Bacteriological And Pathological Studies on Pneumonia in Lambs with Special references to inflammatory markers

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ABSTRACT

This study was carried out to study the most common bacterial causes of pneumonia in lambs in certain farms in Zagazig city, Sharkia Province, to evaluate the acute Phase Proteins as inflammatory markers and the most obvious pathological changes in lungs and bronchi. In this study,50 lambs were used, (aged3-6 months),divided into two groups.Gp1(25 healthy lambs) and Gp2 (25Pneumonic lambs).Nasopharyngeal swabs and two blood samples from each lamb were taken for bacteriological examination and biochemical analysis to determine serum total proteins, albumin, C Reactive Proteins (CRP), Haptoglobin (Hp), and Plasma Fibrinogen (PF). Specimens were taken from lungs and bronchi of lambs in Gp2for histopathological study. The obtained results revealed that *E.coli, Staphylococcus aureus and Pasteurella hemolytica* are the main common causes of pneumonia in lambs. A significant increase in CRP, Hp and PF levels in pneumonic lambs, also, an increase in serum total proteins supplemented with a decrease in serum albumin in pneumonic lambs. Lungs of pneumonic lambs showed severe congestion and thrombosis of pulmonary blood vessels. Thickening of interlobular septa, emphysema associated with consolidated areas in addition to suppurative and fibrinous pneumonia were seen.

From this study, it can be concluded that , *E.coli, S.aureus and P. hemolytica* are the common causes of bacterial pneumonia in certain farms in Zagazig city. The inflammatory markers are indicative of inflammation; lungs were suffering from Fibrinous and Suppurative pneumonia leading to high mortality in lambs in this study.

INTRODUCTION

Pneumonia is one of the most common respiratory problems in small ruminants throughout the world and it is one of the important causes of lamb mortalities. Pneumonia is caused by a complex interaction between the environment which produces stress factors, microorganisms and the host's immune response ***(1,2)***. The most frequent causes of Pneumonia in lambs are *Pasteurella multocida* or *Mannheimia haemolytica* which are commonly found in the upper respiratory tract of healthy goats, under stress factors an outbreak of acute pneumonia was followed. *Escherichia coli*, *Staphylococcus aureus*, *Corynebacterium pyogenes*, *Proteus vulgaris* are the most common causative bacterial agents of Pneumonia in lambs ***(3)***. *Pasteurella Spp.*, *Klebsiella*, *Pseudomonas Spp.*, and *Staphylococcus aureus* are the most common bacterial causes of Sheep pneumonia ***(4)***. *Pasteurella multocida B:6, E.coli, Staphylococcus aureus* are the main bacterial causes of pneumonia in ruminants and young calves.***(5)***. Pneumonia in Sheep may be either Catarrhal due to *E.coli* infection or Purulent due to *Staphylococcus aureus*or Fibrinous due to *Pasteurella Spp.(6)*.Different types of pneumonia including catarrhal, purulent, fibrinous, fibrinonecrotic and even interstitial

forms, caused by *Mannheimia hemolytica, E.coli and Pasteurella multocida*, therefore, it is difficult to identify a pathogenic microorganism responsible for each pneumonia type. *(2)*.

There are several pathologic forms of pneumonia in lambs; mild to severe and acute to chronic. The traditional bacterial pneumonias are characterized by exudation and consolidation and bronchopneumonia of the lungs.*(7)*. Desquamation of the epithelial lining of some bronchi with thickening of interalveolar septa were seen *(8)*. CRP, Hp and PF are the most important indicators of the inflammatory process in sheep subjected to infection, inflammation or stress *(9)*.

The aim of this work is to evaluate the clinical diagnostic value of acute phase proteins (Hp ,CRP ,PF, Albumin ,Globulins and Total proteins) as markers of infection and inflammation and to study the pathological changes associated with bacterial pneumonia.

MATERIAL AND METHODS

Animals

In this study a total of 50 Lambs, 3-6 months of age were used , classified into 2 grops.(Gp-1), consists of 25 healthy lambs proved to be free from infection and external & internal parasitic infestation. No obvious clinical signs of respiratory manifestations were seen. The second group (Gp-2) consistes of 25 diseased Lambs suffering from respiratory manifestations with variable degrees of the clinical signs; from mild to severe.

Nasopharyngeal Swabs

Swabs were collected from Lambs suffering from healthy and diseased lambs (Gp1 &Gp2) for bacteriological examinations. The collected samples were inoculated into nutrient broth and incubated at 37°C for 24 hrs and then subcultured into 7% Sheep blood agar and MacConkey's agar at 37°C for 24-48 hrs. Growing colonies were picked up and purified by subculture on nutrient broth and then cultured on selective media, the isolates were identified for their morphology and biochemical activities.*(10,11)*.

Blood Samples: Blood samples were collected from all examined Lambs (Gp1 &Gp2), through jugular vein puncture using sterile plastic syringe.

The first blood sample from each examined lamb was taken and put in a sterile tube with an anticoagulant (sodium citrate) for measuring the plasma fibrinogen level, *(12)* . The second blood sample was taken and put in a sterile tube without using an anticoagulant to obtain a clear, non- hemolysed sera for detection of, CRP , Serum haptoglobin level, total proteins, albumin and globulins *(13)*.

Pathological examination: The gross lesions of the pneumonic lambs were recorded and tissue samples from lungs, bronchi and bronchial lymph nodes were taken and fixed in a neutral buffered formalin 10% then processed and paraffin sections of 5 micron thickness were prepared and stained with H&E for histopathological examination *(14)*.

Statistical analysis: Statistical analysis of the obtained data were performed *(15)*.

RESULTS

Seven lambs out of 25 were died from bacterial pneumonia with 28% mortality rate . The obtained results were tabulated in Table 1- 3.

Bacteriological examinations

The bacteriological examinations of nasopharyngeal swabs of the examined lambs

The bacteriological examinations of nasopharyngeal swabs of the examined lambs and the biochemical identification of the clear isolates ***(10, 11)***, revealed that, the major bacterial agents of pneumonia in lambs were , *Pasteurella haemolytica (Mannheimia haemolytica)* 5 (20%), *Esherichia coli*4 (16%), and *Staphylococcusaureus* (12%).There were also mixed infection among the examined lambs Table 1.

Biochemical analysis of the Blood serum and plasma

The results of biochemical analysis of the examined serum and plasma are tabulated in Table 2,3.

The levels of acute phase proteins (APP) are very low in healthy lambs comparing with pneumonic ones. The levels of Hp, CRP ,and plasma fibrinogen PF are very low in Gp-1 (Healthy lambs) ,regarding to pneumonic lambs Gp-2, there were a significant increase in the concentration of serum Hp,CRP and Plasma fibrinogen. Regarding to serum albumin, there is a significant decrease in pneumonic lambs accompanied by significant increase in globulins and serum total proteins.

Pathological Findings

Macroscopically: The affected lungs were severely congested with dark grayish areas in pulmonary parenchyma, particularly in the anterior lobes. Others showed consolidated areas. The cut surface revealed frothy exudate, the consistency of most examined lungs was slightly firm and tough. In some cases, multiple hemorrhagic, emphysematous and consolidated areas were found.. The examined bronchial lymph nodes were enlarged ,congested and sometimes hemorrhagic.

Microscopically: The lungs of the examined lambs showed edema, capillary hyperemia and thrombosis in some blood vessels as well asneutrophilic infiltration (with *E.coli* infection) in the alveolar and bronchiallumina, and thickening in the wall of some blood vessel due to fibrosis were seen in few cases.(Fig.1). Hyperemia of the interalveolar capillaries, thickening of the interlobular septa, together with alveolar edema and leukocytic infiltrations, in addition to the presence of emphysematous areas were also seen (Fig.2). In some cases (with *P.hemolytica* infection), lungs showed Fibrinous pneumonia which characterized by accumulation of fibrinous exudate inside most of the lung alveoli with leukocytic infiltration mainly neutrophils and macrophages (Fig.3). Lungs in most cases (with *S.aureus* infection) showed areas of emphysema, others were consolidated with congestion of pulmonary blood vessels (Fig.4). Desquamation of the epithelial lining of the bronchioles in addition to accumulation of pus inside bronchial lumina had been noticed (Fig.5) .Some cases showed multiple focal Suppurative pneumonia represented by abscess formation with central eosinophilic area and leukocytic aggregations (Fig.6). In most of pneumonic lambs the bronchial lymphnodes were congested in addition to depletion of lymphocytes (Fig.7).

Table 1.Types of bacterial isolates from the examined pneumonic lambs

No.	Pure culture			Mixed culture		
	Type of Bacteria	No.	%	Type of Bacteria	No.	%
1	*Pasteurella hemolytica*	5	41.66%	*Staphylococcus aureus* & *Pasteurella hemolytica*	4	30.76
2	*Escherichia coli*	4	33.33%	*Pasteurella hemolytica* & *Escherichia coli*	5	38.46
3	*Staphylococcus auerus*	3	25%	*Escherichia coli* & *Staphylococcus aureus*	4	30.76

Table 2. Mean Values of Serum albumin, Globulins,Total proteins and Plasma fibrinogen in both healthy and Pneumonic lambs.

Parameter	Healthy Lambs Gp- 1	Pneumonic Lambs Gp- 2
Albumin (gm/dl)	.086± 3.45	2.71 ± 0.39*
Globulins (gm/dl)	2.45± 0.51	3.51 ±0.46 *
Total proteins (gm/dl)	0.63±5.90	0.59 ± 6.22*
Plasma fibrinogen (mg/dl)	4.36±365.11	5.45 ±554.21**

Table 3. Mean Values of Serum C-reactive proteins, Haptoglobins in both healthy and Pneumonic lambs.

Parameter	Healthy Lambs Gp- 1	Pneumonic LambsGp-2
C- reactive proteins	3.48± 0.61	7.59±0.96**
Haptoglobins	0.24±0.06	0.98± 0.37*

*Significant at P< 0.05

** Highly significant P < 0.01

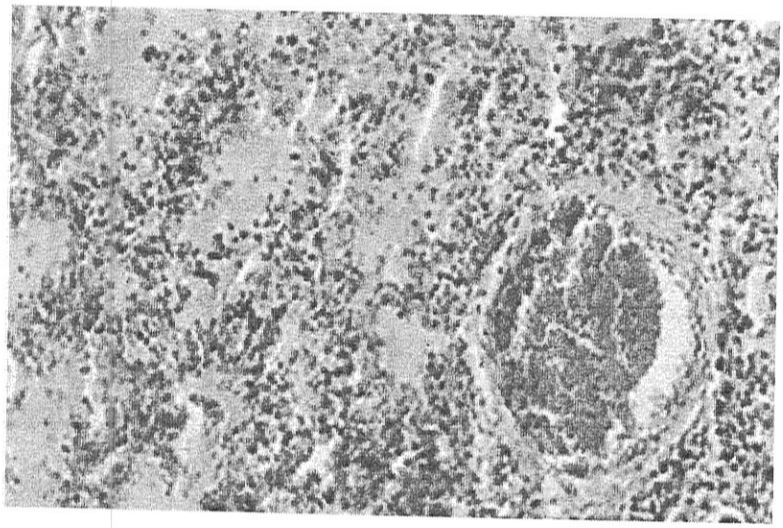

Fig.1. Photomicrograph of lung of pneumonic lamb due to mixed infection with *E.coli* & *P.hemolytica* showing: Thrombosis in blood vessels, serous exudate in some alveoli and neutrophilic infiltration H&E X120.

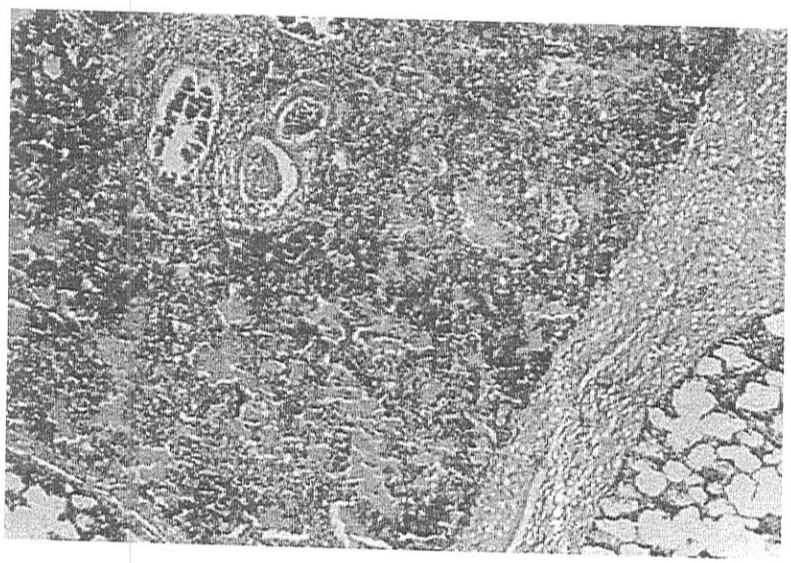

Fig.2. Photomicrograph of lung of pneumonic lamb due to *E.coli* infection showing thickening of the interlobular septa together with alveolar edema in addition to the presence of emphysematous areas and hyperaemia of interalveolar capillaries. H&EX120

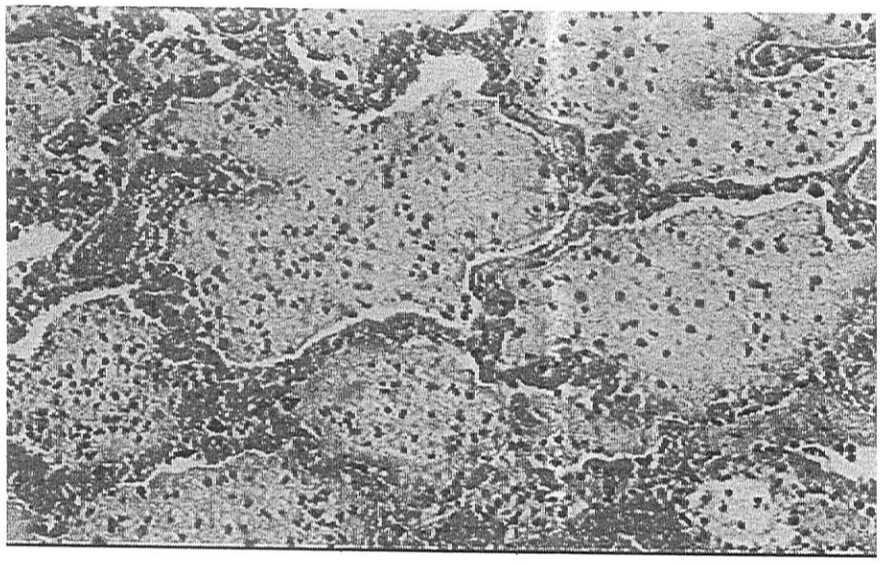

Fig.3. Photomicrograph of lung of pneumonic lamb due to *P.hemolytica* infection showing fibrinous pneumonia which characterized by accumulation of fibrinous exudate inside the lung alveoli with leukocytic infiltrations mainly neutrophils and macrophages. H&EX 300

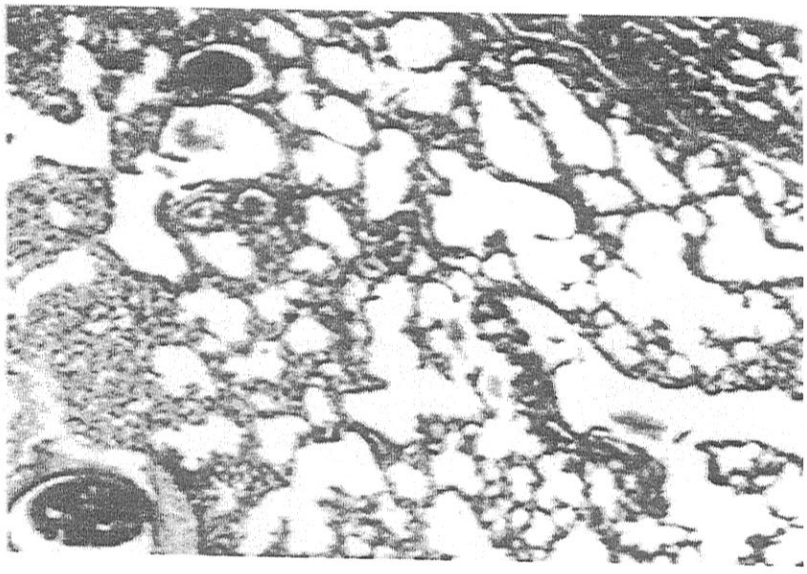

Fig.4. Photomicrograph of lung of pneumonic lamb due to *E.coli &S.aureus* infection showing emphysematous areas together with congestion of pulmonary blood vessels and interalveolar capillaries. H&EX 300

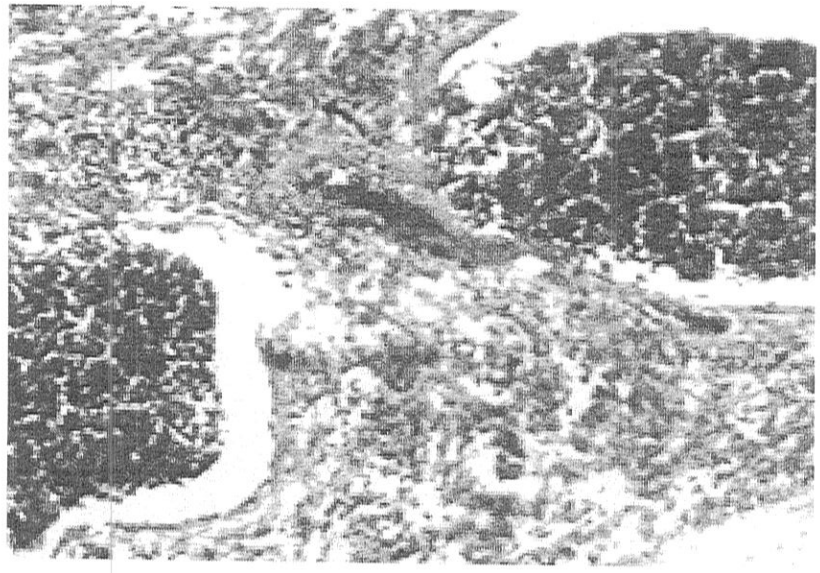

Fig.5. Photomicrograph of lung of pneumonic lamb due to *S.aureus & P.hemolytica* infection showing desquamation of epithelial lining bronchi and accumulation of pus inside bronchial lumina H&EX 200

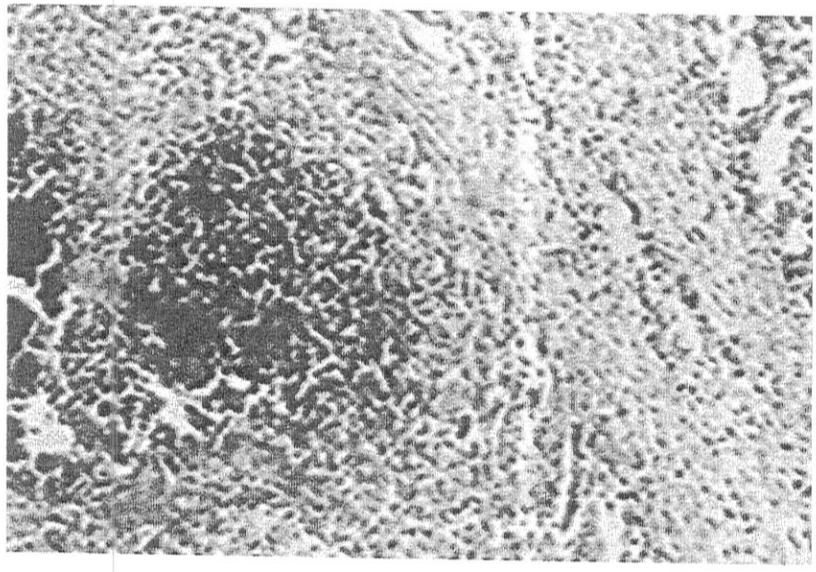

Fig.6. Photomicrograph of lung of pneumonic lamb due to *E.coli & S.aureus* showing focal suppurative pneumonia represented by abscess formation with central eosinphilic area and leukocytic aggregations H&EX100

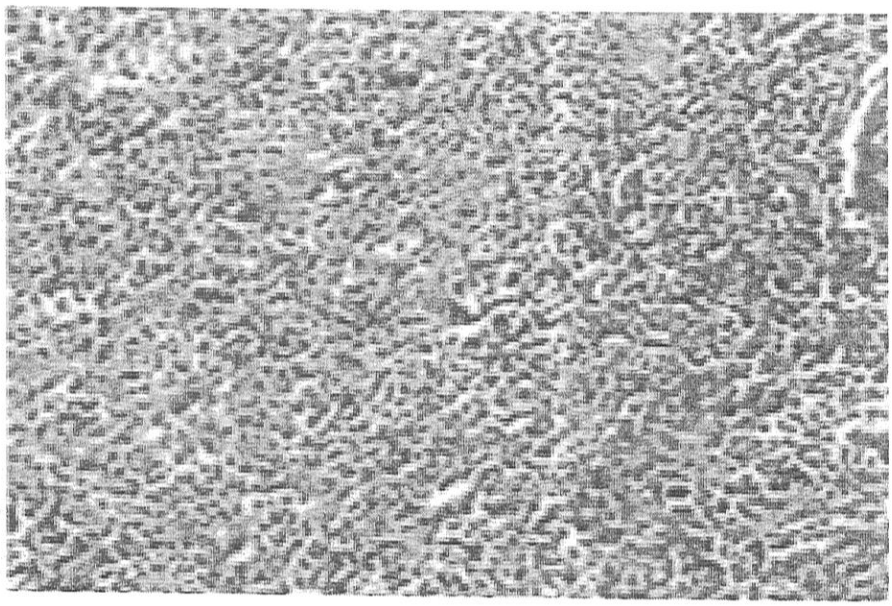

Fig.7. Section of bronchial lymph node of pneumonic lamb showing depletion of lymphocytes H&EX300

DISCUSSION

Sheep pneumonia is one of the most common causes of mortality and morbidityespecially in newborn and feedlot lambs. Pneumonia is caused by a complex interaction between the environment, which produces stress, microorganisms and the immune response of the host ***(16,1,2)***.In the current study the mortality rate (28%) among pneumonic lambs naturally infected with *Staphylococcus areus* , *E.coli* and *Pasteurella haemolytica* was .Previously published records ***(17)*** showed that ,bacterial pneumonia in lambs was caused by *E.coli* (24.70%), *Staphylococcus aureus*(10.40) and *Pasteurella multocida* (15.50%) *Pasteurella hemolytica* constitutes (24.44%) ,mixed bacterial infection was also recorded with different percentages ***(18,19)***. Nearly similar results were recorded in this study where pneumonia caused by E.oli represents 24%, *Pasteurella hemolytica* 24% and *Staphylococcus aureus* represents (52%) ; this higher percentage may be due to the bad environmental stress during the period of this experiment.

APP are serum molecules which are synthesized by many cells categories specially hepatocytes,the quantitative changes of APP are subsequent of hepatocytes dysfunction that correlated with plasma proteins synthesis . CRP, Hp,PF are the most important indicators of the inflammatory process in sheep as these blood proteins change in concentration in animals subjected to infection , inflammation or stress ***(9,20,21)***. During bacterial paumonia APP (Hp,CRP&PF) are associated with the severity of disease and serve as biomarkers and are functionally significant ***(28)***, CRP concentration for example is increased in acute inflammation, this confirms that the extrahepatic cells may secreted CRP. The main function of CRP is to recognize, connect

and detoxify or remove from blood toxic substances that arise from injuried tissues. Fibrinogen is involved in homeostasis which leads to fibrin formation ,it is also used as an indicator of bacterial infection *(29)*. Haptoglobin inhibits bacterial development by binding iron, depriving bacteria of this element *(20)*.

In this study, the biochemical analysis of serum and plasma revealed that the levels of CRP ,Hp,PF are low in the examined healthy lambs compared with pneumonic ones. The values of CRP, Hp, PF are influenced by the severity of pneumonia in the examined lambs. These results were in agreement with the results recorded by several authors *(20,22,23)*, which recorded that, the acute phase proteins in parallel with the inflammatory reactions, they added that the basic function of CRP is to recognize, connect and detoxify or remove the toxic substances arising from the damaged tissues from blood, similar results were obtained previously *(24,25,26)*, as the quantitative changes of CRP, Hp, PF, Albumin and T.proteins are synthesized by many cell categories specially hepatocytes and during inflammatory processes. Our results confirm these findings where the values of CRP, Hp, PF are significantly increased in pneumonic lambs with severe inflammatory reactions.

Gross changes of lungs of pneumonic lambs in this study, were severe congestion with dark grayish areas in pulmonary parenchyma with the presence of emphysematous and consolidated areas besides multiple hemorrhagic areas on the lung surface. The cut surface revealed frothy exudate. Meanwhile, congestion of the lungs was the most pathologic lesions observed in pneumonic sheep, in addition to presence of consolidated areas and voluminous lungs *(17,19)*.

Microscopically, the lungs of the examined pneumonic lambs; showed edema, hyperemia, and thrombosis of the blood vessels with neutrophilic exudation and thickening of the wall of some blood vessels as previously described *(2,27)*. Depending on the nature and pathogenicity of the bacterial agents, the bronchial and bronchiolar epithelium showed several pathological changes; varies from hyperplasia, desquamation and necrosis of their epithelial lining, these findingscome in line with the results of the present study where desquamation of epithelial lining, thickening and hyperplasia of bronchial &bronchiolar wall were parallel with the type and severity of pneumonia in examined lambs *(7)*. The interlobular septa was thickened with purulent or fibrinous exudate filling lung alveoli , some bronchi were filled with pus, while, fibrinous pneumonia in lambs represented by presence of fibrinous exudate in lung alveoli with capillary hyperemia were obtained previously *(19)*.

The present study revealed also accumulation of pus inside bronchial lumina, in addition to multiple focal suppurative pneumonia of lung tissue. Abscesses formation replacing lung alveoli was seen. Similar results were recorded *(8)*, which describe abscesses in lungs of sheep naturally infected with *Staphylococcus aureus* in addition to aggregations of neutrophils or suppuration with central eosinophilic areas.

CONCLUSION

From this study we concluded that: *E.coli, Staph.aureus and P.hemolytica* are the most common causes of pneumonia in lambs in in certain farms in Zagazig city, causing high mortality reached 28%among pneumonic lambs. A significant increase in the level of serum CRP, Hp, T.Proteins and PF. and also a significant decrease in serum albumin in pneumonic lambs were recorded. APP are indicative of inflammation or infection in lambs . Histopathological changes revealed , suppurative and fibrinous pneumonia with severe congestion of pulmonary blood vessels.

Thickening of interlobular septa and, presence of emphysematous and consolidated areas in the lungs with enlarged and inflammed bronchial L.Ns. had been detected.

Abbreviations
APP : Acute Phase Proteins
CRP: C-Reactive Proteins
PF : Plasma fibrinogen.
Hp :Haptoglobins
T.Proteins: Total proteins

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الملخص العربى

دراسات بكتريولوجية وباثولوجية على الالتهاب الرئوى فى الحملان مع الاشارة الى دلالات الالتهاب

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معهد بحوث صحة الحيوان (معمل فرعى الزقازيق)- مركز البحوث الزراعية

أجريت تلك الدراسة على عدد٥٠ من الحملان (عمر ٣-٦ أشهر) ببعض المزارع بمدينة الزقازيق قسمت الى مجموعتين :المجموعة الاولى (مجموعة ضابطة) تتكون من ٢٥ حملا بحالة صحية جيدة. والمجموعة الثانية ايضا تتكون من ٢٥ من الحملان و تعانى من اعراض تنفسية شديدةوارتفاع فى درجة الحرارة .تم اخذ مسحات من التجويف الانفى والبلعومى للفحص البكتريولوجى كما تم سحب عينتين من الدم من كل حمل.

العينة الاولى وضعت على مانع تجلط لقياس مستوى Plasma fibrinogen(PF) .ووضعت العينة الثانية بدون مانع للتجلط لقياس دلالات الالتهاب (Hp-CRP—T.proteins- Albumin) . كما اخذت عينات من اعضاء الحملان النافقة (الرئة- الشعب الهوائية والغدة الليمفاوية الشعبية) للفحص الباثولوجى .وتبين منهذه الدراسة ارتفاع معدل نفوق الحملان الى ٢٨% وان الفحص البكتيريولوجى لمسحات التجويف الانفى والبلعومى قد أثبت أن البكتيريا المسببة للالتهاب الرئوى فى الحملان فى بعض المزارع بمدينة الزقازيق هى :

(*E.coli.,S. aureusandP.hemolytica*) . وتبين من نتائج الدراسة حدوث زيادة معنوية لمستوى دلالات الالتهاب فى مصل الحملان المريضة (Hp-CRP—T.proteins) اضافة الى زيادة فى نسبة الفايبرينوجين فى بلازما الدم مقارنة بحملان المجموعة الضابطة . وذلك كرد فعل اولى لحدوث الالتهاب والعدوى المرضية . كما تبين ايضا حدوث نقص فى مستوى Albumin وزيادة فى مستوى T.proteinsفى الحملان المريضة مقارنةبالمجموعة الضابطة. وقد اكد الفحص الباثولوجى حدوث التهاب شديد بالرئتين والشعب الهوائية مع حدوث التهاب صديدى وفايبرينى بالرئتين .كما وجد تنكس بجدر الشعب الهوائية وتجمع صديدى بتجاويف الشعب الهوائية .وتبين وجود تمدد بالرئتين فى معظم الحالات التى تم فحصها.نستخلص من هذه الدراسة ان اهم المسببات البكتيرية للالتهاب الرئوى فى الحملان بمدينة الزقازيق هى :

E.coli.,S. aureusand P.hemolytica. كما أشارت الدراسة الى اهمية معرفة دلالات الالتهاب كمؤشر تشخيص مبكر للعدوى والالتهاب . وأكد الفحص الباثولوجى وجود التهاب شديد وتمدد بالحويصلات الهوائية بالرئتين اضافة لوجود التهاب صديدى وفايبرينى بالرئتين.