Pathological And Bacteriological Studies On Lung Affections Of Cows At Kaluobia Governorate

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ABSTRACT

This study was carried out on 150 lung samples and its bronchial lymph nodes of cows slaughtered at Kaluobia abattoir during three months (between Aug. 2007 and January 2008).Bacterial isolation was recoded in 32 lung (21.3%) and 13 lymph nodes samples (8.66%). The isolated microorganisms from lung were *Staph. aureus, E. coli, Streptococcus spp., Pseudomonas aeruginosa, Corynebacterium spp. and Pasteurella spp.* in 7(4.7%), 6(4%), 4(2.7%), 4(2.7%), 3(2%) and 3(2%) respectively. Mixed bacterial infection was also found in 5(3.33%).

The isolated microorganisms from bronchial lymph nods were *Staph. Aureus, Pseudomonas aeruginosa, Corynebacterium spp., and Streptococcus pneumoniae* in 5(3.3%),3(2%), 1(0.7%) and 1(0.7%), respectively. ***E. coli*** and ***Pasteurella spp***. didn't isolated from bronchial lymph nodes. Mixed bacterial infection was also found in 3(2%).

The antibiotic sensitivity test for isolated microorganisms revealed that Gentamycin (30 µg) and Erythromycin (15 µg) were the most sensitive antibiotics of choice.

The pathological examination revealed variable histopathological alterations which may be attributed to the immune status of the animal as well as the type and stage of infection. Also in cases of mixed bacterial infection the pathological alteration was comparatively severe. The histopathological changes in correlation to each bacterial infection were described.

From the present study it was concluded that the bacterial causes of pneumonia in cattle were numerous resulted in variable pathological lesions. Animal, housing, feeding are important to minimize the occurrence of infection. Once infection occurred, proper diagnosis of the causative agent and subsequently proper treatment based on antibiotic sensitivity test is the correct way of control.

INTRODUCTION

Lung affections and respiratory diseases are major source of economic losses among cattle , buffaloes and other animal species ***(1)***. The causes of lung affection specially pneumonia are numerous and may be viral, fungal, parasitic, bacterial and / or mixed infection. Five hundred cows and buffaloes slaughtered in Ismailia Governorate, from and their lungs and bronchial lymph nodes were collected and bacteriological examinations revealed the infection of 65 cows (19.34%) and 31 buffaloes (18.9%) with *staph. aureus, streptococcus pneumoniae, corynebacterium bovis, E. coli, Bacillus species, Pseudomonas aeruginosa* and mixed infection ***(2)***. Bacteriological examinations 68 lung samples from 1-3 years old buffalo- calves at Assiut Governorate indicated that 66 (97.06%) samples were positive for mixed bacterial infection, while the other two samples (2.94%) were bacteriologically negative ***(3)***. Another study showed that mortality rate due to pneumonia among dairy cattle was 26% ***(4)***. Respiratory affections specially pneumonic pasteurellosis was the most common cattle respiratory disease caused by *Pasteurella multocida* ***(5)***. *Staph. aureus* and *strept. Pneumoniae* were also isolated from pneumonic lungs ***(6)***. *E. coli* and *Pasteurella multocida* as a main cause of pneumonia were found ***(7,8)***. These bacterial infections caused different pathological changes in infected lung

as hemorrhages, edema, leucocytic infiltration, alveolar emphysema and proliferation of bronchiolar epithelium and trachitis *(2,9,10).*

The present work was aimed to isolate the causative agents of lung affections in cows at Kaluobia Governorate to investigate a relationship between these bacterial agents and their detected pathological lesions *in vitro*, pathogenicity and virulence of the isolated microorganisms in vitro.

MATERIAL AND METHODS

1-Animals

One hundred and fifty cows of 5-12 years old slaughtered at Kaluobia Governorate abattoir, during October 2007 to January 2008, were used for the present study. Tissue samples from lungs and bronchial lymph nodes were subjected to bacteriological and pathological investigations.

2-Samples

Tissue specimens from 150 lungs and 150 bronchial lymph nodes were collected under aseptic condition and divided into two parts. The 1st.part was taken in cold container to the laboratory and within one hour submitted to bacterial isolation and identification. The 2nd.part was fixed in 10% neutral buffered formalin for histopathological examination.

3- Bacteriological studies

Under aseptic conditions the collected samples were cultured into nutrient broth at 37C for 24h, and then sub-cultured into the following nutrient agar (Difico), 5% sheep blood agar , macConkey agar and SS agar. Biochemical tests were performed (Indol production, Methyl red, Voges proskaur,Citrate, urease and sugar fermentation test for identification of *E.coli* and *pseudomonas aeruginosa*, Coagulase test, Dnase test and heamolysis in addition to catalase and oxidase test for identification of *staph.aureus.* The obtained isolates were identified *(11-13).*

a-Antibiotic sensitivity tests

The sensitivity of bacterial isolates against different antibiotic was done using antibiotic disks (Biomerieux). Erythromycin (15 µg), Garamycin (30 µg), Kanamycin (30 µg), Neomycin (30 µg), Oxytetracycline (10 µg), Spectinomycin (10 µg), Chlormphenical (30 µg) and Ampicillin (10 µg) were used for this purpose .

b-Pathogenicity and virulence of some isolates (*in vitro*)

1- *Pasteurella spp. (14)*

Four white mice weighting 18-22 g were used for each isolate. All mice were injected intraperitoneally (I/P) by 0.1 ml of bacterial suspension of $1.5x10^8$ CFU. One mouse was kept as a control for each isolate and injected I/P with 0.1 sterile normal saline.

The mortality rate and post-mortem changes were recorded. From heart blood of dead mice, re-isolation of inoculated strains was carried out. Blood films were prepared and stained with Leshiman´s stain for detected the bipolarity of Pasteurella spp.

2- *Staph. aureus*

Also Staph. Aureus was inoculated in sterile rabbit plasma and observed from 1h., 12hrs, 24hrs and the time of coagulation must be recorded.

4- Pathological studies

Gross examination of lungs and associated lymph nodes was carried out. Samples from lungs and lymph nodes were preserved in 10% neutral buffered formalin washed in running water and dehydrated in different grades of concentrated alcohol, cleared in zylene and embedded in paraffin. Paraffin sections of 5 µ thickness were obtained and stained by Haematoxylin and Eosin *(15)* and examined for histopathological lesions

RESULT

Table 1. The number and percentage of microorganisms isolated from both lungs and lymph nodes of Cows.

Microorganism	No. of bacterial isolates from lungs.		No. of bacterial isolates from bronchial L.N.		Total No. of isolates	
	No	%	No	%	No	%
Single infection *Staph . aureus*	7	4.7	5	3.3	12	8
E. coli	6	4.0	-	-	6	4.0
Strept. pneumonia	4	2.7	1	0.7	5	3.3
Pseudomonas aeruginosa	4	2.7	3	2.0	7	4.7
Pasteurella spp.	3	2.0	-	-	3	2.0
orynebacterium Spp.	3	2.0	1	0.7	4	2.7
Mixed infection *S. aureus + E. coli*	2	1.3	1	0.7	3	2.0
S. aureus + seudomonas aeruginosa	2	1.3	2	1.3	4	2.7
E. coli +Strept. pnumoniae	1	0.7	-	-	1	2.7

The percentage was calculated according to the number of lungs (150) and bronchial lymph nodes (150).

Table 2. Antibiogram of the isolated microorganisms

Isolates	Gara-mycin 30µg	Chlor-amphicol 30µg	Erythro-mycin 15 µg	Kana-mycin 30µg	Neomycin 30µg	Ampi-cillin 10µg	Oxyte-tracycline 30µg	Spectin-omycin 10µg
Staph aureus	+++	-	+++	+	-	+++	+++	-
Corynebacterium spp	+++	-	+++	--	-	+++	++	-
Streptococcus pneumoniae	++	-	++	-	-	+++	+++	-++
Pseudomonas aeruginosa	+++	-	-	+	+	+	-	-
Pasteurella spp.	+++	-	-	-	-	+++	++	-
E. coli	+++	-	+++	+++	+	-	-	-

Table 3. Pathogenicity and Virulence of isolated *Pasteurella spp.* in mice (*in Vitro*)

No. of Isolates	No. of inoculated mice	Time of death post intraperitonial inoculations			Mortality rat	P.M. lesions
		Within 12hrs.	Within 24 hrs	Within 48hrs.		
3	12	8	3	1	100%	Acute septicemia

Table 4. Co- agulas test for staph aureus isolated from lymph nodes of cows.

No. of Isolates	Time of coagulation					
	Less than 1hr		12 hrs		24hrs	
	No.	%	No.	%	No.	%
12	6	50	4	33.35	2	16.65

The pathological findings

A- Gross examination

Most of the examined lungs were apparently normal, except in few cases in which the diaphragmatic and cardiac lobes showed dark red purplish coloured areas of firm texture in one case, the pleura covered these areas was cloudy.

B- Histopathological examination

1- Lungs infected by *Staphylococcs aureus*

In most of the cases examined hyperplasia of mucous cells lining the bronchi with thickened congested alveolar capillaries , filling of alveolar lumen by mucous exudates and few mononuclear inflammatory cells infiltrations were observed (Fig. 1). While in few cases lesions were more sever and characterized by desquamation of bronchial epithelium, pribronchial mononuclear inflammatory cells infiltration, thickened of alveolar epithelium, congested perialveolar capillaries, filling of the alveolar lumen with eosinophilic homogenous exudates and polymorphneuclear leucocytes (Fig.2).

2-Lungs infected by E-coli.

The affected lunges mostly showed congested, dilated alveolar capillaries, focal hemorrhages, filling of some alveoli with homogenous esinophilic exudates with few mononuclear inflammatory cells infiltration (Fig.3).

3-Lungs infected by Streptcoccus spp.

The most of the examined cases showed hyperplasia of bronchial epithelium, peribronchial mononuclear inflammatory cells infiltration, dilated congested perilaveolar capillaries, focal hemorrhages, and focal mononuclear inflammatory cells infiltration (Fig. 4).

In some cases focal areas of loss of cellular details and architectures with severe polymorphneuclear leucocytes aggregation and infiltrating the surrounding alveoli were observed (Fig 5).

4-Lungs infected by *Pseudomonas aurginosa*

Most of the examined lungs showed congested dilated alveolar capillaries, and hyperplasia of bronchial epithelium. In few cases there was filling of alveolar lumen with homogenous esinophilic exudate and massive mononuclear inflammatory cells infiltration (fig 6).

5- Lungs infected by Pasteurella .

Infected lungs showed congested blood vessels, serofibrinous inflammation represented by filling of alveolar lumen by serofibrinous exudat with fibrin threads and mononuclear inflammatory cells infilteration. The bronchial lumen was filled with fibrin mash entangled detached epithelial cells and few mononuclear inflammatory cells which also infiltrated in propria submucosa as well as peribronchial tissues (Fig 7& 8). Edema of interlobular tissues as well as thickened pleura with mononuclear inflammatory cells infiltration were seen in few cases

6-Lungs infected by Corynbacterium spp.

The examined lunges showed thickened edematous interlobular septa, filling of the alveolar lumen by massive number of neutrophils and large foamy macrophage, with congested dilated alveolar capillaries and proliferation of type 2 pneumocytes (Fig.9).

7- Lungs infected by Mixed infection of staph. aureus. with E.coli .

Lesions were sever and showed desquamation of bronchial epithelium. peribronchial mononuclear inflammatory cells infiltration, diffuse hemorrhages, and sever inflammatory cells infiltration (Fig 10).

8-Lungs infected by -mixed *staph aureues* and *Psudeomonas Aurginosa.*

The lesions were so severe with impacted bronchial lumen by neutrophiles and detatched epithelial linings with homogenous esinophilic exudates, the surrounding alveoli showed filling of alveolar lumen by homogenous esinophilic exudate neutrophils and few mononuclear inflammatory cells infiltration (Fig 11).Focal areas showed filling of alveolar lumen by large macrophage and mononuclear inflammatory cells (Fig.12).

9-Lungs infected by Mixed infection of *E.coli* and *Strept. pneumonia*.

Dilatation and edema of interlobular tissues, thickened interalveolar septa, dilated capillaries, filling of alveolar lumen by neutrophils and perivascular mononuclear inflammatory cells infiltration were observed (Fig 13&14).

Affection in bronchial lymph nodes

The pathological alteration in examined lymph nodes were nearly similar and mostly revealed serous lymphadenitis with edema of sinuses and cellular infiltration in the cases infected with *Staphylococcs aureus* , (Fig.15), neutrophiles were seen in some cases infected with *Pseudomonas aurginosa*,and mixed *staph aureues* and *Psudeomonas aurginosa*. Depletion of lymphocytes or hyperplasia were also recorded in cases infected with *Corynbacterium spp, Strept coccus spp* . *and Pseudomonas aurginosa* (Fig.16).

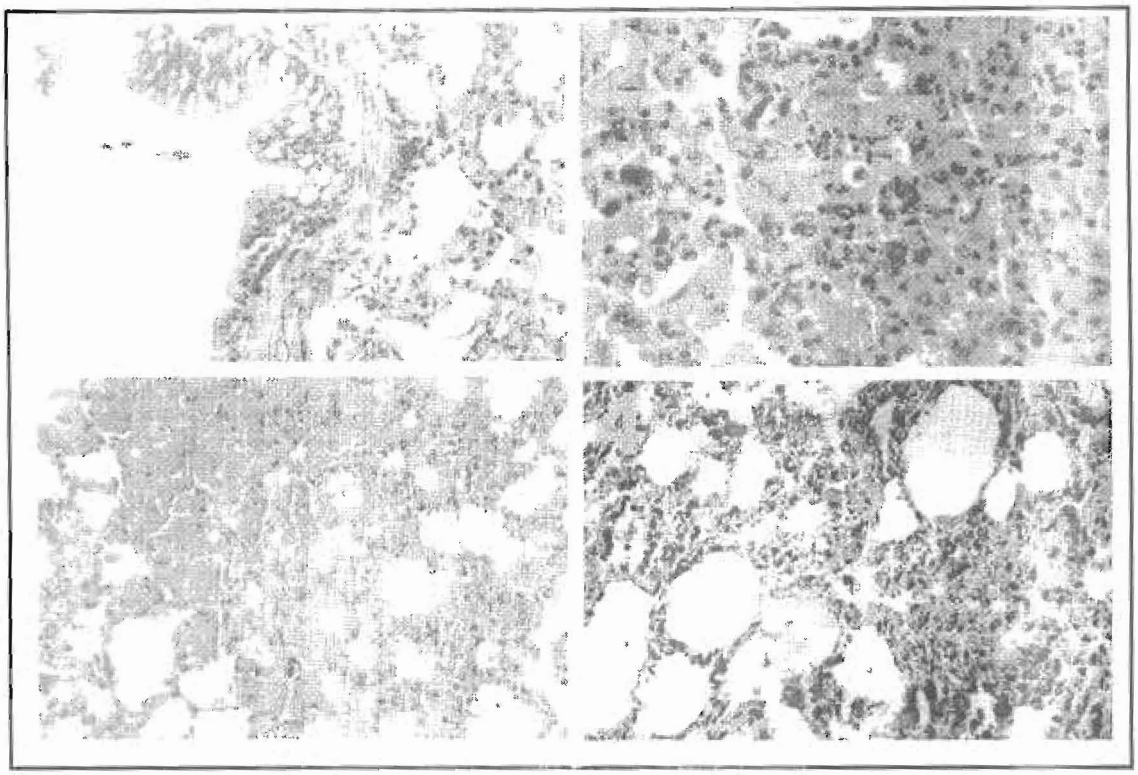

Fig. 1. Lungs of cow infected with *staph.aureus* showing hyperplasia of mucous bronchiolar secreating cells with congestion of interalveolar capillaries.(H&E × 200).

Fig. 2. Lungs of cow infected with *staph.aureus* showing thickning of alveolar epithelium, filling of the alveolar lumen with eosinophilic homogenous exudates and infilteration of polymorphneuclear leucocytes.(H&E × 400).

Fig. 3. Lungs of cow infected with *E-coli*. . showing congested, dilated alveolar capillaries, focal hemorrhages, filling of some alveoli with homogenous esinophilic exudates with few mononuclear inflammatory cells infiltrations (H&E × 200).

Fig. 4. Lungs of cow infected with *Strept coccus* showing dilated congested perilaveolar capillaries and focal hemorrhages,.(H&E × 200).

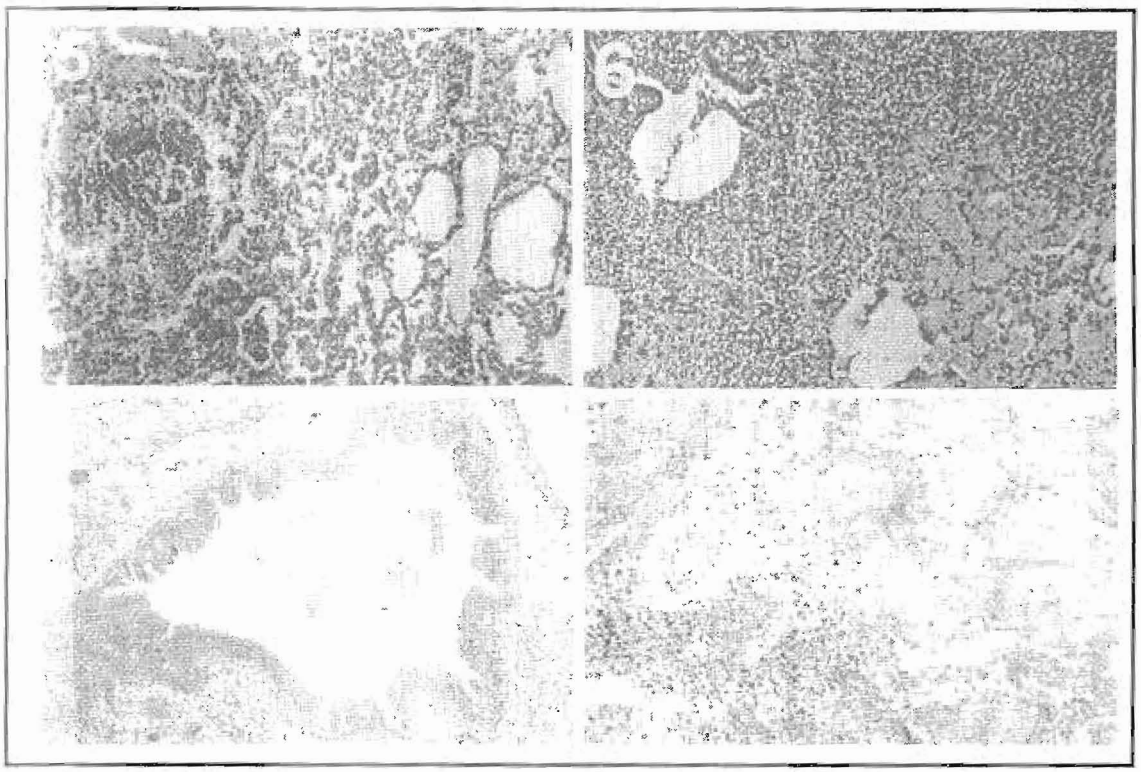

Fig. 5 Lungs of cow infected with *Strept coccus spp*. showing focal areas of loss of cellular details and architectures with sever polymorphneuclear leucocytes aggregation and infiltrating the surrounding alveoli (H&E × 200).

Fig. 6. Lungs of cow infected with *Pseudomonas aurginosa* showing filling of alveolar lumen with homogenous esinophilic exudate and massive mononuclear inflammatory cells infiltration.. (H&E × 100).

Fig. 7 Lungs of cow infected with *Pasteurella* .. showing filling of the bronchial lumen with fibrin mash entangled detached epithelial cells and few mononuclear inflammatory cells which also infiltrated in propria sub mucosa as well as peribronchial tissues (H&E × 100).

Fig. 8. Lungs of cow infected with *Pasteurella* showing serofibrinous inflammation represented by filling of alveolar lumen by serofibrinous exudat with fibrin threads and mononuclear inflammatory cells infilteration (H&E × 400).

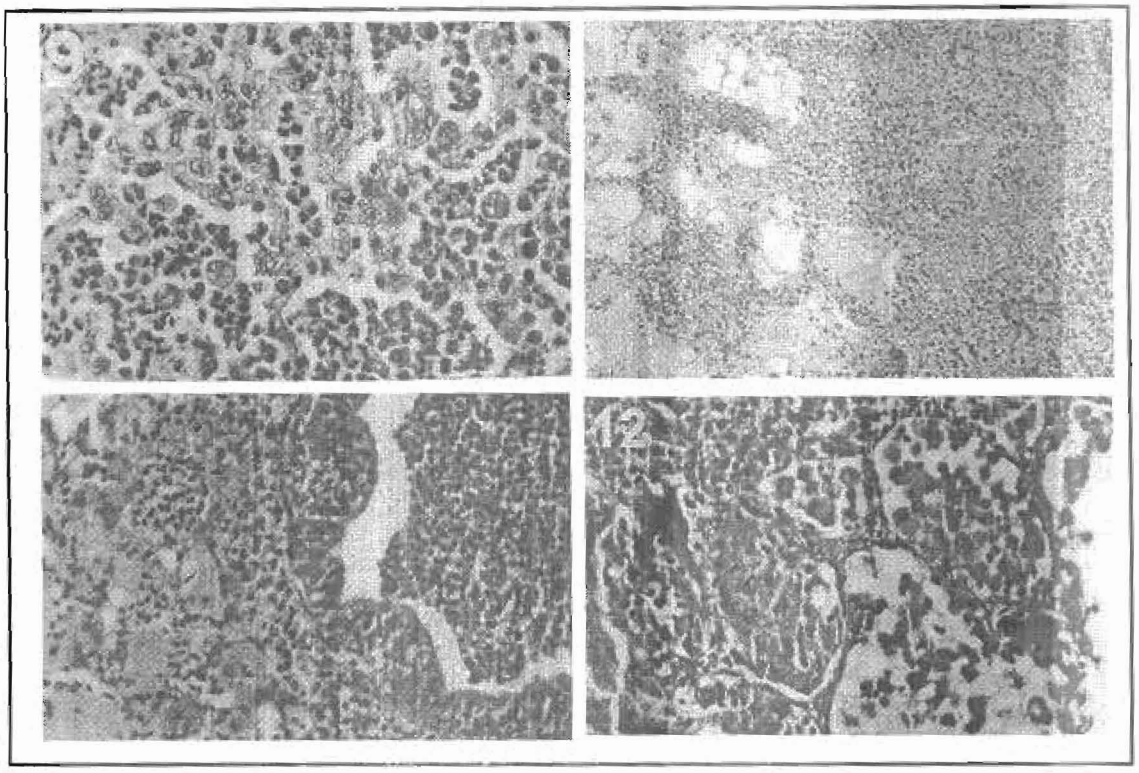

Fig. 9. Lungs of cow infected with *Coryn-bacterium spp* showing filling of the alveolar lumina by massive number of neutrophils and large foamy macrophage, with congested dilated alveolar capillaries and proliferation of type 2 pneumocytes.(H&E × 650).

Fig. 10. Lungs of cow infected with mixed *infection of Staph. aureus. with E.coli* showing diffuse hemorrhages, filling of alveoli with homogenous esinophilic exudate and sever inflammatory cells infiltration (H&E × 200).

Fig. 11. Lungs of cow infected with mixed *staph aureues and pseudomonas aurginosa* showing impacted bronchial lumen by neutrophils and detatched epithelial lininges with homogenous esinophilic exudates. The surrounding alveoli showed filling of alveolar lumen by homogenous esinophilic exudate neutrophils and few mononuclear inflammatory cells infiltration.. (H&E × 400).

Fig. 12. Lungs of cow infected with mixed *staph aureues and pseudomonas aurginosa* showing filling of alveolar lumen by large macrophage and mononuclear inflammatory cells.. (H&E × 400).

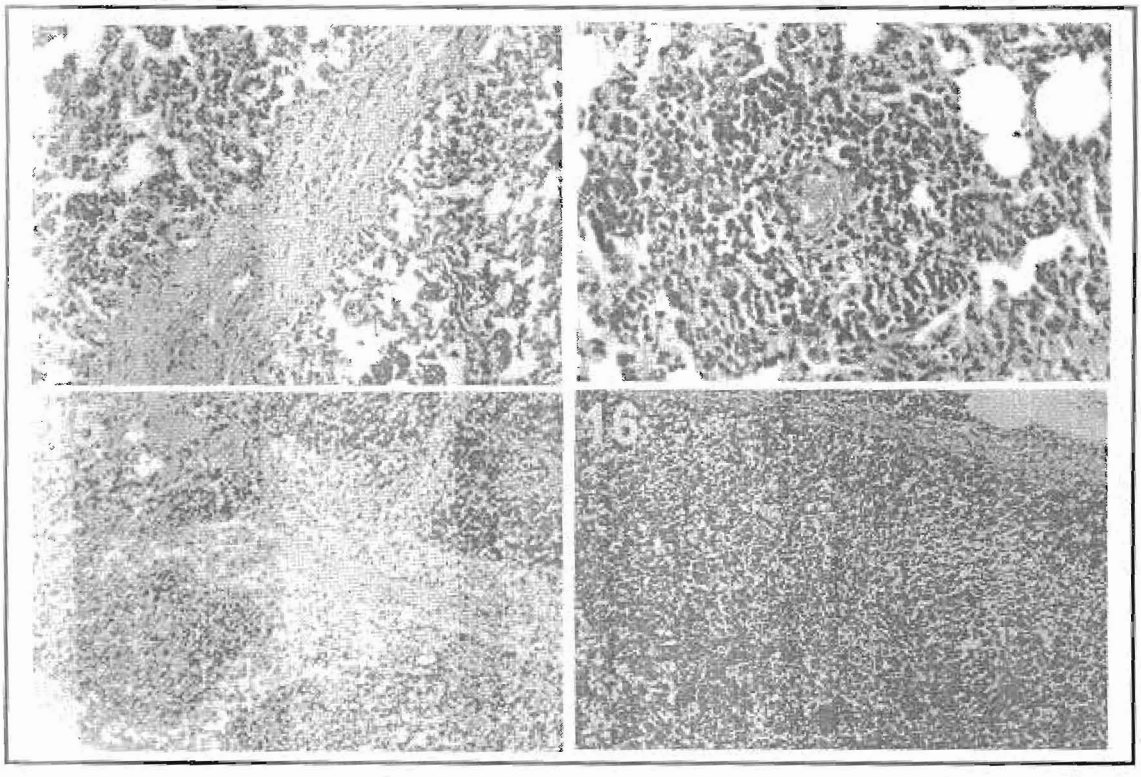

Fig. 13. Lungs of cow infected with mixed infection of *E.coli with Strept. pneumonia.* showing dilatation and edema of interlobular tissues, thickened interalveolar septa , dilated capillaries and filling of alveolar lumen by neutrophils (H&E × 100).

Fig. 14. Lunges of cow infected with mixed infection *of E.coli with Strept. pneumonia.* showing perivascular mononuclear inflammatory cells infiltration (H&E × 400).

Fig. 15. Bronchial lymph node of cow showing serous lymphadenitis with edema of sinuses and infiltration , (H&E × 100).

Fig. 16. Bronchial lymph node of cow showing lymphoid hyperplasia (H&E × 100).

DISCUSSION

Bacteriological examination of one hundred and fifty lungs and their corresponding bronchial lymph nodes of cows slaughtered at Kaluobia Governorate revealed isolation of different pathogenic bacteria with different percentages, as shown in Table 1; a findinges which coincides with pathogens previously isolated *(2).*

Antibiogram of the isolated microorganisms revealed that Gentamycin (30 µg) and Erythromycin (15 µg) were the most sensitive antibiotic of choice (Table 2) . These results are consistent with the previous study *(16).*

The results in Table 3 revealed the virulence and pathogenicity test of *pasteurella spp.* in mice. All the isolates were highly pathogenic to mice producing acute septicemia and death within 24-48 hours post inoculation. Similar result was cited in different studies *(17-22).*

Regarding the virulence of *Staph. aureus* which resulted in clot formation in rabbit plasma (Table 4), these results agree with that previously obtained *(23).*

The pathological examination revealed varied histopathological alterations which may be attributed to the immune status of the animal as well as the stage of infection *(24)*.Also in cases with mixed bacterial infection the pathological alteration were comparatively sever.

The bronchial alterations observed were hyperplasia of epithelial lining and mucous secreting cells, with peribronchial infiltration by mononuclear inflammatory cells. These changes were associated to infection with *staph. aureus, streptococcus, Pseud.aurginosa, mixed staph. aureus. and Ecoli*, while in *pasteurella* infection, the bronchial lesions showed in association filling of alveolar lumen by desquamated epithelium, fibrinouse exudate and mononuclear inflammatory cells. Also in cases of *Pseud.aurginosa and mixed Staph. aureus. and Pseud.aurginosa* neutrophiles were impact the bronchial lumen with homogenous esionophilic exudate. The previous lesion could be attributed to the chronic irritation of the invading microorganism *(25)*.

In the pneumonic areas the most prominent alteration observed were thickened alveolar wall with congested capillaries, filling of alveolar lumen with homogenous esinophilic material. In cases with *E.coli* infection, in association with neutrophilic infiltration (micro absseces formation) there was few mononuclear inflammatory cells infiltration associated to infection with *staph aureus , strept spp, Pseud.aurginosa, mixed staph. aureus. With Pseud.aurginosa, mixed staph. Aureus* with *Ecoli and mixed Ecoli with strept. Staphylococcs aureus* adher to epithelial cells lining bronchi with lysis of erythrocytes due to their toxin represent sever irritation to pulmonary tissues results in previously mentioned lesions as described previously *(26)*. Filling of alveolar lumen with massive number of neutrophiles associated with large numberes of foamy macrophages were observed in cases with *Coryne bacterium* infection. In *pasteurella* infection, alveoli were mostly filled with serofibrinous exudate, neutophils and mononuclear inflammatory cells. Influx of neutrophils accompanied by accumulation of fibrin after fuliminant proliferation of pasteurella in upper respiratory tract and colonization of the microorganism in lower respiratory tract and in interalveolar spaces produing leuktoxine *(27)*. The changes could be attributed to the liable leuktoxine of pasteurella which is potently cytotoxic *(28)*.

These results are consistent with those described in several studies *(2,10,25,29)*. Similar lesions of chronic necrotizing pneumonia associated to *E coli* and *Coryn bacterium pyogene* infection was described by *(30, 31)*.

The lesions in the associated lymph nodes were mostly edema and serous lymphoid inflammation , lymphoid depletion and hyperplasia. The change in the lymph node reaction may be attributed to the action of the microorganism and their toxins or to the stage and severity of infection as previously mentioned *(32)*.

From the present study it could be concluded that bacterial causes of pneumonia in cattle were numerous resulted in variable pathological lesions. Proper animal caring, housing, feeding are important to minimize the occurrence of infection. Once infection occurs proper diagnosis of the causative agent and using of proper treatment based on antibiotic sensitivity test is the way of treatment to avoid complications .

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الملخص العربي

دراسات باثولوجية وبكتيريولوجية عن إصابات الرئة في الأبقار بمحافظة القليوبية

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تم أجراء البحث على عدد ١٥٠ رئة وعدد ١٥٠ غدة ليمفاوية من الأبقار المذبوحة بمجازر محافظة القليوبية .

وأوضحت الفحوص البكتريولوجية أن عدد ٣٢ رئة ايجابية للعزل البكتيري بنسبة ٢١,٣%وعدد ١٣ غدة ليمفاوية بنسبة ٨,٦٦% وأنواع البكتيريا التي تم عزلها من الرئة هي المكور الذهبي والأشر يشيا كولاى وأنواع من المكور العنقودي والسيدوموناس اورجينوزا والكورينى باكتيريم والباستيريلا وبنسب ٤,٧%و ٤% و٢,٧ % و ٢%و٢% على التوالي وكان هناك عزل لإصابات مزدوجة بأكثر من نوع من البكتريا بنسبة ٣,٣٣%. وبالنسبة للبكتريا المعزولة من الغدد الليمفاوية المصاحبة للرئة كانت المكور الذهبى وأنواع من والكورينى باكتيريم و المكور العنقودي والسيدوموناس اورجينوزا بنسب ٤٥و ٢,٧%و ٢%و ٢% على التوالي وكان هناك عزل لإصابات مزدوجة بأكثر من نوع من البكتريا بنسبة٢% من الغدد الليمفاوية. وتم عمل اختبار الحساسية على المعزولات حيث كان الجينتاميسين والأريثرومايسين أكبر تأثيرا على البكتيريا .

وبأجراء الفحوص الباثولوجية على عينات الرئة والغدد الليمفاوية المصاحبة تبين وجود تغيرات هستوباثولوجية متنوعة وربما ترجع إلى الحالة المناعية للحيوان إثناء الإصابة وشدة الإصابة ونوع الميكروب . وأوضحت النتائج أن التغيرات الباثولوجية كانت شديدة في حالات الإصابات المزدوجة بأكثر من نوع من البكتريا.

من هذه الدراسة يتضح أن البكتيريا المسببة للالتهاب الرئوي في الأبقار عديدة والتغيرات الباثولوجية المصاحبة متنوعة وان اختيار المضاد الحيوي المناسب لكل نوع من المعزولات البكتيرية يمثل أساس العلاج للأبقار المصابة.