Pneumonia in Sheep: Bacteriological and Clinicopathological Studies

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Abstract

This study aimed to first, isolate the most common bacterial pathogens causing pneumonia in sheep second, to study the hematological, biochemical, immunological and histopathological changes associated with the disease. Twenty apparently healthy Barki ewes in addition to 30 respiratory-distressed cases with typical respiratory manifestations were used in this study. Nasal swabs for bacteriological examination, blood samples for hemogram and serum biochemical assays and tissue samples for histopathology were collected from healthy and diseased ewes. The results implicated that the most common bacterial pathogens isolated from both apparently healthy and diseased ewes included *Klebsiella pneumoniae* and *Staphylococcus aureus*. The results of hematological parameters, showed a significant decrease in all red blood cell parameters. Diseased ewes also showed a significant increase in total leukocytic and absolute neutrophilic, monocytic and eosinophilic counts and a significant decrease in lymphocyte count. Data of selected biochemical parameters implicated a significant decrease in blood pH and serum concentrations of albumin, A/G ratio, calcium, inorganic phosphorus, sodium and chloride while a significant increase was seen in the levels of total protein, globulin, blood urea nitrogen, creatinine, potassium, enzymatic activity of ALT and AST and plasma bicarbonate and fibrinogen concentrations. The results of immunological parameters, showed a significant increase in alpha2 and gamma globulins in diseased animals. The main histopathological alterations induced by respiratory infections were bronchitis, bronchiolitis, alveolar emphysema, inflammatory and non-inflammatory edema, serous bronchopneumonia and interstitial pneumonia. The results of this study can conclude that first, *Klebsiella pneumoniae* and *Staphylococcus aureus* appeared to be the main bacterial causes of pneumonia in sheep. Second, pneumonia in sheep is associated with significant hematological, biochemical, immunological

and histopathological alterations which upon understanding can provide us with a good knowledge about the disease process in sheep and thus lead to better management and proper treatment.

Key words: Sheep, Pneumonia, Bacteriology, Immunity, Hematology, Clinical Pathology

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Introduction

Sheep are considered the most important future growth of livestock in Egypt that play a vital economic role and support the survival of millions of people in our country (Hatem *et al*., 2003). Sheep have the ability to convert and diversify different types of forages into valuable products for mankind, such as mutton, milk and wool (Galal *et al.,* 2005). The importance of sheep, to the socioeconomic wellbeing of people in developing countries cannot be emphasized hence it is necessary to study the diseases and syndromes that affect this species in order to enhance and sustain their productivity to meet the demand of the human population places upon them (Baker and Grey, 2004).

Respiratory diseases of sheep particularly pneumonia continues to be a major problem commonly encountered in sheep flocks, affecting groups or individuals of all ages and types (Naveed *et al.,*1999). The disease is multifactorial in origin often involves a combination of infectious causes as well as predisposing environmental and managerial factors (Barghouth, 1999). Respiratory diseases in sheep result in poor live weight gain and mortality, thus causing considerable financial losses for lamb producers. Economic losses include unthrifitness, coast of treatment and preventive measures of non-fatal cases (Barghouth, 1999 and Edwards *et al.*, 1999). The disease is also an important animal welfare concern (Naveed *et al.*, 1999).

Pneumonia is the most common problem associated with the lower respiratory tract of sheep that can be acute, chronic, or progressive and can caused by bacteria, viruses, or parasites. Among infectious agents, bacterial pneumoniae are responsible for outbreaks, sudden death and high mortality in lambs ((Naveed *et al.*, 1999).

Because of the clinical economic importance of the disease in sheep, it was a topic of interest of many researchers in the field of small ruminant practice. But in many instances, most studies were critically focused on the causes of the disease, the diagnostic procedures that can be performed, checklist of potential pathogens to improve diagnosis and assess the potential of therapeutic and preventative strategies, clinical diagnostic methods and treatment options and control measures. Nonetheless, to our knowledge, little data about the clinicopathological changes associated with pneumonia in sheep are available. Therefore, the present investigation aimed to study some clinicopathological changes which involve comparing hematological, biochemical, immunological and histopathological changes between healthy and pneumonic animals with special reference to the bacterial causes of the disease in sheep.

Materials and Methods

Animals

Fifty female Barki ewes with ages from 2-4 years were used in this study (in the period from 16th of December 2011 till 30th of April 2012). Thirty ewes were suffering from respiratory manifestations (20 sporadic cases from different farms and 10 from slaughter house showing signs of respiratory distress). The reset (20 ewes) were apparently healthy and were considered as control group.

Samples

Nasal swabs were collected aseptically from both apparently healthy as well as diseased ewes for bacteriological examination. Blood samples were collected from both groups and were divided into three parts. The first part was collected on disodium ethylene diamine tetracetic acid (EDTA) for hemogram. The second part was collected on heparin (20 IU/ ml) for measuring of plasma values of fibrinogen (F), pH and bicarbonate (HCO_3). The third part was placed in a plain

centrifuge tubes for separation of serum and serum samples were stored at -20ºC until assayed for the rest biochemical parameters. For histopathology, the lung tissues were collected from the slaughtered animals showing signs of respiratory diseases at abattoir and were sectioned. Sections of adjacent grossly uninvolved tissue were also collected.

Analytical methods

Bacteriological studies

The collected swabs were cultivated under aseptic condition into media like blood agar, MacConkey's bile salt agar, Salmonella Shigella agar, mannitol salt agar and selenite F broth. All inoculated media were incubated aerobically at 37^{o}C for 24 hours for selective isolation of Salmonella (Frobes *et al.,* 1998). Suspected colonies onto the fermented media were identified morphologically according to its staining reaction, shape, size and arrangement and were confirmed by full biochemical identification at the department of bacteriology, mycology and immunology, faculty of veterinary medicine, university of Sadat City.

Hematological studies

The evaluated hematological parameters in this study included estimation of red blood cell count (RBCs), hemoglobin concentration (Hb), packed cell volume (PCV), total (TLC) and differential leukocytic counts. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated from RBCs, Hb and PCV values. These parameters were performed according to the routine hematological procedures adopted by Feldman *et al*. (2000).

Serum biochemical studies

Serum samples were assayed for the concentrations of total protein (TP), albumin (Alb), blood urea nitrogen (BUN), creatinine (Cr), calcium (Ca), inorganic phosphorus (iP) and serum enzymatic activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Total globulin was determined by subtracting albumin from serum total protein and then A/G ratio was estimated. These parameters were determined by spectrophotometric method using commercially available test kits supplied by Biodiagnostics (Egypt) and following the manufacturer’s instructions. Serum concentrations of sodium (Na), potassium (K) and chloride

(Cl) were determined by flame photometer and diagnostic kits of Randox (Randox Laboratories Ltd, Crumlin, Co. Antrim, UK). Plasma concentration of fibrinogen was detected by Spectrophotometric method using commercial kits of Boehringer Ingelheim (Germany). Blood pH and Plasma bicarbonate concentrations were measured by Rapid point 340® Blood Gas Analyzer (England) using kits supplied by Synbiotics Corporation (11011 via Frontera, San Diego).

Immunological studies

Serum protein electrophoretic fractionation profile was carried out by a Polyacrylamide Gel Electrophoresis according to Lewis *et al.* (2006).

Histopathological studies

For histological sections, the collected lung samples were rapidly fixed in 10% neutral buffered formalin solution and paraffin sections were prepared and stained with haematoxylin and eosin according to Bancroft and Gamble (2002).

Statistical analysis procedures

The values were presented as mean ± standard deviation (SD). Mean values of diseased and control groups were compared by student's t test at 0.05 level of probability (Sndecor and Cochran, 1980).

Results

Bacteriology

As shown in Table 1, 80 isolates were recovered from the 50 nasal swabs which included 24 *Klebsiella pneumonae* (*Kl. pneumonae*), 22 *Staphylococcus aureus* (*Staph. aureus*), 10 *Proteus species* (*Proteus spp*), 9 *Shigella species* (*Shigella spp*), 5 *Pseudomonas aeroginosa* (*Ps. aeroginosa*), 4 *Escherichia. coli* (*E. coli*), 3 *Pasteurella species* (*Pasteurella spp*), 2 *Histophilus*

somnus (H. somnus) and 1 *Enterobacter species* (*Enterobacter spp*) with percentages of (48%, 44%, 20%, 18%, 10%, 8%, 6%, 4% and 2%) respectively. The prevalence rate of bacterial isolates included 1 isolate from apparently healthy ewes while the rate in diseased animals revealed 1 isolate from 11 cases, 2 isolates from 8 cases and 3 isolates from 11 cases (Table 2).

Table 1: Prevalence rate of bacterial isolates from apparently healthy and pneumonic ewes

Bacterial isolate	Nasal swabs		Total	(%)
	control	diseased		
Kl. pneumonae	6	18	24	48
Staph. aureus	6	16	22	44
Proteus spp	2	8	10	20
Shigella spp	2	7	9	18
Ps. aeroginosa	2	3	5	10
E. coli	1	3	4	8
Pasteurella spp	1	2	3	6
H. somnus	-	2	2	4
Enterobacter spp	-	1	1	2

Table 2: The prevalence of single and mixed infection in the respiratory disease-affected ewes

Group	Samples	Number of bacterial isolates					
		One	%	Two	%	Three	%
Control	20	20	100	-	-	-	-
Diseased	30	11	36.67%	8	26.67%	11	36.67%

Hematology

Table (3) reveals that there was a significant (P<0.05) decrease in RBCs count and Hb, PCV, MCV, MCH and MCHC values in the diseased group compared to the control. As shown in Table (4), significant (P< 0.05) increase in TLC was seen in the diseased group with significant (P<0.05) increase in the mean values of neutrophilic, eosinophilic and monocytic counts. The mean values of lymphocytic count showed a significant (P< 0.05) decrease in the diseased group while no changes were observed in the count of basophils.

Table 3: Blood cell parameters of the pneumonic ewes compared to the control healthy group

Values are mean ± SD

RBC Parameters	Group (N=15)		WBC Parameters ($\times10^3$/µl)	Group (N=15)	
	Control	Diseased		Control	Diseased
RBCs ($\times10^6$ /µl)	10.57± 0.11	8.38±0.35*	TLC	7.55±0.46	12.06±1.20*
Hb (g/dl)	12.00±0.43	8.81±0.30*	Neutrophils	2.30±0.22	7.00±0.82*
PCV (%)	39.00±0.82	30.00±1.50*	Eosinophils	0.07±0.01	0.40±0.05*
MCV (fl)	38.62±0.88	33.97±0.74*	Basophils	0.04±0.04	0.11±0.02
MCH (pg)	12.03±0.33	9.61±0.36*	Lymphocytes	4.70±0.17	3.19±0.22*
MCHC (%)	31.98±1.35	26.82±1.24*	Monocytes	0.13±0.02	0.38±0.07*

Significant differences in the values between the diseased and the control groups were indicated by (*) at P< 0.05.

Serum biochemistry

The results of serum biochemical changes as shown in Table (4) clarified that significant (P<0.05) decreases were noticed in serum concentration of albumin and A/G ratio in diseased group compared to the control one but serum values of total proteins and globulins showed a significant increase. Serum concentrations of blood urea nitrogen, creatinine and serum enzymatic activities of ALT and AST were significantly (P<0.05) increased in the diseased ewes. The mean values of serum levels of Ca, iP, Na and Cl were significantly (P<0.05) lower in

the diseased ewes while, serum K levels showed a significant (P<0.05) increase. Comparison the mean values of plasma HCO_3 concentrations and blood pH between the two groups revealed a significant increase in HCO_3 concentrations and a significant decrease in pH in the diseased animals (Table 4). A significantly (P<0.05) higher values of plasma fibrinogen concentrations were detected in the diseased ewes compared to the respective control group (Table 4).

Table 4: Changes in serum biochemical parameters and acid-base status of the pneumonic ewes compared to the control healthy group

Values are mean ±SD.

Parameter	Group (N=15)		Parameter	Group (N=15)	
	Control	Diseased		Control	Diseased
TP (g/dl)	4.91±0.11	6.70±0.11*	Ca (mg/dl)	10.75±0.99	7.80±0.38*
Alb (g/dl)	2.73±0.21	2.03±0.01*	iP (mg/dl)	3.30±0.27	1.96±0.40*
Glob (g/dl)	2.18±0.29	4.66±0.18*	Na (mmol/L)	146.43±1.40	133.00±1.41*
A/G ratio	1.25±0.30	0.42±0.12*	K (mmol/L)	4.36±0.31	6.05±0.34*
BUN (mg/dl)	31.26±2.70	40.97±1.19*	Cl (mmol/L)	96.83±1.69	85.00±2.83*
Cr (mg/dl)	1.00±0.20	1.34±0.20*	pH	7.14±0.15	5.82±0.10*
ALT (U/L)	24.53±1.31	28.62±0.84*	HCO3 (mmol/L)	7.97±1.10	15.73±0.95*
AST (U/L)	30.00±0.77	39.21±1.03*	F (mg/dl)	430.00± 17.32	623.33±20.82*

Significant differences in the values between the diseased and the control groups were indicated by (*) at P< 0.05.

Serum protein electrophoretic fractionation profile

The results of serum protein electrophoretic fractionation profile as presented in Table (5) showed that there was a significant (P<0.05) decreases in serum concentration of albumin but serum levels of alpha$_2$ (α_2-Glob) and gamma (γ-Glob) globulins demonstrated a significant increase. On the other hand, serum concentrations of alpha$_1$ (α_1-Glob) and beta (β-Glob) globulins were not significantly changed.

Table 5: Serum protein (total and electrophoretic) concentrations (g/dl) in the pneumonic ewes compared to the control group

Variable	Group (N=20)	
	Control	Diseased
TP	4.90±0.36	6.70±0.46*
Albumin	2.73±0.21	2.03±0.27*
Alpha 1 globulins	0.34±0.20	0.50±0.15
Alpha 2 globulins	0.76±0.15	1.18±0.19*
Beta globulins	0.58±0.21	0.70±0.11
Gamma globulins	0.47±0.21	2.29±0.31*

Significant differences in the values between the diseased and control groups were indicated by (*) at $P < 0.05$.

Histopathology

Macroscopic examination of cases obtained from slaughtered and dead sheep showed that some affected lungs were swollen, wet and upon sectioning, yellowish fluid exude freely down. Various degrees of congestion indicated by dark red patches were seen in some lungs. Emphysema was evident in many cases. The emphysematous areas were higher, lighter and paler than the surrounding tissue. Upon palpation, there were crepitating sounds. Other affected lungs were pale gray in color, firm in texture and exhibited multiple small abscesses or large one abscess which, on sectioning, showed thick creamy or greenish pus and gritting sound.

Histopathological examination of lungs revealed catarrhal bronchitis with the lumen contained viscid mucus and inflammatory cells (Fig.1 A). Edema was associated with vascular dilatation and hemorrhages that varied from excessive extra vascular accumulation of pale eosinophilic homogenous fluid extending to the pulmonary interlobular, interstitial, within the alveoli and bronchial lumen (Fig.1 A).There was congestion of alveolar wall with active alveolar hyperemia (Fig. 1 B). Interstitial pneumonia was evident in some cases with the alveolar septa were infiltrated with mononuclear cells (Fig. 1 C). Alveolar emphysema was seen either alone or most commonly in the pulmonary tissue surrounding or immediately in the vicinity of the previously mentioned lesions (Fig.1 A, D).

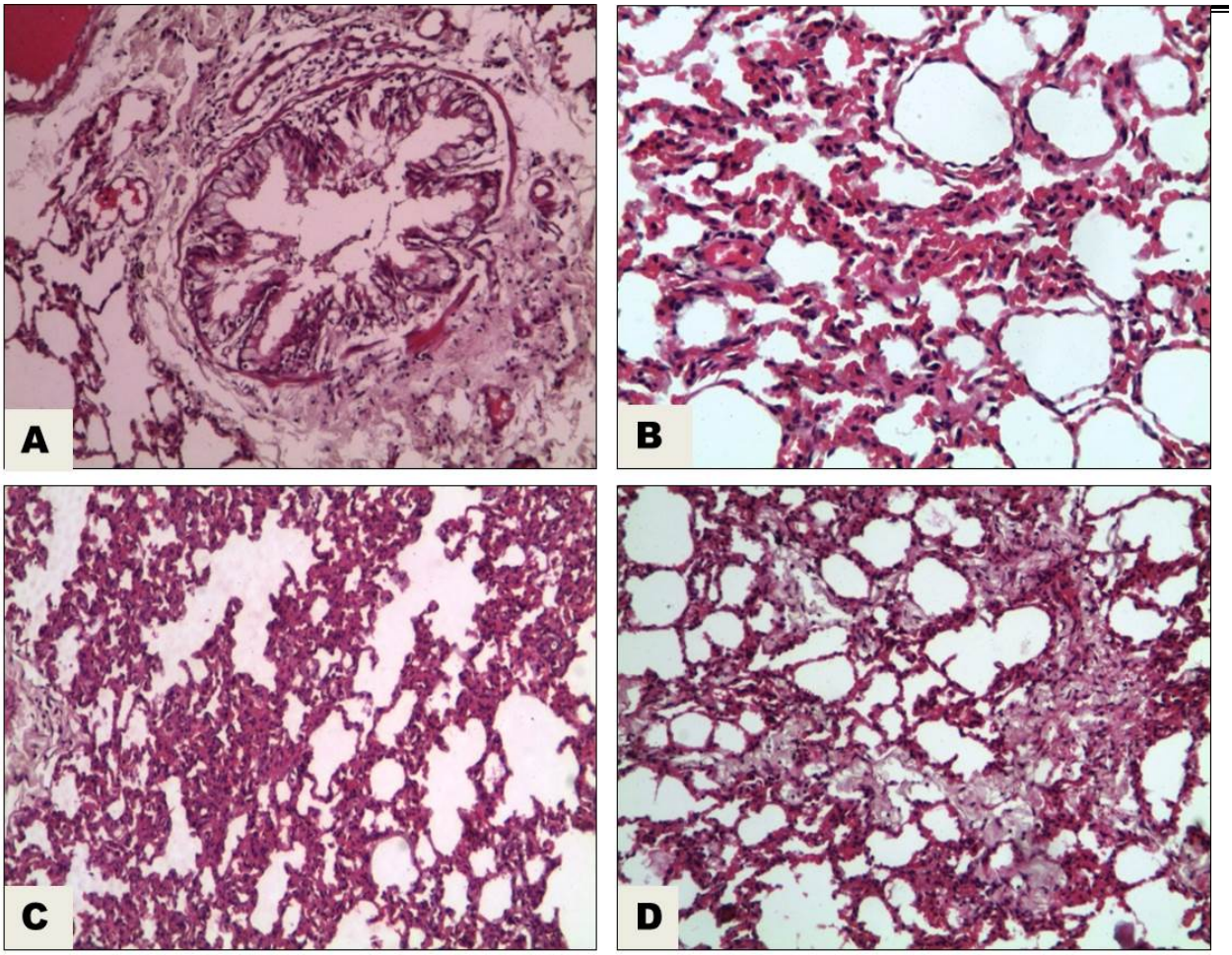

Fig. 1: Light micrograph of lung of ewe stained by (H&E) showing: **A)** Catarrhal bronchitis, peribronchiolar edema, lymphocytic infiltration, congestion of the B.V and alveolar emphysema; **B)** Congestion of alveolar wall with active alveolar hyperemia; **C)** Interstitial pneumonia with area of alveolar emphysema, the alveolar septa are infiltrated with mononuclear cells; **D)** Serous pneumonia where the alveolar wall filled with serous secretion and inflammatory cells, the surrounding area showing alveolar emphysema. (A, C, D, x10 & B x20).

Discussion

Sheep play a vital economic role as they are raised mainly for lamb production, followed by wool and milk for large section of population especially in village and desert areas. Thus they

can support the survival of millions of people in many countries all over the world including Egypt (Hatem *et al.*, 2003 and Ali *et al.*, 2009).

Respiratory disorders are still serious problem facing sheep raring (Hatem *et al.*, 2003).The importance of respiratory diseases of sheep depends on their prevalence, their effect on productivity, the value of the animal and for some diseases, their international spread (Ali *et al.*, 2009). The disease is a complex and multifactorial in which bacterial, viral, mycoplasmal and fungal infections combine with other factors such as stress of weaning, transportation, commingling and parasitism to produce acute respiratory diseases (Louis, 1996).

In this study, bacteriological examination of 20 swabs from apparently healthy and 30 nasal swabs from diseased adult Barki sheep was carried out. The bacterial isolates recovered from apparently healthy sheep were *Kl. pneumonae*, *Staph. aureus*, *Proteus spp*, *Shigella spp*, *Ps. aeroginosa*, *E. coli* and *Pasteurella spp*, which agrees with the finding of Elgwoud (1998). The recovery of these bacterial isolates from apparently healthy sheep is consistent with what was well-known that about 40% of healthy sheep carry infectious agents in the upper respiratory tract that under the stress factors cause respiratory disease (Radostitis *et al.*, 2000).

Concerning the diseased sheep, the results revealed the presence of many bacterial species as single or mixed isolates. *Klebsiella pneumonae* were the most predominant bacteria isolated at a percentage of 48%followed by *Staph. aureus* (44%), *Proteus* (20%), *Shigella* (18%) and *Ps. aeroginosa* (10%). Other bacterial causes isolated were *E. coli* (8%), *Pasteurella Spp* (6%), *H. somnus* (4%) and Enterobacter (2%). Similar results more or less were reported by previous studies (Elgwoud, 1998; Zaitoun, 2001 and Mahmoud *et al.*, 2005). The differences between the records were mainly due to the geographical distribution at which the investigator was adopted.

The effect of pneumonia on red cell parameters seen in this study included a significant decrease in RBCs count, Hb, PCV, MCV, MCH and MCHC values in the diseased group indicating the presence of microcytic hypochromic anemia. These changes could be attributed to the mononuclear phagocytic system under the circumstances of inflammatory conditions becomes hyperplastic trapping free iron and hence increases iron storage in phagocytic cells. Decreases iron transfer to developing erythroid cells in bone marrow leading to reduction of Hb

synthesis and production of microcytic hypochromic RBCs (El-Naser and Khamis, 2009 and Aytekin *et al.,* 2011).

In regard to the white blood cell parameters, results of the present study showed a significant increase in total leukocytic count which may be explained by absolute neutrophilia, eosinophilia and monocytosis. Total leukocytic count was reported to increase in acute inflammatory diseases particularly those due to bacterial infections. This could be attributed to that infectious agents and products of tissue injury stimulate a variety of cells to release growth factors, cytokines, and other mediators of inflammation that act as prompt stimuli and are all interrelated in causing the increase in total white blood cells count and more production, proliferation, maturation and bone marrow release of mature and immature neutrophils (Sayed *et al.,* 2002). Another possible explanation for this leukocytosis represented by a neutrophilia is the stress to which the animal exposed during the course of the respiratory illness that results in endogenous release of corticosteroids which have major role in regulating circulating concentration of leukocytes in moderate and severe pneumonia (El-Naser and Khamis, 2009). Eosinophilia could be the result of hypersensitivity effect produced by microorganisms and the resultant release of histamine as reported in previous studies (Raghib *et al.,* 2004).

In consistence with the previous reports, marked reduction in lymphocyte count was observed in the diseased ewes. The lymphopenia might be due 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 (Aytekin *et al.,* 2011). Furthermore, generalized infection and the influence of allergen that induce type-1 hypersensitivity and release of histamine could be a possible cause for this lymphocytopenia as mentioned by Azzam and Aly (2006). These results were further confirmed by the reported histopathological findings shown in this work which demonstrated perbronchial and peribronchiolar lymphocytic infiltration seen in most cases of microscopically examined lung sections that might explain the changes that occurred in lymphocytes.

Serum biochemical alterations in respiratory diseases were common and might display reasonably predictable changes in response to inflammation. In this regard, the results of the

present investigation revealed that there was a significant increase in serum values of total protein and globulin and a significant decrease in serum concentrations of albumin.

Hypoalbuminemia could be due to anorexia and inability of liver to synthesize protein (El-Seidy *et al.,* 2003). Others suggested that certain bacteria or bacterial toxins increase capillary permeability and permit escape of plasma proteins in tissues so osmotic pressure of proteins is increased in the tissue fluids and at the same time decreased in the blood (Omran *et al.,* 2005). Albumin is also considered a negative acute phase protein and its value frequently and markedly declines during inflammation (Ceron *et al.,* 2005 and Georgieva *et al.,* 2011). Further, Kaneko *et al.* (1997) stated that albumin is particularly general metabolic transporter protein, any decrease in albumin may enhance the protective and healing function of inflammation by focusing the animals' metabolic activities toward synthesis protective proteins such as fibrinogen, haptoglobin and serum amyloid acid.

The Hyperproteinemia seen in this investigation may be attributed to hyperglobulinemia resulting from increased γ globulins as declared by the results of serum protein electrophoresis presented in this study (El-Seidy *et al.,* 2003).

As markers of renal function, serum concentrations of creatinine and blood urea were measured. The results revealed that the mean values of serum creatinine and urea blood urea concentrations were significantly higher in the diseased ewes compared to control. The increase in urea concentration could be explained by the accelerated catabolism of body protein and could result as a response to infection while the increase in serum creatinine might be attributed to kidney dysfunction after infection (Radostitis *et al.,* 2000).

Data obtained from the present study implicated a significantly high serum enzymatic activity of ALT and AST in the diseased group. These changes could be attributed to dysfunction of various organs including liver due to hepatic degenerative and necrotic changes caused by bacterial infection and toxins (Raghib *et al.,* 2004; Talkhan *et al.,* 2009 and Aytekin *et al.,* 2011).

Taking in consideration the important role of respiratory system in regulating acid-base status and subsequently the serum concentrations of some minerals, electrolytes and blood gases, alterations in these parameters were always important criteria to make comments on the

diagnosis, treatment and prognosis of the diseases affecting respiratory system (Tanritanir *et al.*, 2010). In this respect, the results of this study showed a significant decrease in serum calcium and phosphorous concentrations. The decrease in serum calcium might be the result of anorexia, decreased intestinal absorption or increased renal excretion (Radostitis *et al.*, 2002). As about 40-45% of calcium is protein bound mainly to albumin, so hypoalbuminemia might be a possible cause for this hypocalcemia (Kaneko *et al.*, 1997). The significant decrease in serum phosphorous concentrations seemed to be secondary to reduced phosphorus absorption from the gut and reduced phosphorus resorption from the tissues (Orr *et al.*, 1990).

In respect to the changes in serum electrolytes, the results of this study implicated that there was a significant decrease in serum concentrations of sodium and chloride while serum values of potassium were significantly increased (Gaber *et al.*, 2000). Some studies attributed the changes occurring in serum electrolytes during the course of respiratory disease to the hyperpyrexia in the acute course of the disease and metastatic infection of liver and kidneys resulting in hepatic and renal dysfunction (Novert, 2004). High serum potassium concentrations could be seen in respiratory diseases particularly if acidosis is present because H^+ ions accumulated in the extracellular fluid is exchanging with potassium present in the intracellular fluid leading to hyperkalemia (Kaneko *et al.*, 1997).

In this work, significant decrease in blood pH values was detected in affected ewes indicating respiratory acidosis. Respiratory acidosis usually accompanied the respiratory affection in which the signs of respiratory dyspnea were markedly observed and was evidenced throughout a significant decrease in the blood pH and a significant increase in the circulating proton concentrations. Possible explanation for such alteration could be attributed to decreased pulmonary ventilation and reduction in the elimination of CO_2 (hypercapnia) with the resultant increase in carbonic acid concentrations and base excess (Tanritanir *et al.*, 2010). Plasma bicarbonate concentrations in the present investigation were found to be markedly enhanced in the affected ewes confirming the insufficient CO2 elimination (Ozkanlar *et al.*, 2012). Others explained the elevation in the levels of HCO_3 accompanying respiratory acidosis by the partial metabolic compensation due to the fact that the kidney responds to respiratory acidosis by renal retention of bicarbonate (secondary metabolic alkalosis) in exchange with chloride to maintain

electrical neutrality (Cambier *et al.*, 2002). This explanation was supported in this study by testing of serum levels of chloride which showed a significant decrease in the pneumonic ewes.

To monitor the effect of pneumonia on acute phase response, plasma fibrinogen concentrations were measured which demonstrated a significant increase in the diseased group. Fibrinogen is one of the most important acute phase reactant proteins that was always considered a reliable indicator of the presence of inflammation in cattle and sheep (Cheryk *et al.*, 1998). As a part of acute-phase response, fibrinogen was found to be linked to adhesion and migration of neutrophils and activation of their defense functions (Smiley *et al.*, 2001). In bacterial pneumonia, acute phase proteins were found to correlate with the severity of the disease, serve as biomarkers and were functionally significant in such cases (Quinton *et al.*, 2009 and Fathi *et al.*, 2011).

The current findings of serum protein electrophoresis in diseased ewes revealed a significant increase in serum values of γ and α_2 globulins while no changes were observed in α_1, and β globulins. The increase in α_2 globulins implicates a systemic acute phase response to inflammation as most important proteins of the positive acute-phase response to inflammation are in alpha and beta globulins (Apaydin and Dede, 2010). On the other hand, the increase in **γ** globulins may reflect increased antibodies production in response to antigenic stimulation caused by the microorganisms (Gaber *et al.*, 2000).

Histopathologically, the affected lungs sections revealed catarrhal bronchitis and bronchiolitis with the mucosa of affected bronchi was hyperemic, markedly thickened and slightly sloughed out with presence of viscid mucus. The epithelium showed degenerative and necrotic changes and was desquamated besides ulceration of the walls and peribronchial lymphocytic aggregation Alveolar emphysema was evident in many cases. Emphysematous areas showed no vascular reaction or inflammation. Edema was associated with vascular dilatation and hemorrhages that varied from excessive extra vascular accumulation of pale eosinophilic homogenous fluid extending to the pulmonary interlobular, interstitial, within the alveoli and bronchial lumen were dilated and filled with edematous fluid (Samah, 2004 and Yani, 2009).

Based on information obtained from this study we have opinion that bacterial agents

appeared to be the main cause of pneumonia in sheep with the high incidence was recorded for *Klebsiella pneumoniae* and *Staphylococcus aureus*. Hematologically, pneumonic ewes had microcytic hypochromic anemia and significant inflammatory leukocytosis. The most important biochemical alterations included respiratory acidosis in addition to significant alterations seen in many serum variables implicating different degrees of organs dysfunction caused by the disease. The disease also appeared to have pronounced systemic acute phase reaction and strong humoral immune response. The histopathological findings in this study clarified the presence of different types of pathological lesions in the affected tissues depending on type of microorganisms and stage and severeity of infection. Overall then, this work can improve our understanding and provide a good knowledge about of the disease process in sheep that can lead to better management and proper treatment.

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