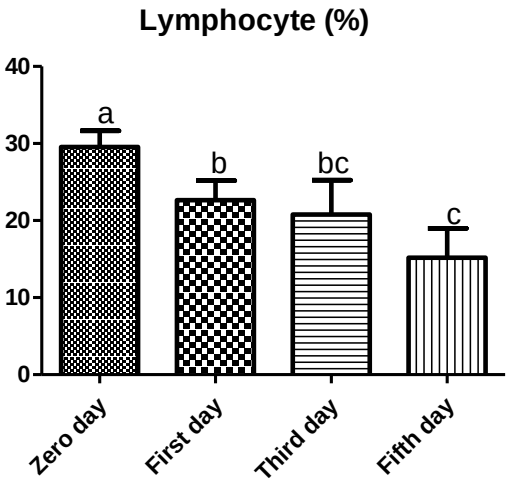
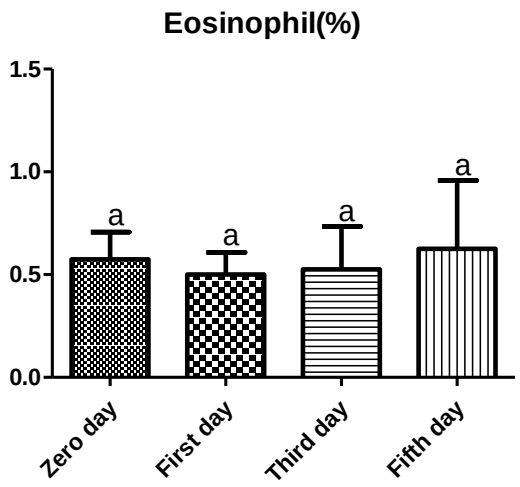


Figure (3-10): Changes in PCV% of infected dogs at zero day before induction and during 1st, 3rd and 5th day after induction.



Figure(3-11): Changes in lymphocytes% of infected dogs at zero day before induction and during 1st , 3rd and 5th day after induction.



Figure(3-12): Changes in Eosinophils % of infected dogs at zero day before induction and during 1st ,3rd and 5th day after induction.

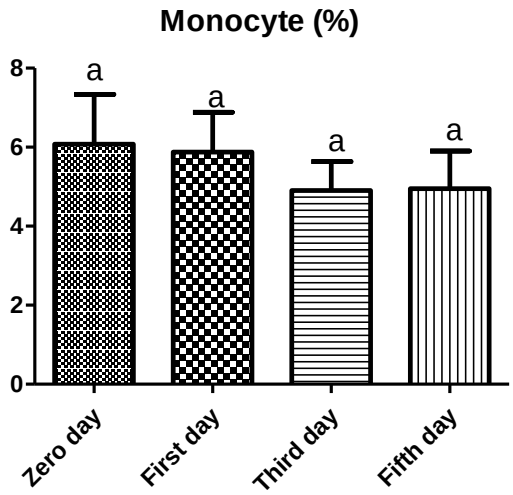


Figure (3-13): Changes in Monocytes % of infected dogs at zero day before induction and during 1st ,3rd and 5th day after induction.

4.3.Biochemical parameters :

The biochemical changes in SAA, CRP, HP, IL6, total protein, albumin, globulin, cortisol and IgG are represented in **Table (3-3).**

4.3.1.Changes in acute phase proteins and pro-inflammatory cytokine:

The pneumonic dogs showed a slight increase in SAA on the 1st day, significantly increase ($P<0.05$) on the 3rd day then reach to its maximum value on the 5th day post intra-tracheal instillation**(Fig. 3-14).** There was a significant increase ($P<0.05$) in CRP and HP on the 1st day reach to its maximum value at 5th day post intra-tracheal instillation **(Fig. 3-15&3-16).**The pneumonic dogs showed a significant increase($P<0.05$) in IL6 on the 3rd day reach to its maximum value on the 5th day post intra-tracheal instillation **(Fig. 3-17)**. The increase in CRP level was more than 6 folds on the 1st day post intra-tracheal instillation compared to its level on zero day before intra-tracheal instillation, but the increase in HP level was more than 3 folds on the 1st day post intra-tracheal instillation compared to its level on zero day before intra-tracheal instillation.

4.3.2. Changes in serum proteins:

The pneumonic dogs showed a significant increase (P<0.05) in total protein on the 5th day **(Fig. 3-18)** and a slight decrease in albumin on the 1st day then significantly decrease (P<0.05) on the 3rd day, reach to its lowest value on the 5th day post intra-tracheal instillation **(Fig.3-19)**. There was a slight increase in globulin on the 1st day then significantly increase(P<0.05) on the 3rd day, reach to its maximum value on the 5th day post intra-tracheal instillation **(Fig. 3-20)**, and a significant decrease(P<0.05) in A/G ratio on the 1st day, reach to its lowest value on the 5th day post intra-tracheal instillation **(Fig.3- 21)**.

4.3.3 Changes in serum cortisol and immunoglobulin:

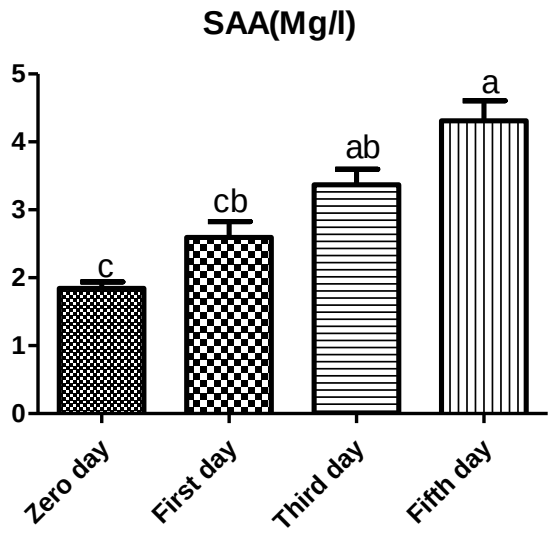
The pneumonic dogs showed a slight increase in cortisol level on the 1st day then gradually increase till reach to its maximum value on the 5th day (P<0.05) **(Fig.3- 22)** and there was a slight increase in IgG on the 1st day then significantly increase(P<.05) on the 3rd day post intra-tracheal instillation **(Fig. 3-23)**.

Table (3-3) : **Biochemical parameters in dogs on zero day and on the 1st ,3rd and 5th day post intra-tracheal instillation of bacterial broth**.

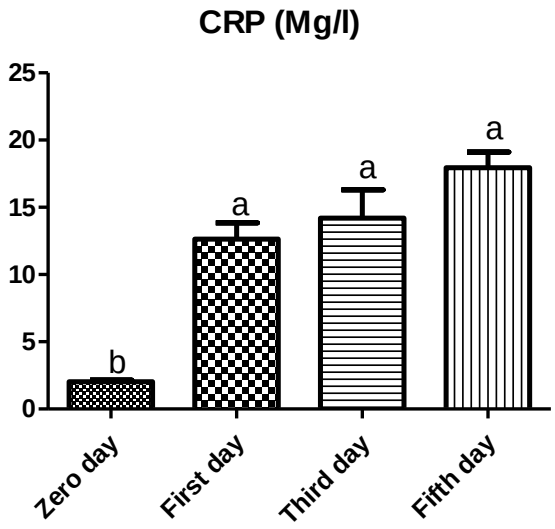
	Zero day	First day	Third day	Fifth day	*P-value*
SAA (mg/l)	**$1.85^{c} \pm 0.09$**	**$2.59^{bc} \pm 0.23$**	**$3.37^{ab} \pm 0.23$**	**$4.31^{a} \pm 0.29$**	**0.013***
CRP (mg/l)	**$2.02^{b} \pm 0.13$**	**$12.64^{a} \pm 1.21$**	**$14.21^{a} \pm 2.09$**	**$17.95^{a} \pm 1.16$**	**0.000*****
HP (mg/dl)	**$11.33^{c} \pm 0.62$**	**$33.74^{b} \pm 2.01$**	**$75.65^{a} \pm 4.7$**	**$83.98^{a} \pm 3.9$**	**0.002****
IL6 (Pg/ml)	**$0.38^{c} \pm 0.02$**	**$0.67^{bc} \pm 0.12$**	**$1.02^{b} \pm 0.11$**	**$1.5^{a} \pm 0.13$**	**0.000*****
Total protein (g/dl)	**$5.75^{b} \pm 0.09$**	**$6.18^{b} \pm 0.08$**	**$6.12^{b} \pm 0.01$**	**$7.2^{a} \pm 0.002$**	**0.001****
Albumin (g/dl)	**$3.29^{a} \pm 0.08$**	**$2.87^{ab} \pm 0.02$**	**$2.5^{bc} \pm 0.18$**	**$2.4^{c} \pm 0.04$**	**0.02***
Globulin (g/dl)	**$2.51^{c} \pm 0.18$**	**$3.29^{bc} \pm 0.06$**	**$3.61^{b} \pm 0.17$**	**$4.79^{a} \pm 0.33$**	**0.023***
A/G ratio	**$1.34^{a} \pm 0.14$**	**$0.87^{b} \pm 0.01$**	**$0.7^{b} \pm 0.09$**	**$0.55^{b} \pm 0.07$**	**0.03***

Cortisol (µg/dl)	**3.26^{b} ± 0.45**	**7.29^{ab} ± 0.33**	**9.36^{a} ± 0.81**	**10.31^{a} ± 0.9**	**0.01***
IgG (Mg/dl)	**448.5^{b} ± 7.9**	**485.5^{ab} ± 9.4**	**523.5^{a} ± 8.8**	**463.5^{ab} ± 5.9**	**0.007****

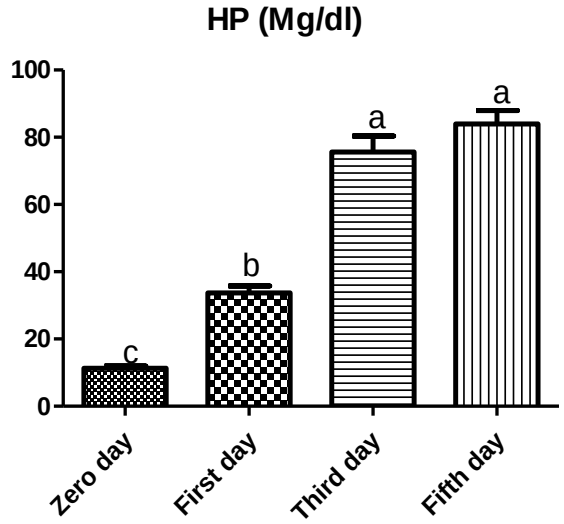
Results are presented as (mean ± S.E.). The mean values of different superscript in the same raw are significantly different at (*P-value* < 0.05).



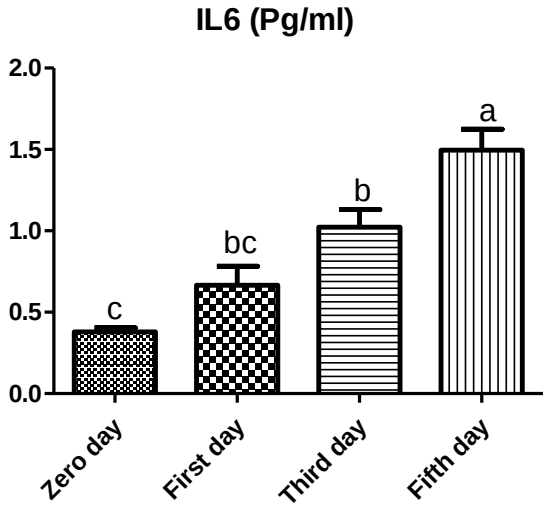
Figure(3-14): Changes in SAA value of infected dogs at zero day before induction and during 1st,3rd and 5th day after induction.



Figure(3-15): Changes in CRP value of infected dogs at zero day before induction and during1st,3rd and 5th day after induction.



Figure(3-16): Changes in HP value of infected dogs at zero day before induction and during1st,3rd and 5th day after induction.



Figure(3-17): Changes in IL6 value of infected dogs at zero day before induction and during 1st,3rd and 5th day after induction.

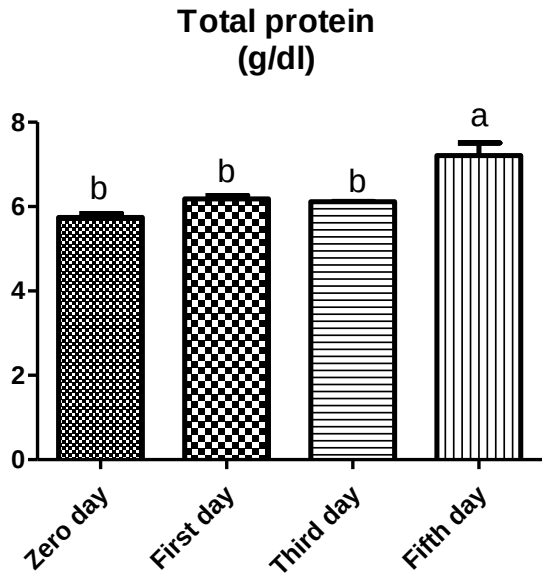


Figure (2-18): Changes in total protein value of infected dogs at zero day before induction and during 1st,3rd and 5th day after induction.

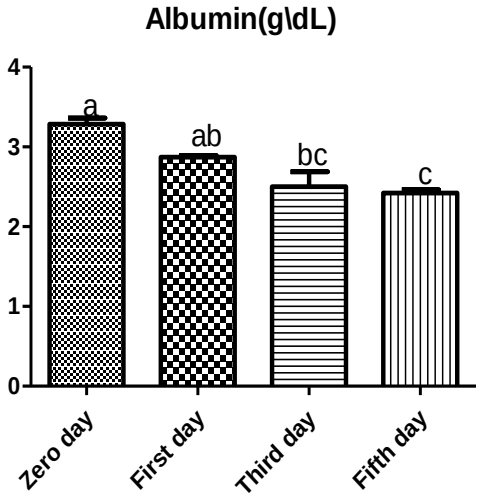
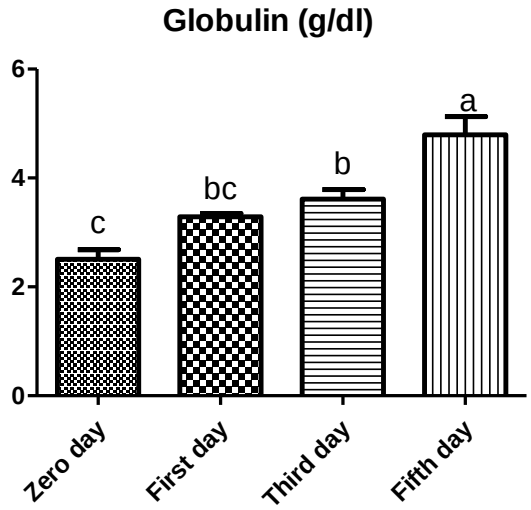
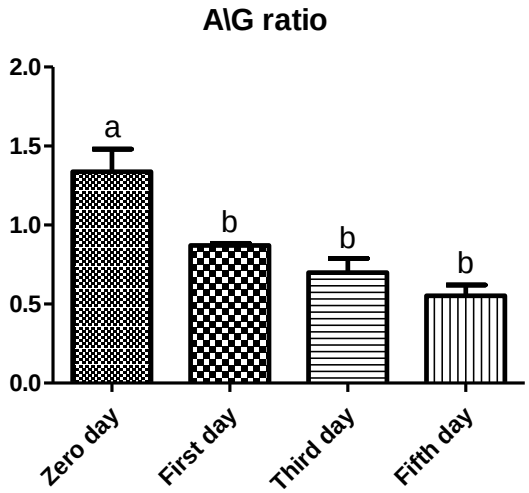


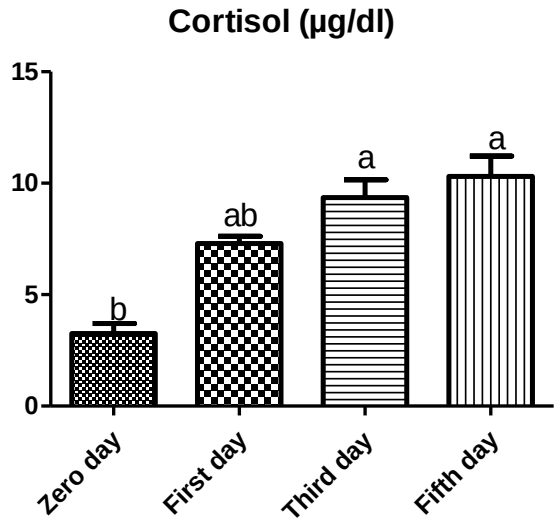
Figure (2-19): Changes in serum albumin value of infected dogs at zero day before induction and during 1st,3rd and 5th day after induction.



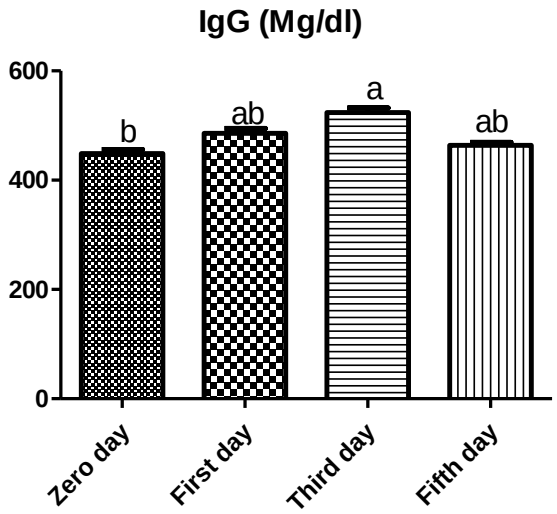
Figure(2-20): Changes in serum globulin value of infected dogs at zero day before induction and during 1st,3rd, and 5th day after induction.



Figure(2-21): Changes in A/G ratio of infected dogs at zero day before induction and during 1st,3rd, and 5th day after induction.



Figure(2-22): Changes in serum cortisol level of infected dogs at zero day before induction and during 1st,3rd and 5th day after induction.



Figure(2-23): Changes in serum IgG level of infected dogs at zero day before induction and during 1st,3rd and 5th day after induction.

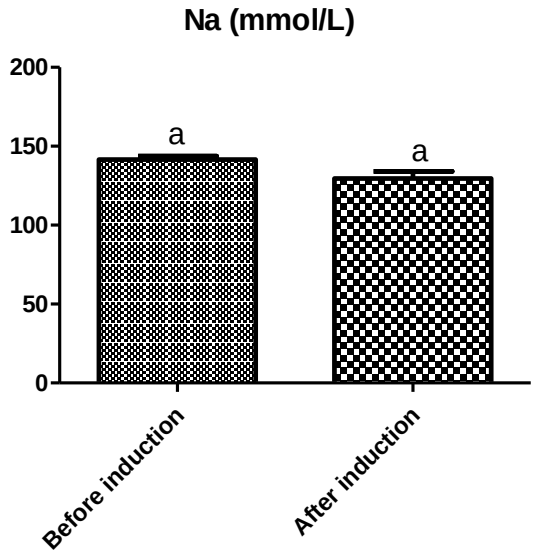
4.3.4 Changes in serum minerals and electrolytes:

The pneumonic dogs showed non-significant decrease (P<0.05) in Na**(Fig. 3-24)** and Ca ions **(Fig.3- 27)** and a significant decrease in K**(Fig. 3-25)**, Cl**(Fig. 3-26)** , P **(Fig. 3-28)** and Mg **(Fig. 3-29)** ions on the 5th day post induction **Table (3-4).**

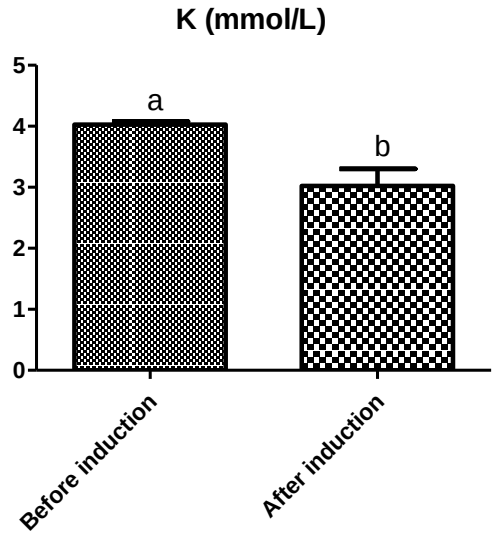
Table(3-4): **Serum electrolytes and minerals changes in dogs serum before and after induction.**

	Before induction	**After induction**	***P-value***
Na (mmol/L)	141.6[a] ± 2.2	129.6[a] ± 4.4	0.1[NS]
K (mmol/L)	4.03[a] ± 0.05	3.02[b] ± 0.28	0.04*
CL (mmol/L)	102.2[a] ± 3.1	60.3[b] ± 6.6	0.01*
Ca (Mg/dl)	9.56[a] ± 0.52	8.58[a] ± 0.5	0.4[NS]
P (Mg/dl)	5.17[a] ± 0.43	3.83[b] ± 0.44	0.02*
Mg (Mg/dl)	2.7[a] ± 0.1	1.42[b] ± 0.1	0.003**

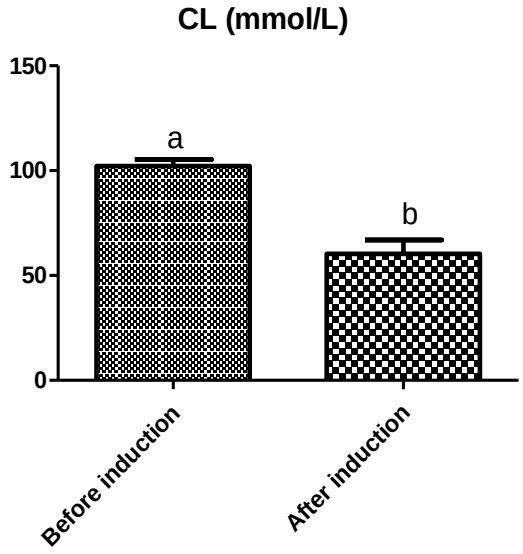
Results in the following table are presented as mean ± S.E. The mean values of different superscript in the same raw are significantly different at *P-value* < 0.05 .NS: non-significant, * : Significant at *P* < 0.05, ** : Significant at *P* < 0.01



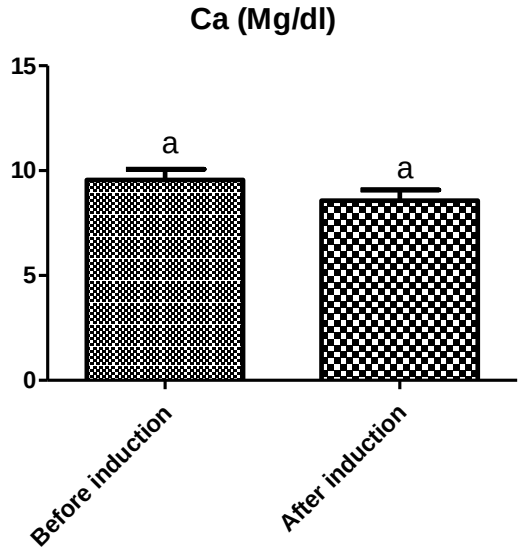
Figure(3-24): Na level before and after induction.



Figure(3-25): K level before and after induction.



Figure(3-26): Cl level before and after induction.



Figure(3-27): Ca level before and after induction.

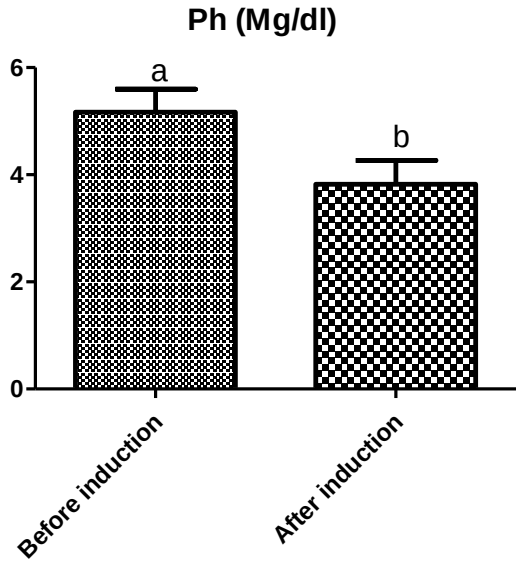


Figure (3-28): P level before and after induction.

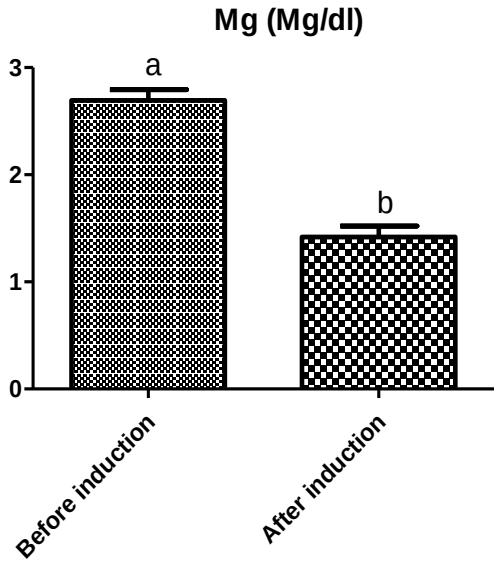
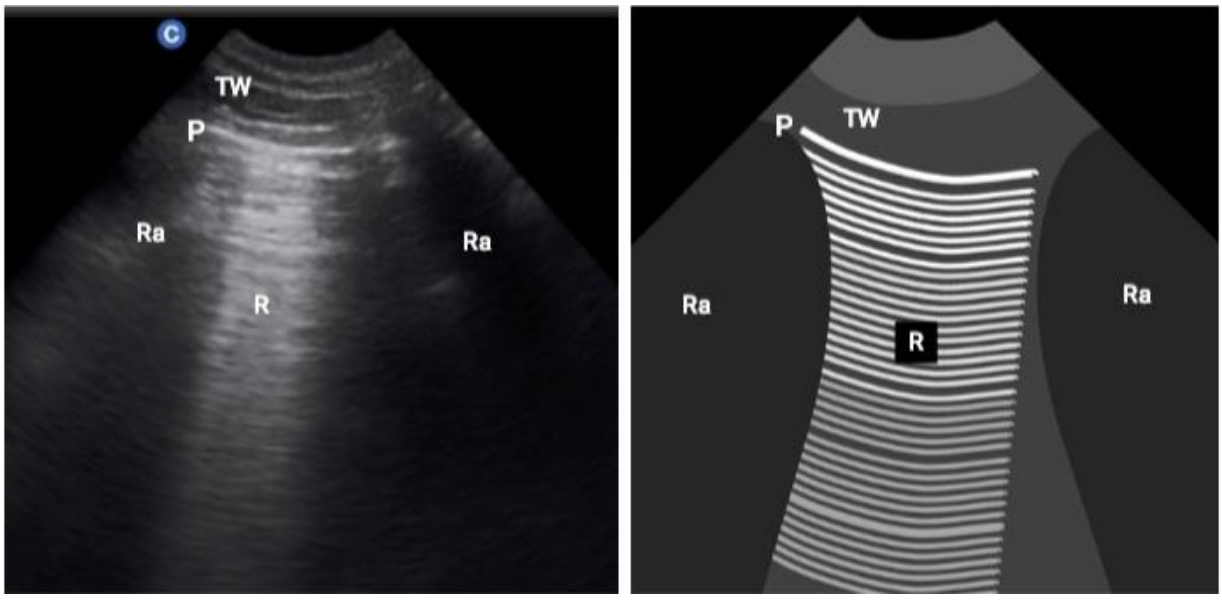


Figure (3-29): Mg level before and after induction.

4.4 Ultrasonographic findings:

Normal lung tissue appeared as reverberation artifacts in the form of echogenic bands that running parallel to the surface of the lung. Costal and pulmonary pleura were represented by a smooth hyperechoic line between the surface of the lungs and the musculature of the thoracic wall**(Fig. 3-30).** The pneumonic dogs lungs ultrasound on the 1st and the 2nd day post induction showed no changes, but the changes appeared on the 3rd day post induction .On the 3rd day the Pleural surface appeared as thick irregular hyperechoic band and there was focal consolidation of lung tissue represented by a heterogeneous hypoechoic area with hepatic like echogenicity**(Fig.3-31)**. On the 5thday the Pleural surface appeared as thick irregular hyperechoic band, there was diffuse consolidation of lung tissue represented by a heterogeneous hypoechoic area with hepatic like echogenicity and focal bronchopneumonia represented by small, round, ramified and tubular anechoic zones with partially echogenic wall represent transverse and sagittal sections of blood and fluid bronchograms **(Fig.3-32).** On the 7thday Lung surface appeared as thick hyperechoic band and displaced up to 1.5cm from the thickened pleural surface by the hypoechoic fluid representing pleural effusion, Cellular content of pleural effusion fluid appeared as echogenic clusters or granular appearance(white arrow) and there was diffuse bronchopneumonia represented by small, round, ramified and tubular anechoic zones with partially echogenic wall represent transverse and sagittal sections of blood and fluid bronchograms **(Fig. 3-33)**.



Figure(3-30): Ultrasonography of a dog chest with normal lung on zero day before induction : comet-tail reverberation artifacts (R) pulmonary pleura (P) the thoracic wall (TW) Rib artifact (Ra).

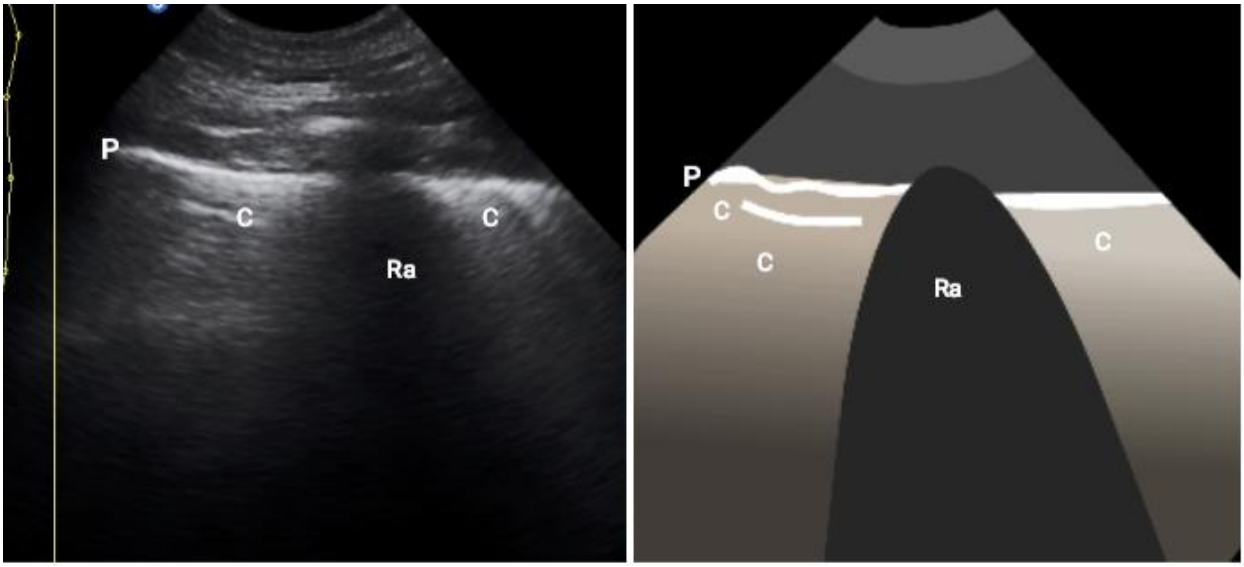


Figure (3-31): Ultrasonography of dog chest on the 3rd day post induction of bacterial pneumonia: Pleural surface(P) appeared as thick irregular hyperechoic band, focal consolidation of lung tissue(C) represented by a heterogeneous hypoechoic area with hepatic like echogenicity.

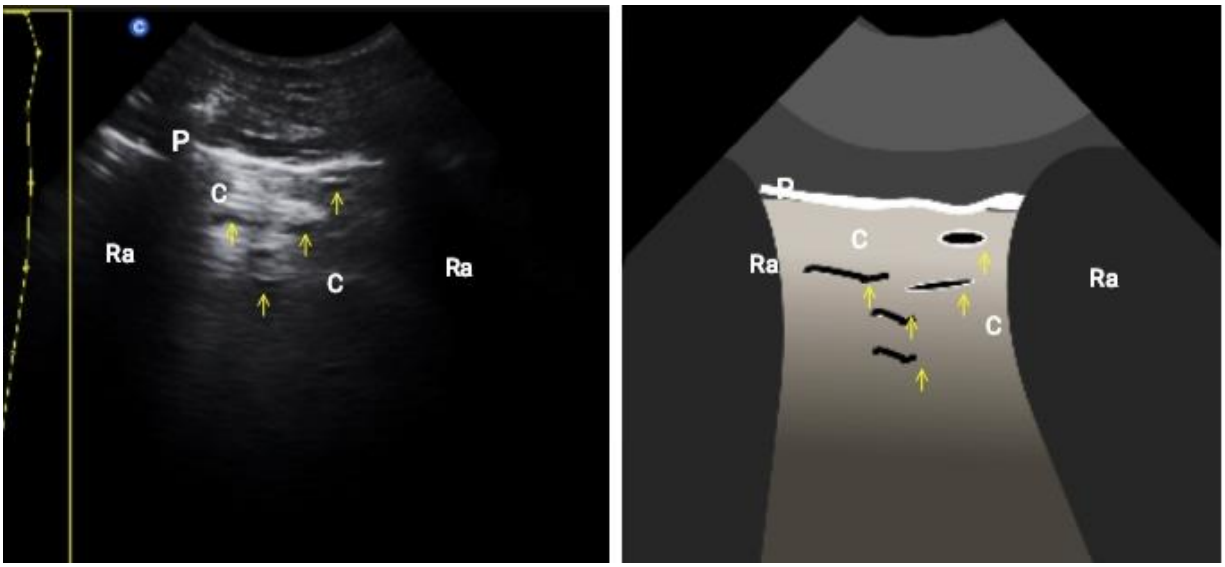


Figure (3-32): Ultrasonography of dog chest on the 5th day post induction of bacterial pneumonia: Pleural surface (P) appeared as thick irregular hyperechoic band, diffuse consolidation of lung tissue (C) represented by a heterogeneous hypoechoic area with hepatic like echogenicity. Focal bronchopneumonia represented by small, round, ramified and tubular anechoic zones with partially echogenic wall represent transverse and sagittal sections of blood and fluid bronchograms (containing exudate) (yellow arrows).

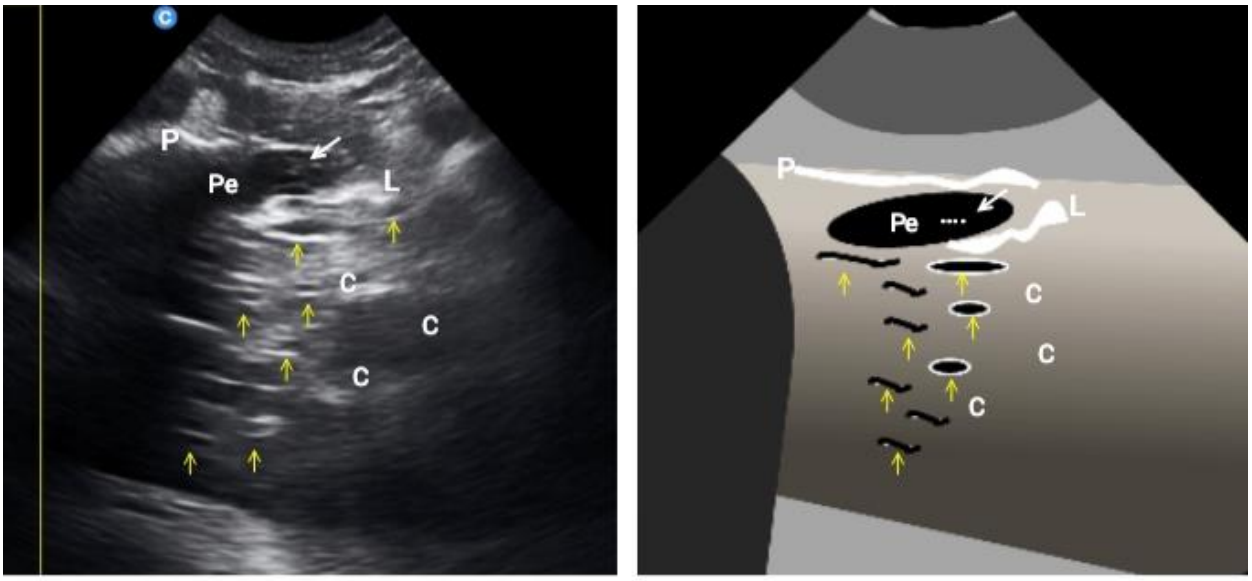


Figure (3-33): **<u>Ultrasonography of dog chest on the 7th day post induction of bacterial pneumonia</u>: Lung surface (L) appeared as thick hyperechoic band and displaced up to 1.5 cm from the thickened pleural surface (P) by the hypoechoic fluid representing pleural effusion (Pe); Cellular content of pleural effusion fluid appeared as echogenic clusters or granular appearance (white arrow). Diffuse bronchopneumonia represented by small, round, ramified and tubular anechoic zones with partially echogenic wall represent transverse and sagittal sections of blood and fluid bronchograms (containing exudate) (yellow arrows).**

4.5 Pathological findings:

4.5.1 PM examination

Dogs were euthanized and lungs were removed and examined by visual examination. The PM examination of affected lungs revealed pneumonia represented by pulmonary congestion and consolidation appeared as dark red to brownish areas. Also there was Pleurisy with abundant pleural fluid **(Fig. 3-34).**

4.5.2 Histopathological examination

Histopathological examination of the lung tissue of the pneumonic dogs showed Focal wide area of the collapsed air alveoli and inflammatory cells infiltration **(Fig.3-35)** thickening in

the pleura by inflammatory cells infiltration, hemosiderosis and inflammatory edema with underlying collapsed and emphysematous air alveoli **(Fig.3-36&3-37)**.There was congestion in the interalveolar blood vessels associated with inflammatory cells infiltration in between the alveoli **(Fig.3-38&3-39)**. Acute catarrhal bronchopneumonia was detected ,represented by mild desquamation of their lining epithelium with presence of faint bluish mucous in the bronchial lumen with few mononuclear leukocytic infiltration , more over the epithelial cell of pulmonary bronchiole showing sever degree of vacculation and contain various amount of blood **(Fig.3-40).**

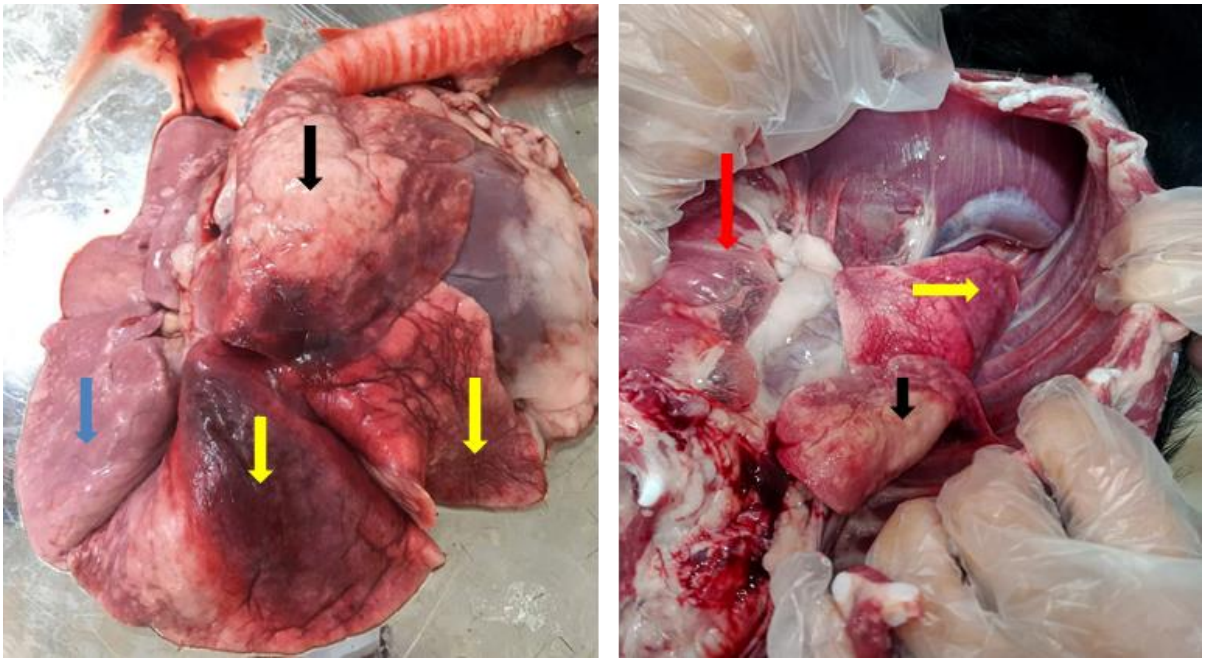
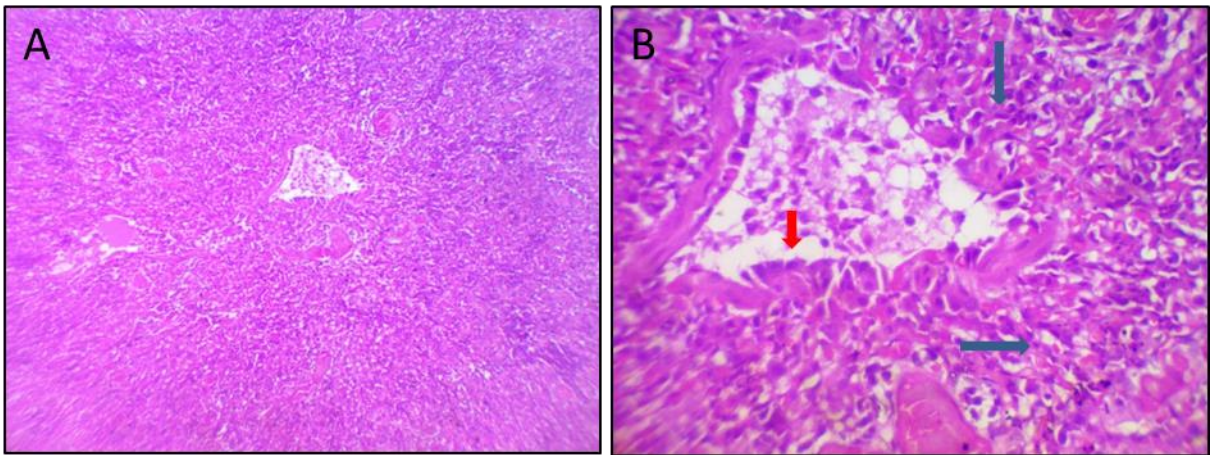


Figure (3-34): PM lesion of pneumonic dogs showing dark red to brownish areas of congestion (yellow arrows) and consolidation(blue arrow) of lung lobes, areas of emphysema (black arrows), and thickening of pleura (red arrow).



Figure(3-35): The lung tissue in examined cases showing sever degree of bronchopneumonia(A)in which the small sized bronchioles showing degenerative changes in their lining epithelium (red arrow) with heavy leukocytic cellular infiltration (blue arrow) in the alveoli (H&E,X100 , 400).

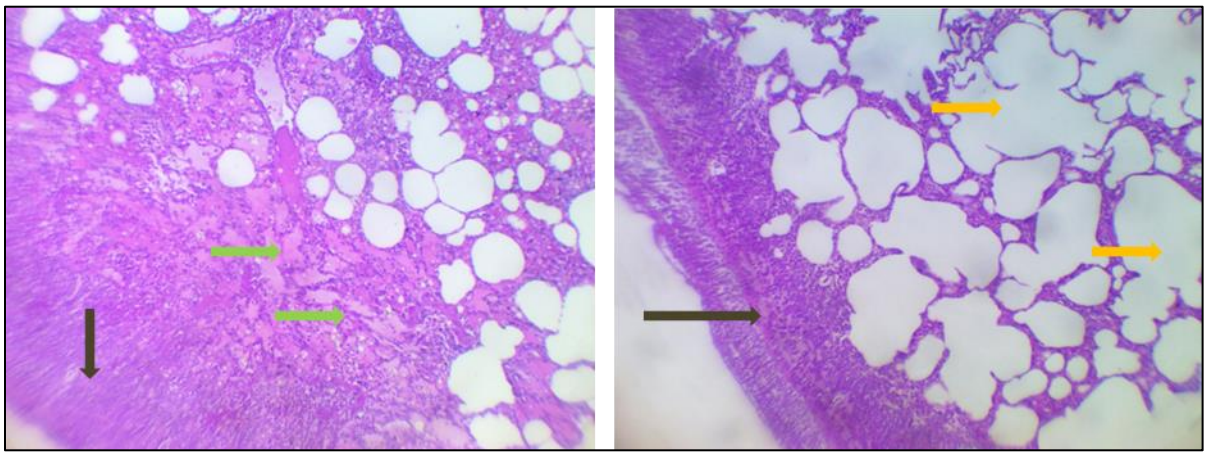
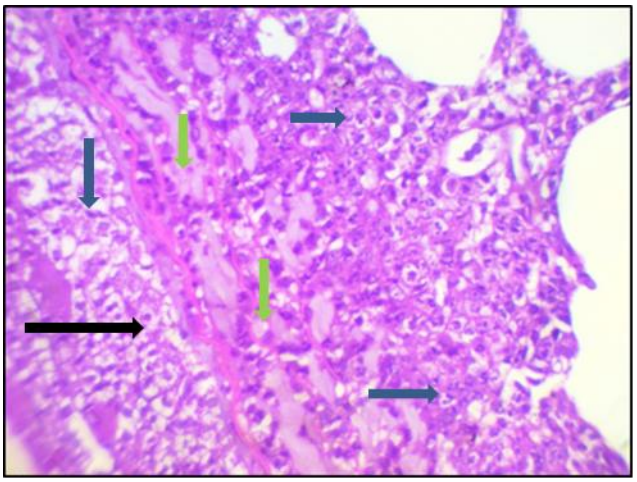


Figure (3-36): Lung tissue showing sever thickening of the pleura with mononuclear leukocytic infiltration (black arrow) the under lining pulmonary tissue showing emphysema in which the pulmonary alveoli were ruptured and form a wide space with swollen rounded ends (yellow arrow), mild pulmonary edema was also detected in which the inflammatory fluid appear as faint eosinophilic coloration infiltrated with few inflammatory cells (green arrow) (H&E,X100).



Figure(3-37): Lung tissue showing sever thickening of the pleura (black arrow) with mononuclear leukocytic infiltration (blue arrow), mild pulmonary edema (green arrow) was also detected in which the inflammatory fluid appear as faint eosinophilic coloration infiltrated with few inflammatory cells (blue arrow) (H&E,X400).

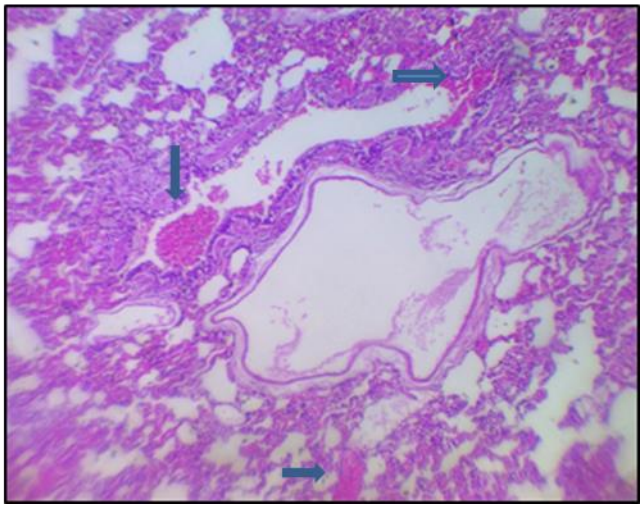


Figure (3-38): Pulmonary tissue in showing active hyperemia represented by sever congestion and dilatation of both pulmonary blood vessels and interalveolar blood capillary (blue arrows) (H&E,X100).

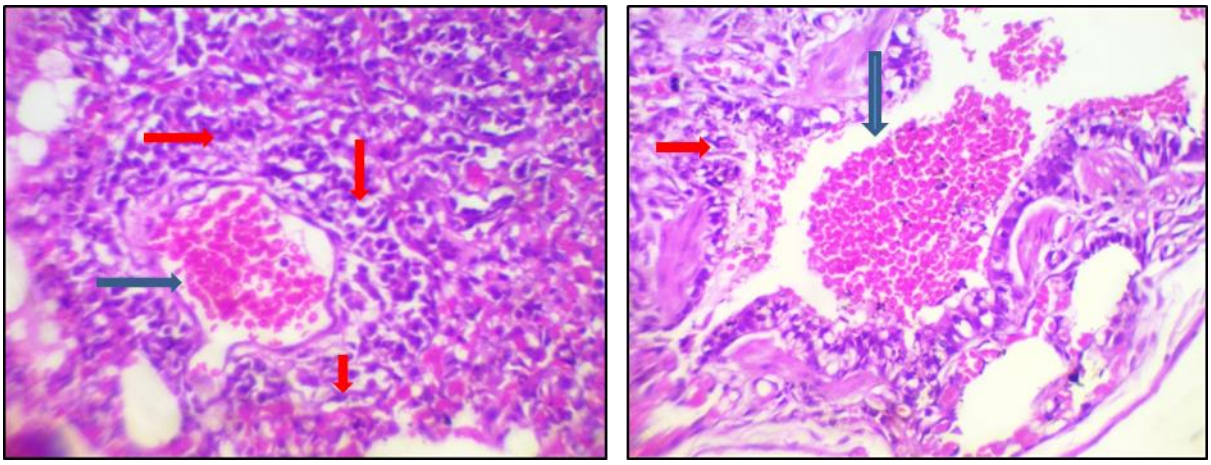


Figure (2-39): Most of the pulmonary blood vessels in the examined lungs showing congestion ,dilatation and filled with blood (blue arrow) in addition to perivascular mononuclear leukocytic infiltration (red arrows) (H&E,X400).

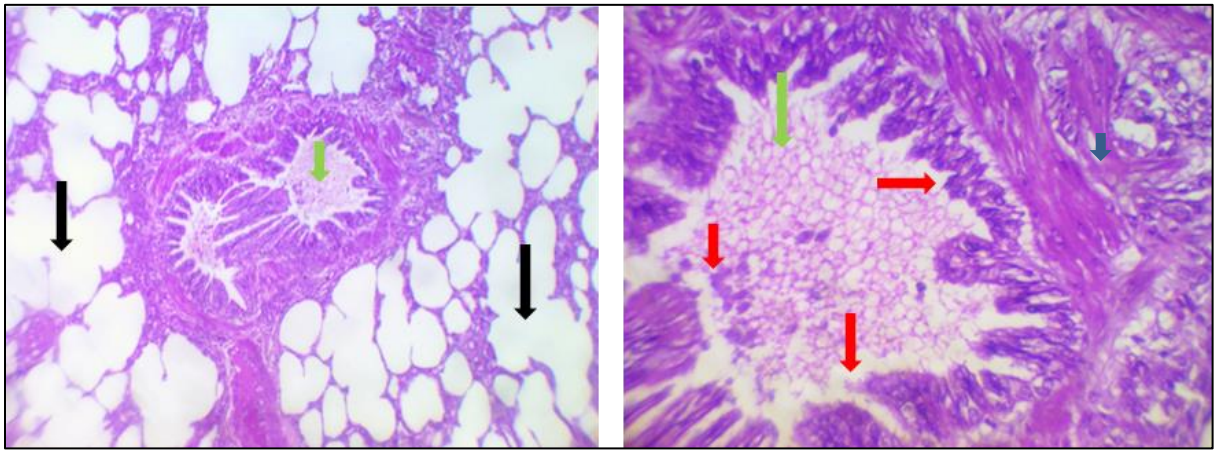


Figure (3-40): Showing acute catarrhal bronchopneumonia with pulmonary emphysema (black arrow) also detected, the bronchioles showing mild desquamation of their lining epithelium with presence of faint bluish mucous in the bronchial lumen with few mononuclear leukocytic infiltration (red arrows), more over the epithelial cell of pulmonary bronchiole showing sever degree of vacculation and contain various amount of blood (green arrow) (H&E,X100 , X400).

5.Discussion:

Dogs suffered from bacterial pneumonia showed variable clinical signs including general signs such as fever, anorexia, congested mucous membrane, ocular discharge, depression. In addition to respiratory manifestations approved as cough, nasal discharge, rapid respiration, dyspnea and lung sound abnormalities including harsh loud pulmonary sound and wheezes. The pneumonic dogs showed significant increase in pulse rate, respiratory rate and body temperature, similar findings were previously recorded by **Brady et al.,(2000); Radhakrishnan et al.,(2007); Ali,(2011); Dear.,(2014) and Viitanen (2017).** Fever occurred due to a substance called lipopolysaccharide present in the cell wall of some bacteria, it is an example of an exogenous pyrogen which causes a release of prostaglandin E2 (PGE2). PGE2 stimulates hypothalamus that stimulate the thermoregulatory center which generates a systemic reaction through increasing rate of heat production and decreasing rate of heat loss causing increase in the body temperature. The high body temperature accompanied with increasing in pulse and respiratory rate, also respiratory and heart rate increase to compensate hypoxia **(Brady et al.,2000)**. Cough, tachypnea, respiratory distress, fever, and abnormal auscultation are not always present in all dogs with BP, and the absence of a single feature, such as fever or cough, does not rule out the diagnosis of BP according to **Radhakrishnan et al., (2007) ; Kogan et al., (2008); Dear,(2014) and Viitanen (2017)**.

The pneumonic dogs showed a significant decrease in RBCs count, Hb content and PCV%. Similar result was described by **Kogan et al ., (2008); Viitanen (2017) and Dear., (2020)**. The decrease in these parameters was attributed to, respiratory affections especially pneumonia. The phagocytic cells increase in case of inflammation and make trapping to large amount of iron that required for RBCs synthesis in bone marrow, resulting in a decrease in Hb production and the development of anemia **(Ismael et al., 2017).** These anemia could be caused by a reduction in calorie and protein intake, or by the sequestration of iron in bone marrow macrophages and hepatocytes during infection, rendering it unavailable for use in haemoglobin production resulting in erythropoiesis suppression (**El-Naser and Khamis., 2009)and (El-Sharkawy., 2018).** From our result, there was a significant increase in WBCs count and neutrophils in the pneumonic dogs. These results agreed with **Kogan et al ., (2008); Ali.,(2011); Viitanen (2017) and Dear., (2020).** The increase of WBCs, mainly neutrophils is a frequent

finding in many diseases because of acute inflammatory response due to presence of bacterial infection**(Kogan., 2008).** On other hand, there was a significant decrease in lymphocytes .These results agreed with **Kogan et al ., (2008)**; **Ali .,(2011); Viitanen (2017) and Dear., (2020).** Lymphopenia could be attributed to high cortisol level that may leading to lymphocyte sequestration in the lymphoid tissue rather than its releasing into the blood stream to participate in the inflammatory reaction(**El-Naser and Khamis., 2009); and(El-Sharkawy., 2018).** The mean value of monocyte% and eosinophil% were not significantly changed during the experiment that agreed with **Brady et al .,(2000) and Ali .,(2011).**

serum biochemical analysis of the pneumonic dogs showed a significant increase in total protein and globulin and a significant decrease in albumin and A/G ratio. These results agreed with **Ceron et al. (2005) ;Kogan et al. (2008) and Hong et al.(2021).** The changes in protein profile during acute phase response were caused by increasing in synthesis of acute phase proteins, complement proteins, and immunoglobulins, so hyperproteinemia is usually associated with infection and inflammation **(Evans and Duncan, 2011) and (El-Sharkawy, 2018).** Serum albumin is the major negative acute phase protein **(Kumar et al., 2018)**. It has been observed that 30–40% of hepatic protein anabolic capacity is utilised for the creation of positive acute phase proteins during the acute phase response, resulting in a reduction in the production of other proteins such as albumin, causing hypoalbuminemia **(Tóthová et al., 2013); (Šoltésová et al., 2015).** Hypoalbuminemia could be suggestive of lung inflammation and vasculitis that resulted in leakage of albumin into the alveolar space**(Kogan et al., 2008).** The significant decrease in A/G values occurs mainly due to the increased immunoglobulin synthesis following antigenic stimulation and decrease of albumin production **(Evans and Duncan., 2011).**

The acute phase response is a nonspecific inflammatory response of the host that develops quickly after tissue damage or inflammation. Changes in plasma proteins known as acute phase proteins (APPs) include some that decrease in concentration (negative APPs), such as albumin, and others that rise in concentration (positive APPs), such as CRP, SAA, and HP. The majority of positive APPs are glycoproteins produced mostly by hepatocytes in response to pro-inflammatory cytokines like IL6 that released from leukocytes at the site of inflammation into the bloodstream **(Ceron et al .,2005) and(jain et al., 2011).** Serum biochemical analysis of the pneumonic dogs showed a significant increase in CRP ,SAA , HP and IL6 throughout 24

hours from induction, these results agreed with **Ceron et al**.,**(2005)**; **Quinton et al . (2009)** ; **jain et al.,(2011)and Viitanen (2017)**. APPs are responsible for protecting the organism from additional injury, eliminating infectious agents, removing toxic compounds and residues, and initiating the repair process required for the organism to return to normal function and restore homeostasis **(jain et al., 2011).** Both hepatocytes and peripheral tissues produce APPs **(Ceciliani et al., 2012).**

The major positive APPs, such as CRP and SAA in dogs, have low physiological levels, but rise rapidly within hours after inflammatory stimulus and normalize quickly when inflammatory stimulus ceases **(Eckersall and Bell, 2010).** Due to these properties, the major positive APPs have received the most attention as inflammatory biomarkers than the intermediate positive APPs, such as Hp. **(Ceron et al., 2005).** serum CRP has been found to be very useful in aiding the diagnosis in humans with CAP, and the measurement of CRP is currently recommended by human guidelines **(Woodhead et al., 2005) and (Lim et al., 2009)**. Our aim was to assess whether CRP could also be applied as a diagnostic biomarker in dogs with BP. Results showed that dogs with BP had significantly higher serum CRP concentrations on the 1st day post intra-tracheal instillation, its concentration was 6 folds more than zero day. The significant increase of SAA could be attributed to role of SAA in host immunity. It binds with Gram-negative bacteria and allows their destruction by phagocytic cells **(Orro et al., 2011)**. The pro-inflammatory cytokines such as IL6 are proteins that secreted by inflammatory cells at the site of inflammation. These cytokines play an important role in elimination of the infection through stimulation of the phagocytosis and the production of APPs from hepatocyte**(Ditchkoff et al., 2001)**;**(Ridpath, 2010)and (jain et al., 2011).** That agree with the result of this study which showed a significant increase in IL6 level in the serum of pneumonic dogs on the 1st day post induction.

The pneumonic dogs showed a slight increase in IgG throughout the five days of examination that agreed with **Nordbring et al ., (1969),** that may attributed to stimulation of immunity system by antigenic reaction of bacteria to create immunoglobulin, but IgG concentration reach to its maximum concentration after 3 to 4 weeks of infection**(de la Torre et al .,2016) and (Nordbring et al ., 1969).**

Cortisol is the main hormone released in case of stress to restore homeostasis and physiological conditions **(Fujiwara et al ., 1996).** The pneumonic dogs showed a significant increase in serum cortisol level, that agreed with **Burkitt et al ., (2007); Gotoh et al ., 2008 and Cortés-Puch et al ., (2014)** , that may attributed to stress resulted from clinical and biochemical disturbance in the pneumonic dogs

The serum minerals and electrolyte levels revealed decrease of Ca, Na and a significant decrease in P, K, Cl and Mg level. This result is in agreement with **Sankaran et al ., (1997); Kumar et al., (2018); Nasser et al., (2018)and Ravioli et al.,(2021).** Inappetance and anorexia might be explaining the lower plasma concentrations of estimated minerals**(Kumar et al., 2018)**. The decrease in serum calcium might be due to high cortisol level that act to depress intestinal Ca absorption and increase urinary Ca excretion **(Compston., 2018).** About 40-45% of calcium is protein bound mainly to albumin, so hypoalbuminemia might be a possible cause for this hypocalcaemia **(De Witte et al., 2021).** Hypokalemia resulted from high cortisol level that act to depress intestinal K absorption and increase urinary K excretion **Fan et al.,(2020).** The decrease in Na and Cl level may be go back to that respiratory disorders have adverse effect on electrolytes, body fluids and acid base balance. Hyponatremia may results from false hyperglycemia caused by stressful effect of respiratory diseases, which may leads to gluconeogenesis **Ravioli et al .,(2021).**

Normal lung tissue could not be shown due to its air content but reverberation artifacts in the form of echogenic bands running parallel to the surface of the lungs were visible. Costal and pulmonary pleura could not always be distinguished and were represented by smooth hyperechoic line between the surface of the lung and the musculature of the thoracic wall, similar findings were previously recorded by **Jung and Bostedt .,(2004) ; Mannion .,(2008); Larson .,(2009); Lisciandro et al .,(2014); Penninck and d'Anjou., (2015) and Soldati et al ., (2019).** The pneumonic dogs lungs showed consolidation and pleural effusion, the same result was previously recorded by **Copetti et al .,(2008).** If the alveolar air is exchanged by fluid, the lung tissue appears hypoechoic**(Scott, 2013).** The irregularity of the visceral pleural surface of the lung due to unbalanced air content of the lung periphery can be a first sign of consolidation. Small accumulations of exudate, blood, mucus, edema fluid, or tumor cells, as well as scarring from a past bout of pneumonia or pleuritis, cause comet-tail artefacts to radiate from these non-

aerated locations**(Rabeling et al.,1998 and Tharwat and Oikawa., 2011)** and also they have reported that consolidations appeared as echogenic regions with comet-tail artifacts, Comet-tail artifacts and consolidated areas of mild severity represented mild to moderate abnormalities.

Ultrasonographic diagnosis of pulmonary parenchymal consolidation was based upon the detection of hypoechoic pulmonary parenchyma and bronchograms or vessels seen within it **(Zeineldin et al., 2016).** Consolidated lung areas have echogenicity similar to that of liver but differentiated from it by presence of irregular hyperechoic areas which correspond to small pockets of remaining gas .Pulmonary consolidation occurred due to infection and inflammation **(Mannion .,2008).** The affected lung surface appeared as thick hyperechoic band and displaced up to 1.5 cm from the thickened pleural surface by the hypoechoic fluid representing pleural effusion. That may attributed to Initial bacterial infection that caused local inflammatory reaction resulting in increased capillary microvascular permeability and a rapid escaping of fluid containing inflammatory cells into the pleural space resulting in pleural effusion**(McCauley and Dean .,2015).**

Histopathological examination of the lung tissue of the pneumonic dogs showed sever degree of bronchopneumonia in which the small sized bronchioles showing degenerative changes in their lining epithelium with heavy leukocytic cellular infiltration in the alveoli ,also showing sever thickening of the pleura with mononuclear leukocytic infiltration. The under lining pulmonary tissue showing emphysema in which the pulmonary alveoli were ruptured and form a wide space with swollen rounded ends. Mild pulmonary edema was also detected in which the inflammatory fluid appear as faint eosinophilic coloration infiltrated with few inflammatory cells. Most of the pulmonary blood vessels in the examined lungs showing congestion, dilatation and filled with blood in addition to perivascular mononuclear leukocytic infiltration. Acute catarrhal bronchopneumonia with pulmonary emphysema also detected, the bronchioles showing mild desquamation of their lining epithelium with presence of faint bluish mucous in the bronchial lumen with few mononuclear leukocytic infiltration, more over the epithelial cell of pulmonary bronchiole showing sever degree of vacculation and contain various amount of blood, similar findings were previously recorded by **Irifun ., (1987) and Mikerov et al ., (2011).** As demonstrated in histopathology of induced pneumonia, increasing the leukocytes

infiltration at the pulmonary tissue was due to inflammatory reaction that resulted from bacterial instillation.

6.Conclusion:

The results of this study concluded that, pneumonia in dogs associated with clinical, hemato-biochemical, ultrasonographic and histopathological changes that can aid in accurate diagnosis of the disease. The ultrasonography, acute phase proteins and pro-inflammatory cytokines could be used as valuable tools for early prediction and diagnosis of pneumonia in dogs.

Respiratory affections are one of the most common complicated affections in dogs and cats that causing high morbidity and mortality, also affecting animal health. Bacterial pneumonia is still one of the most prevalent clinical diagnosis in dogs with acute or chronic respiratory diseases. This study aimed to evaluate the role of acute phase proteins, pro-inflammatory cytokines and ultrasonography in the early diagnosis of bacterial pneumonia in dogs. Also aimed to assess the clinical features, hematological, acute phase proteins and radiographic changes in case of different respiratory affections in dogs and cats, in addition to isolation and identification of the most common bacterial agents that may be included.

In chapter 2: This study was applied on total of 84 animals including 32 dogs and 52cats. These cases suffered from different respiratory affections. The diagnostic evaluation included history, physical examination (84/84), hematology (25/84), serum biochemistry (25/84), thoracic radiographs (38/84), bacterial isolation and identification (24/84). The affected cases showed variable respiratory signs including dyspnea, nasal and ocular discharge, sneezing, cough, abnormal respiratory sound and abnormal lung sound. Hematological changes showed inflammatory leukogram represented by significant increasing in WBCs and neutrophil count. Serum analysis showed marked increase in CRP, SAA and HP levels, with hyperproteinemia and hypoalbuminemia compared to reference value. The most common bacteria isolated from pneumonic cases were Klebsiella, E.coli, Staph., Pseudomonas, Pasteurella, Proteus and Serratia. Radiographic examinations revealed abnormal radiographic patterns associated with the different affections.

In chapter 3: 10 healthy baladi dogs were infected by intra-tracheal instillation of 2ml of klebsiella broth. Clinical, hematological, biochemical and ultrasonographic examinations of the pneumonic dogs were performed on zero day before induction and on the 1^{st}, 3^{rd},5^{th} and 7^{th} day post induction. The affected dogs showed a significant increase in acute phase proteins and Il6 within 24 hours post induction. There was a significant increase in total protein and globulin and a significant decrease in albumin and A/G ratio. The infected lungs showed ultrasonographic changes on the 3^{rd} day post induction.

-Based up on the result of this work, it could be concluded that:

1. Clinical, hemato-biochemical, ultrasonographic and radiographic changes are diagnostic for different respiratory affections in dogs and cats

2. Measurement of acute phase proteins and Il6 can be used as early indicator of infection and inflammation.

-Recommendations:

1. Using of thoracic imaging (sonography and radiography) and acute phase proteins measurement should be applied as main regimen in diagnosis of different respiratory diseases in dogs and cats.

2. The effect of bacterial instillation in different location need further investigation.

English Summary

Respiratory affections are one of the most common affections in dogs and cats affecting animal health, causing high morbidity and mortality. This study is divided into 2 parts. Part 1 focused on bacterial pneumonia in dogs. Part 2 including field study on different respiratory affections in dogs and cats.

Part 1: Field study was carried out on a total of 84 animals including 32 dogs and 52 cats of both sexes and different breeds suffering from different respiratory affections including pneumonia, aspiration pneumonia, Feline upper respiratory diseases and Canine infectious respiratory disease. Clinical, hematological, biochemical and radiographic changes during these affections were evaluated. In addition the most common incriminated bacteria in case of pneumonia was isolated and identified.

1. **Clinical findings:**

   The affected cases showed variable respiratory signs including dyspnea, nasal and ocular discharge, sneezing, cough, abnormal respiratory sound and abnormal lung sound.

2. **Hematological examination:**

   The mean value of RBCs count, Hb content and PCV% were within normal reference value in cases affected with aspiration pneumonia, CIRD and FURI but lower than reference value in case of dogs and cats affected with pneumonia. The mean value of WBCs count and neutrophils were higher than normal reference value in all affections. The mean value of lymphocyte was lower than normal reference value in case of pneumonia, FURI and CIRD but within normal level in case of aspiration pneumonia.

3. **Serum Proteins analysis**:

   The mean value of SAA and HP were higher than normal reference value in case of pneumonia and FURD but within the normal level in case of CIRD. The mean value of CRP was higher than normal reference value in all cases especially pneumonic cases. The affected cases showed changes in total protein, albumin, globulin and A/G ratio.

4. **Radiographic examination:**

   The radiographic views of cases suffering from pneumonia and aspiration pneumonia revealed a predominantly interstitial pattern and alveolar infiltrate, some cases showing lung consolidation. The radiographic views of some cases suffering from kennel cough revealed pneumonia represented by bronchial pattern mainly, but some cases showed mixed pattern.

5. **Bacteriological isolation:**

   The bacteriological examination of the cultured swab collected from the pneumonic animals revealed that, the most common isolated pathogens were Klebsiella, E.Coli, Pasteurella, Pseudomonas, Staphylococcus spp. and other types of bacteria.

Part 2: Ten healthy baladi dogs were anesthetized then infected by intra-tracheal instillation of 2ml of klebsiella pneumoniae broth. Clinical, hematological, biochemical and ultrasonographic examinations of the pneumonic dogs were performed on zero day before induction and on the 1^{st}, 3^{rd},5^{th} and 7^{th} day post induction.

1. **Clinical findings:**

   The pneumonic dogs showed variable clinical signs including respiratory signs in addition to signs of systemic infection. Also showed significant increase in temperature, pulse and respiratory rate.

2. **Hematological examination:**

   The pneumonic dogs showed significant decrease in RBCs count, Hb content and PCV%. Also showed significant increase in WBCs and Neutrophil count and significanr decrease in lymphocyte %.

3. **Biochemical analysis:**

   The pneumonic dogs showed significant increase in acute phase proteins and pro-inflammatory cytokines, significant increase in total protein and globulin and significant decrease an albumin and globulin ratio. Also showed increase in IgG and cortisol level, changes in serum minerals and electrolyte levels.

4. **Ultrasonographic examination:**

   ultrasonographic examination of the pneumonic dogs showed bronchopneumonia represented by thickening of the pleura, lung consolidation and collapse.

5. **Histopathological examination :**

   Macroscopic appearance of the affected lungs showed dark red to brownish areas of consolidation, pleurisy and areas of emphysema, while the microscopic pictures showed sever bronchopneumonia represented by sever thickening of the pleura, collapsed air alveoli with leukocytic cellular infiltration inside the alveoli and in the interstitial tissue, emphysema, inflammatory edema and congested blood vessels.

الملخص العربي

تعد الامراض التنفسية من أكثر العوامل المؤثرة شيوعًا في الحيوانات الأليفة والتي تؤثر على صحة الحيوان ، مما يؤدي إلى ارتفاع معدلات الإصابة بالأمراض والوفيات. هذه الدراسة مقسمة إلى جزئين . ركز الجزء الأول على الالتهاب الرئوي البكتيري في الكلاب. يتضمن الجزء الثاني دراسة حقلية حول مختلف أنواع أمراض الجهاز التنفسي لدى الحيوانات الأليفة.

البحث الاول :

أجريت الدراسة الحقلية على 84 حيوانا منها 32 كلبًا و 52 قطًا من كلا الجنسين وسلالات مختلفة تعاني من أمراض تنفسية مختلفة (التهاب رئوي ، التهاب رئوي شفطي ، أمراض الجهاز التنفسي العلوي لدى القطط ، أمراض الجهاز التنفسي المعدي في الكلاب). تم تقييم التغيرات الاكلينيكية والدموية والكيميائية الحيوية والتصوير الشعاعي خلال هذه التأثيرات. بالإضافة إلى ذلك ، تم عزل وتحديد أكثر أنواع البكتيريا المسببة للإصابة .

1. **نتائج الفحص الإكلينيكي** :

ظهرت على الحالات المصابة علامات تنفسية متغيرة تشمل ضيق التنفس ، وإفرازات من الأنف والعين ، والعطس ، والسعال ، وصوت الجهاز التنفسي غير الطبيعي ، وصوت الرئة غير الطبيعي.

2. فحص صورة الدم:

كانت القيمة المتوسطة لعدد كرات الدم الحمراء ومحتوى Hb ونسبة PCV ضمن القيمة المرجعية الطبيعية في الحالات المصابة بالالتهاب الرئوي الشفطي و CIRD و FURI ولكنها أقل من القيمة المرجعية في حالة الكلاب والقطط المصابة بالالتهاب الرئوي. كانت القيمة المتوسطة لعدد كرات الدم البيضاء والعدلات أعلى من القيمة المرجعية العادية في جميع التأثيرات. كانت القيمة المتوسطة للخلايا الليمفاوية أقل من القيمة المرجعية الطبيعية في حالة الالتهاب الرئوي و FURI و CIRD ولكن ضمن المستوى الطبيعي في حالة الالتهاب الرئوي التنفسي.

3. **تحليل نسبة البروتينات:**

كانت القيمة المتوسطة لـ SAA و HP أعلى من القيمة المرجعية العادية في حالة الالتهاب الرئوي و FURD ولكن ضمن المستوى الطبيعي في حالة CIRD. كانت القيمة المتوسطة لـ CRP أعلى من القيمة المرجعية العادية في جميع الحالات خاصة حالات الالتهاب الرئوي. أظهرت الحالات المصابة تغيرات في نسبة البروتين الكلي ، الألبومين ، الجلوبيولين ونسبة A / G.

4. **الفحص الشعاعي:**

أظهرت الصور الشعاعية للحالات التي تعاني من الالتهاب الرئوي والالتهاب الرئوي الشفطي نمطًا خلاليًا في الغالب وارتشاحًا سنخيًا ، حيث أظهرت بعض الحالات تماسكًا في الرئة. أظهرت الصور الشعاعية لبعض الحالات التي تعاني من سعال بيت الكلب وجود التهاب رئوي يتمثل في نمط الشعب الهوائية بشكل رئيسي ، لكن بعض الحالات أظهرت نمطًا مختلطًا.

5. **العزل البكتريولوجي:**

أظهر الفحص البكتيريولوجي للمسحة التي تم جمعها من الحيوانات المصابة بالالتهاب الرئوى أن اشهر العوامل الممرضة المعزولة هي Klebsiella و E.Coli و Pasteurella و Pseudomonas و Staphylococcus spp. وأنواع أخرى من البكتيريا.

البحث الثانى:

تم تخدير عشرة كلاب بلدية سليمة اكلينيكيا ثم تم احداث اصابتها بالتقطير داخل القصبة الهوائية بمقدار 2 مل من مرق الكلبسيلا . ثم تم إجراء الفحوصات الاكلينيكية والدموية والكيميائية الحيوية والموجات فوق الصوتية للكلاب المصابة في اليوم ما قبل الاصابة وفي اليوم الأول والثالث والخامس والسابع بعد الاصابة.

1. نتائج الفحص الإكلينيكي :

أظهرت الكلاب المصابة بالتهاب رئوي علامات متغيرة بما في ذلك علامات تنفسية بالإضافة إلى علامات عدوى جهازية. كما أظهرت زيادة معنوية في درجة الحرارة والنبض ومعدل التنفس.

2. فحص صورة الدم :

أظهرت الكلاب المصابة بالتهاب رئوي انخفاضًا معنويًا في عدد كرات الدم الحمراء ومحتوى الهيموغلوبين ونسبة PCV. كما أظهرت زيادة معنوية في عدد كرات الدم البيضاء وعدد العدلات وانخفاض كبير في نسبة الخلايا الليمفاوية.

3. التحليل البيو كيميائي :

أظهرت الكلاب المصابة بالتهاب رئوي زيادة معنوية في بروتينات المرحلة الحادة والسيتوكينات المسببة للالتهابات ، وزيادة معنوية في البروتين الكلي والجلوبيولين وانخفاض معنوي في نسبة الألبومين والجلوبيولين. كما أظهرت زيادة في مستويات الاجسام المضادة والكورتيزون .

4. الفحص بالموجات فوق الصوتية:

وأظهر الفحص بالموجات فوق الصوتية للكلاب المصابة بالتهاب رئوي وجود التهاب رئوي في القصبات يتمثل في سماكة الغشاء المحيط للرئة وتماسك الرئة وانهيارها.

5. فحص الأنسجة المرضية:

أظهر المظهر العياني للرئتين المصابة وجود مناطق ذات اللون الأحمر الداكن إلى البني الداكن والتهاب الجنبة ومناطق انتفاخ الرئة ، بينما أظهرت الصور المجهرية التهابًا قصبيًا رئويًا حادًا يمثله سماكة شديدة في غشاء الجنب ، وحويصلات هوائية منهارة مع تسلل خلوي للخلايا الالتهابية داخل الحويصلات الهوائية وفي النسيج بينها. انتفاخ الرئة والوذمة الالتهابية واحتقان الأوعية الدموية.

وقد خلصت هذه الرسالة الى:

1- التغييرات السريرية والكيميائية الحيوية والدموية والموجات فوق الصوتية والتصوير الشعاعي هي تشخيصية لمختلف أمراض الجهاز التنفسي في الكلاب والقطط.
2- يمكن استخدام قياس بروتينات المرحلة الحادة و Il6 كمؤشر مبكر للعدوى والالتهاب.

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Curriculum Vitae

- The researcher was born on 9/8/1995, in Toukh , Qalyubia, Egypt.
- Her primary education was completed in Arab Alghadery Primary School in 2007.
- Her preparatory education was completed in Arab Alghadery preparatory school in 2010.
- Her secondary education was completed in Elder secondary school in 2013 .
- Her undergraduate and professional education was completed in Faculty of Veterinary Medicine, Moshtohour, Benha University in 2018.
- She worked as a demonstrator of internal medicine at the Faculty of Veterinary, Benha University in 2018.
- She registered for M.V.Sc in 2019 at Faculty of Veterinary Medicine, Benha University .
- Email: Gehad.Elgalfy@fvtm.bu.edu.eg
gehadelsayed936@gmail.com

Buffers and reagents, mixtures

- Klebsiella broth ($5x10^9$ organism/ml)
- Propofol anesthetic drug: Importing the Egyptian Company for Pharmaceutical Trade

Equipment and apparatus:

• Endotracheal tube (its length selected according to the distance from the oral commissure of the animal to the thoracic inlet)

• Clean test tubes (vacuum tube). • Eppendorf tubes. • Sterile syringes

• Spectrophotometer: spin lab from Spinreact S.A. Model 2003

•Thermometer. •Stethoscope.

•Deep freezer. • Centrifuge •Slides and cover slides. •Electrical research microscope.

• Ultrasonography: Ultrasonographic examination was performed by using a portable ultrasound machine (4-6 MHZ linear array probe, Eickemeyer Magic 2200, Germany and 3.5 MHZ curved linear probe,WED- 380V,Veterinary Ultrasound Scanner).

• Biological Safety Cabinets Class II A2: S@femate, S@fevision, EuroClone S.P.A. Produzione/ Mfg. Plant: Via Lombardia, 12-27010 Siziano (PV), Italy.

• Hematology Analyzer: XF9080 Perlong Medical Machine CO., Ltd. No. 28-1 Dajiaochang Road, Nanjing 21007, China. 1-4-16-Hot air oven: manufactured by LAB-Line instruments, INC designer and manufacturers LAB-Line Plaza, Mel. Rose Park, ILL 60160. Model 3511-1.

• Spectrophotometer: spin lab from Spinreact S.A. Model 2003. •EDTA

• MacConkey agar, Blood agar, Baired parker agar, Muller Hinton agar. • Nutrient broth.

• Gram stain and Wright's-Giemsa stain. •Peptone water, indole reagent, voges proskeur reagent, lactose and citrate reagents

Publication List

1.Title: Clinical, Hematological, Acute phase proteins and Radiographic Changes In Different Respiratory Affections In Dogs and cats.

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