

Pathological and biochemical studies on bacterial respiratory diseases of broilers in El-Sharkia province

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Abstract

Several respiratory diseases of broiler chickens cause losses to the poultry industry. The probable bacterial microorganisms associated with complicated respiratory diseases among broiler chickens in El-sharkia province were isolated and studied pathologically and biochemically. As Ten chickens from each different ten commercial poultry farms, 5 healthy and 5 had respiratory manifestations including weakness, gasping, dyspnea and mucous discharge at age of 30- 42 days old. A total of 140 chickens samples were processed bacteriologically as swabs and homogenized tissue samples (trachea, lungs, air sacs, hearts and hepatic tissues). Two blood samples were collected from each chicken for studying some biochemical analysis and blood gases. Coughing, depression, conjunctivitis, sneezing, frothy oculo-nasal discharge, increase respiratory rates, facial oedema, nervous signs including tremors in head & neck and variable mortalities reach up to 70% were the most detected signs. Specimens from hearts, trachea, lungs, air sacs, livers of diseased chickens were collected and prepared for histological technique. Our study revealed the prevalence of *E. coli* and *Staph. aureus* in broiler chickens in El-Sharkia Governorate. Out of a total of 140 examined samples (swabs and tissue samples), 135 (96.4%) yielded growth, of which 88 (65.1%) were *E. coli* and 69 (51.1%) were *S. aureus*. Liver function test revealed a significant change between all groups when compared with each other, meanwhile kidney function test revealed a significant increase in the mean value of creatinine compared with control group. Blood gases showed significant decrease in pH, PaO₂, while significant increase in PaCO₂ & HCO₃⁻. Severe lesions were obvious in mixed infected groups with *E. coli* and *Staph. aureus* at which macroscopically petechial haemorrhage in all internal organs with congested carcass, fibrinous airsacculitis, pericarditis were observed. While, microscopically, congested sinusoidal blood Vessels, destruction of tracheal mucosa with degenerative changes of some submucosal glands. Congestion of the cardiac bl. vs. with mononuclear cells infiltrating the pericardium, myocardium and air sacs of affected necropsied chickens. The Livers also showed fibrinous hepatitis, while lungs suffered from congestion and perivascular haemorrhage. Focal areas of caseous necrosis of pulmonary tissues with congestion also observed.

Key words: Lesser grain borer, enterococcus, *E. coli*, salmonella, staphylococcus.

Introduction

Escherichia coli (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) infections constitute one of the most important bacterial diseases affecting the poultry industry (Raji *et al.*, 2003). *E. coli* is the predominant facultative anaerobic normal bacterial flora in the avian intestine and plays an important role in maintaining intesti-

nal haemostasis. The organism is a major pathogen of worldwide importance in commercially raised poultry, contributing significantly to economic losses in both chickens and turkeys (Raji *et al.*, 2003). *S. aureus* occurs among the normal flora of live poultry and broiler chicks become contaminated with the organism within the first few days after being

hatched (**Norttermans *et al.*, 1982**). The elaboration of entero-and invasive-toxins, cytotoxic necrotizing factor (CNF), attaching and effacing genes, fimbrial adhesins and production of haemolysins, siderophores, plasmids and integrons (**Gonzalez and Blanco, 1996; Pass *et al.*, 2000**) by *E. coli* and the specific production of coagulase, hyaluronidase, staphylokinase, nuclease and penicillinase by *S. aureus* (**John, 2006**) constitute some of the pathogenic-markers by which these agents produce diseases in their hosts. Some of these markers contribute to the emergence of multiple drug resistant strains (**Laura *et al.*, 2002**) in the two organisms and consequently to therapeutic failure in humans and animals (**Van Denbogard *et al.*, 2001**). The infected chicken with *E. coli* 0157:H7 cause injured liver and kidney, the changes were more severe in days of infection in AST, ALT and Creatinine levels (**Mona *et al.*, 2012**). The challenged non treated chicken with *E. coli* developed significant increase in the activity of AST, ALT and creatinine (**Abd El-Ghany and Ismail, 2014**). Several respiratory diseases of fowls cause losses to the poultry industry throughout the country. Several authors investigated the probable bacterial agents associated with complicated respiratory diseases among broiler chickens in El -Sharkia governorate (**Saif *et al.*, 2003**). Mucous discharge, gasping sinusitis, dyspnea, swelling of sinuses, facial oedema, tracheitis, pleuritis, exudative pneumonia, airsacculitis and pericarditis characterized the respiratory infection (**Canal *et al.*, 2005**).

The aim of this study was isolate and identify the prevalence of bacteria among broiler chickens and determine the pathological lesions as well as biochemical changes associated with respiratory manifestations.

Materials and Methods

The study was carried out in 10 commercial poultry farms, ten chickens from each farm (5 healthy and 5 had respiratory manifestation) including weakness, gasping, pump-handled

respiration, dyspnea and mucous discharge at age of 30- 42 days old.

Samples for bacteriological studies:

A total of 140 chicken samples from control and diseased chickens were collected aseptically and prepared for bacteriological examination (swabs and homogenized tissue samples from trachea, Lungs, air sacs, hearts and hepatic tissues) were inoculated into 10 ml of Trypticase Soya Broth (TSB). Following 24 hours incubation at 35°C, a loopfull of vortexed broth was inoculated into nutrient agar, MacConkey agar, Eosin Methylene Blue agar and Mannitol salt agar and incubated aerobically at 37°C for 24-48 hours. The obtained isolates were identified morphologically and biochemically based on standard bacteriological methods as described by **Barrow and Feltham, (1993)** and **Garcia and Isenberg, (2007)**. Successively serological identification of the obtained isolates of *E. coli* were carried out according to **Finegold *et al.*, (1982)**.

The O-Serotyping of *E. coli* isolates was carried out according to **Denka Seiken CO., LTD., 3-4-2 Nihonbashikaya-cho, Chuo-ku, Tokyo, Japan**. The interpretation of O serogrouping results regarding to polyvalent and monovalent sera done by slide agglutination test.

The in-vitro antibiotic sensitivity test was done using disc diffusion method, the isolates were suspended in Muller Hinton broth and the suspension was adjusted to a turbidity equivalent to 0.5 McFarland standard. The Antibiotic discs were applied aseptically to the surface of the inoculated plates at an appropriate special arrangement with the help of a sterile forceps. The plates were then inverted and incubated at 37 °C for 24 hours, according to **Finegold *et al.*, (1982)**. Muller Hinton broth & agar and antibiotic discs (AMC) amoxicillin & clavulanic acid, (CIP) ciprofloxacin, (SXT) sulfamethoxazole-trimethoprim, (CRO) ceftriaxone, (S) streptomycin, (DO) doxycycline, (C) chloramphenicol, (E) erythromycin, (DA) clindamycin, (CN) gentamicin were used.

After incubation, the plates were examined and

the diameters of the inhibition zones were measured and recorded to choose the most effective drug to be recommended for use in the affected farms. The diameters of inhibition zones were interpreted according to the **NCCLS (National Committee for Clinical Laboratory Standards, 2002)**.

Biochemical studies:

Blood samples: Two blood samples were collected from each bird. The first sample was taken from the wing vein under aseptic precautions, 3 ml of blood taken without anticoagulant in a clean and dry centrifuge tube, left to clot at room temperature and centrifuged at 3000 rpm for five min. Serum samples were drawn in dry clean capped tubes and freeze at -20°C for biochemical analysis. The second blood sample was collected from brachial artery, one ml of blood on heparin in sterile syringe for blood gases analysis. Test kits were used for colorimetric estimation of the following parameters using spectrophotometer. The liver transferases (alanine aminotransferase "ALT" and aspartate aminotransferase "AST") activities (**Murray, 1984**). The serum creatinine was estimated according to **Henry, (1974)**.

Arterial blood gases analysis (PaO₂, PaCO₂ and HCO₃⁻) and pH were determined by gas analyzer, model AVL990 blood Sweden (**Samah, 2008**).

Statistical analysis:

The data obtained from this investigation were statistically analyzed by F-test according to **Tamhane and Dunlop, (2000)** using MSTAT-C computer program.

Pathological studies:

Diseased chickens with respiratory manifestations were necropsied, specimens from (trachea, Lungs, air sacs, hearts and hepatic tissues) were collected and fixed in 10% neutral buffered formalin solution. Paraffin sections of 5µ thickness were prepared and stained with haematoxylin and eosin for histopathological examination (**Bancroft and Stevens., 1996**).

Results and Discussion

In this study the prevalence of *E. coli* and *S. aureus* in broiler chickens in El-Sharkia Governorate were studied, out of a total of 140 examined samples (swabs and tissue samples), 135 (96.4%) yielded growth, of which 88 (65.1%) were *E. coli* and 69 (51.1%) were *S. aureus*, this results are in accordance with **Mamza et al., (2010)** who reported that the Prevalence of *E. coli* (64.1%) and *S. aureus* (52.5%) in chickens in Maiduguri, Nigeria. Also similar findings were obtained by **Norhan, (2011)** in El-Sharkia governorates, who recorded that the prevalence rates of *E. coli* was (63.3%), 70% of them was in 34 days old till marketing age. The author also reported that the highest prevalence rate of *E. coli* in broilers in relation to the seasons which reach 76% in summer. The prevalence of *S. aureus* in broilers chickens in this study is similar to the results detected by **Youssef and Dalia, (2012)** and agreed with the findings of **Rashied, (2011)** who detected 50.98% isolation rate. Our isolates from broiler chickens at the finishing and marketing age explained high percentage of isolation is in accordance with **McNamee and Smyth, (2000)** and **Youssef and Dalia, (2012)** where they isolated *S. aureus* from birds ranging from 14 to 70 days of age but most cases occurred around 35 days old. However, the onset of the systemic infection appeared to be between 3 and 4 weeks of age (**Awan and Matsumoto, 1998**).

Biochemical reactions of the examined gram negative isolates showed the following results: indole positive (red ring), methyl red positive (red color), Voges Proskauer negative (copper like color) and citrate negative (green color or no change) while triple sugar iron agar medium gave yellow butt/yellow slant H₂S^{-ve} and gas production, they were identified to be *E. coli*. These results are in line with those recorded by **Rahman et al., (2004)**, **Mamza et al., (2010)** and **Norhan, (2011)**.

In this work, the obtained *E. coli* isolates recovered from broilers with respiratory manifestations were serotyped by slide agglutination test and the results showed that they were be-

longed to the strain O78. These results are agreed with those recorded by **Norhan, (2011)** in El-Sharkia Governorate. where she concluded that the *E. coli* O78 strain was the most predominant serotype recovered from broilers with respiratory manifestations and also are in line with those recorded by **Zhao *et al.*, (2005)** where they recovered ninety-five APEC isolates from diagnosed cases of avian colibacillosis from North Georgia. They stated that the most common *E. coli* serotype was O78. ,and **Jin *et al.*, (2008)** who stated that the most common *E. coli* serotype in avian colibacillosis cases was O78 and **McPeake *et al.*, (2005)**, they recovered *E. coli* O78 serogroup from cases of colisepticaemia in broilers with a percentage of 55%.

The results of the in-vitro antimicrobial susceptibility test by disc diffusion method with 10 chosen antimicrobials using 10 *E. coli* isolates and 10 *Staph. aureus* isolates revealed that :

In case of the 10 *E. coli* isolates, 49% of the isolates were resistant to all tested antibiotics, however, 21% of the isolates were moderately sensitive, while 30% of the isolates were sensitive to all tested antibiotics. A high sensitivity of the isolates to sulfamethoxazole-trimethoprim (80%) followed by gentamicin and amoxicillin, clavulanic (50%), ceftriaxone (40%), chloramphenicol, and ciprofloxacin (30%) and doxycycline (20%) were recorded. But the resistance was as follows, (100%) were resistant to erythromycin, (80%) were resistant to erythromycin and naldixic acid, 60% chloramphenicol, (50%) were resistant to ceftriaxone and doxycycline, 30% were resistant to amoxicillin, clavulanic. Furthermore, 20% were resistant to ciprofloxacin and gentamicin, while the intermediate resistant to neomycin, ciprofloxacin 50% which were very close to the findings of **Tuhin-Al-Ferdous *et al.*, (2012)** where they recorded that antimicrobial susceptibility test, out of 22 *E. coli* isolates, (100%) were resistant to erythromycin, (50%) were resistant to doxycycline, (54.55%) were intermediate resistant to ciprofloxacin. On the contrary, (100%) of their isolates were susceptible to sulfamethoxazole-

trimethoprim.

While, in case of 10 *Staphylococcus aureus* isolates to 10 antibiotic revealed that 56% of the isolates were completely resistant to all tested antibiotic, however, 33% of the isolates were moderately sensitive, while 11% of the isolates were sensitive to some tested antibiotics. Antibiotic resistance to erythromycin 100%, clindamycin 90%, sulfamethoxazole-trimethoprim 80%, and doxycycline, chloramphenicol 60% were observed. A high sensitivity of the isolates to gentamicin (60%) followed by amoxicillin, clavulanic (20%), and ciprofloxacin, ceftriaxone, chloramphenicol (10%) were recorded (Table 1). These results were similar to the findings reported by **Youssef and Dalia (2012)** who reported The in-vitro antibiotic sensitivity test of 10 isolates of *Staphylococcus aureus* to 17 antibiotics which revealed that 58.2% of the isolates were resistant to all tested antibiotic, however, 35.3% of the isolates were moderately sensitive, while 6.5% of the isolates were sensitive to all tested antibiotics. They also reported the sensitivity of the isolates to amoxicillin, clavulanic (20%), and ciprofloxacin (10%) by the same ratio obtained in this study. The high resistance rate of the isolated *staphylococci* to antibiotics in our study was inconsistent with **Persoons *et al.*, (2009)**.

The present investigation of biochemical analyses of liver function test revealed that the infected chickens belong to the group of *E. Coli* and mixed infection demonstrated a significant increase ($P \geq 0.05$) in the mean values of the serum ALT and AST compared with control, also there was a significant change between all groups when compared with each other (table 2). The elevation of AST and ALT values in our study are in agreement with the findings of **Campbell and Coles, (1986)**, **Omaima, (1987)**, **Waffa and Maha, (2004)** and **Mona *et al.*, (2012)**.

They suggested that infected chicken with *E. coli* injured the liver and the changes of ALT, AST levels were more sever in days of infection. However, **(Naglaa *et al.*, 2011)** demonstrated that chickens infected with *E. Coli*

showed significant increase in the mean values of ALT and AST levels on the 15th and 22nd days of age while at 36th day no significance compared with the negative control group. The kidney function test results of the infected groups (*E. coli* and mixed one) displayed a significant increase ($P \geq 0.05$) in the mean value of creatinine compared with control group or with each other as present in table (2). The elevation of creatinine level in our study agreed with **EL-Boushy et al., (2006)** & **Mona et al., (2012)** and also **Abd El-Ghany and Ismail, (2014)**. The increase in serum creatinine level was attributed to the endotoxin of *E. coli* which induced hypotension and altered renal microcirculation, lead to tubular injury particularly at the hypotonic outer medulla and glomerular filtration rate and filtration fraction which markedly decreased creatinine clearance with no tubular damage. (**Heyman et al., 2000; Waffa and Maha, 2004 and Cotran et al., 2005**) reported that altered creatinine level can be used as indicator of kidney dysfunction or may be associated with other condition that result in decreased renal blood flow (**Naglaa et al., 2011**) demonstrated that chicken infected with *E. Coli* showed significant increase in mean value of creatinine compared with the control negative group on the 15th and 22nd days of age while at 36th day of infection no significance difference with the control.

The effect on arterial blood gases are demonstrated in table (3). The results compared with the control group demonstrated that there was a significant decrease in pH, PaO₂ while significant increase in PaCO₂ and HCO₃⁻. Our results agreed with **Samah, (2008)** who reported that the exposed rats to polyaromatic hydrocarbon 5 hours daily for 15th and 30th days showed dyspnea during smoke inhalation and increase respiratory rate immediately after exposure then gradually decrease and dyspnea occurred while increase nasal secretion and mouth breathing, sneezing were seen in late stage, lowering the blood pH, significant increase in PaCO₂ and decrease PaO₂ with decrease level of HCO₃⁻ ion had been noticed. Also our results partially agreed with **Rehab et al., (2012)**

as there was a significant increase in HCO₃⁻ that may be due to the stage of partial respiratory compensation of the chickens (low pH, high in PaCO₂ and HCO₃⁻).

The clinical signs were coughing, depression, conjunctivitis, sneezing, frothy oculo-nasal discharge, increase respiratory rates, facial oedema, nervous signs including, tremors in head and neck and variable mortalities reach up to 70%. Variable signs of illness, respiratory distress, reduction in feed intake and water consumption, cyanosis of comb and wattle, oedema in head and neck, general signs of weakness, ruffled feather and high mortalities reach 100% were detected in severe cases. Most of these signs are similar with those mentioned by **John, (2006)**. Macroscopically, Postmortem examination revealed congested trachea, mucous filled sinuses, petechial haemorrhage in all internal organs with congested carcass as congestion of breast muscles specially in infected chickens with only *E. coli* isolates (**fig.1**). Fibrinous airsacculitis, pericarditis and pneumonia also observed specially in chickens with *E. coli* isolates (**fig.2**). The clinical signs and pathological signs are not pathognomic because both Newcastle (ND) and avian influenza (AI) can cause similar signs with severe outbreak (**Gado, 2014**). While, microscopically chickens with only *E. coli* isolates showed congestion of sinusoidal blood vessels (**fig.3**). Destruction of tracheal mucosa with degenerative changes of some submucosal glands (**fig.4**). Sloughing of tracheal epithelia associated with submucosal oedema (**fig.5**). Heart showed myocarditis, the predominant cells are round cells (**fig.6**) and interstitial oedema also observed (**fig.7**). Air sacs of affected necropsied birds showed airsacculitis, mononuclear cells infiltrating interstitially and around bl. vs. specially in 5-6 weeks old chickens (**fig.8**). Liver showed area of lymphocytic hepatitis accompanied with congestion (**fig.9**). Most of these colisepticemic lesions were similar to that reported in chickens and also turkeys specially in respiratory tissues in subacute polyserositis stage by **Saif et al., (2003)**. While, chickens with mixed infection

with *E. coli* and *S. aureus* revealed congestion of pulmonary blood vessels accompanied with collapse of air sac and emphysema (**fig.10**). Lung also suffered from focal area of caseous necrosis (**fig.11**). While some cases showed haemorrhage and congestion of pulmonary vasculatures accompanied with collapsing and emphysema of air sac (**fig.12**), as well as focal areas of caseous necrosis, the predominant infiltrating cells are polymorphonuclear round cells (**fig.13**). Staphylococcosis is a world wide disease because *staphylococci* are found almost everywhere in the environment. The disease occurs in one of the several forms as in *E. coli* infection as haemorrhagic or gangrenous septicemia, arthritis or sternal bursitis (**Ghauhan,**

2012).

The fact that the etiology of respiratory diseases is very complex and it usually involves more than one pathogen so presence of other agents as co-infected pathogens ,bad management, immunosuppressive agents have a role in aggravation of respiratory disease (**Tamer, 2014**).

Table (1). Antimicrobial susceptibility test by disc diffusion method using 10 *E. coli* isolates and 10 *Staph. aureus* isolates.

	susceptibility	AMC (20/10) mcg	CIP 5 mcg	SXT (1.25/23 .75)mcg	CRO 5 mcg	S 10 mcg	DO 30 mcg	C 30 mcg	E 15 mcg	DA 2 mcg	CN 10 mcg	Total
<i>E. coli</i> isolates	Sensitive	5 (50%)	3 (30%)	8 (80%)	4 (40%)	----	2 (20%)	3 (30%)	----	----	5 (50%)	30%
	intermediate sensitive	2 (20%)	5 (50%)	2 (20%)	1 (10%)	2 (20%)	3 (30%)	1 (10%)	----	2 (20%)	3 (30%)	21%
	Resistant	3 (30%)	2 (20%)	----	5 (50%)	8 (80%)	5 (50%)	6 (60%)	10 (100%)	8 (80%)	2 (20%)	49%
<i>Staph.</i> <i>aureus</i>	Sensitive	2 (20%)	1 (10%)	----	1 (10%)	----	----	1 (10%)	----	----	6 (60%)	11%
	Intermediate sensitive	4 (40%)	5 (50%)	2 (20%)	7 (70%)	6 (60%)	4 (40%)	3 (30%)	----	1 (10%)	1 (10%)	33%
	Resistant	4 (40%)	4 (40%)	8 (80%)	2 (20%)	4 (40%)	6 (60%)	6 (60%)	10 (100%)	9 (90%)	3 (30%)	56%

AMC: amoxicillin & clavulanic acid, CIP: ciprofloxacin, SXT: sulfamethoxazole –trimethoprim, CRO: ceftriaxone, S: streptomycin, DO: doxycycline, C: chloramphenicol, E: erythromycin, DA: clindamycin, CN: gentamicin.

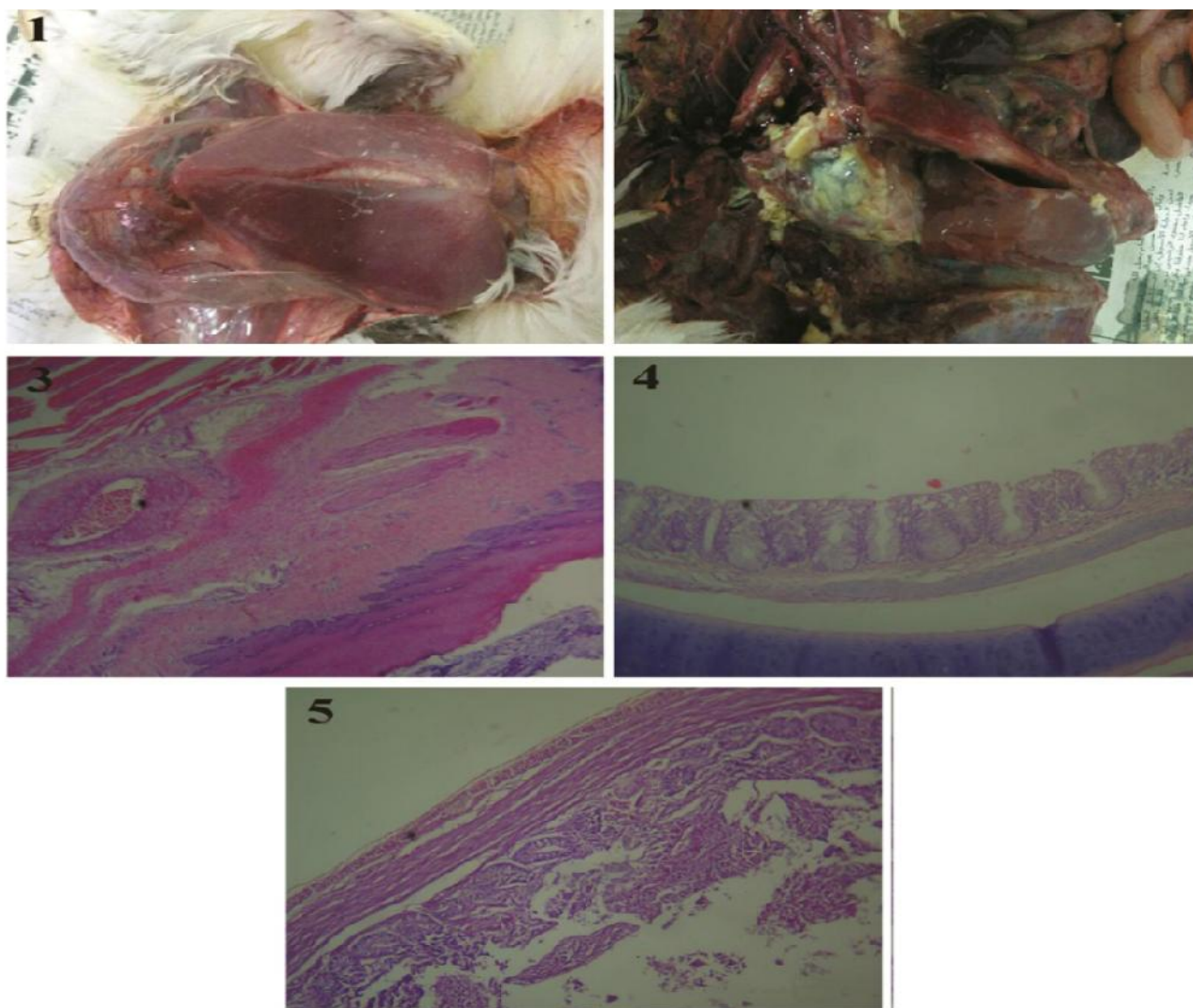
Table (2). Some biochemical parameters tests (mean values $\pm$ S E) of different chicken groups.

Groups	Parameters		
	AST U/L	ALT U/L	Creatinine Mg/dl
Control	33.0 $\pm$ 0.71 ^C	22.8 $\pm$ 0.9 ^C	0.63 $\pm$ 0.02 ^C
<i>E. coli</i>	52.0 $\pm$ 1.9 ^b	37.0 $\pm$ 1.4 ^b	1.0 $\pm$ 0.03 ^b
Mixed	66.6 $\pm$ 3.4 ^a	49.4 $\pm$ 2.6 ^a	1.52 $\pm$ 0.14 ^a

Significance change $P \leq 0.05$

Table (3). Arterial blood gases (mean values $\pm$ S E) of chickens at different groups

Groups	parameters			
	pH	PaCO ₂ (K pa)	PaO ₂ (K pa)	HCO ₃ ⁻ m mol/L
Control	7.32 $\pm$ 0.2 ^a	35.58 $\pm$ 0.18 ^C	60.22 $\pm$ 0.23 ^a	19.42 $\pm$ 0.12 ^C
<i>E. Coli</i>	7.21 $\pm$ 0.01 ^b	37.42 $\pm$ 0.07 ^b	59.76 $\pm$ 0.16 ^b	20.96 $\pm$ 0.10 ^b
Mixed	6.92 $\pm$ 0.1 ^C	38.16 $\pm$ 0.14 ^a	43.32 $\pm$ 0.19 ^C	22.52 $\pm$ 0.10 ^a

Significance change $P \leq 0.05$ **Chickens with colibacillosis (*E. coli* isolates)****Fig (1):** Congestion of breast muscles**Fig (2):** Fibrinous airsacculitis, pericarditis and congested lungs.**Fig (3):** Sinuses showing congestion of sinusoidal bl.vs. (H&Ex100)**Fig (4):** Trachea showing destruction of tracheal mucosa with degenerative changes of Some submucosal glands and submucosal oedema (H&Ex100)**Fig (5):** Trachea showing epithelial sloughing associated with submucosal oedema (H&EX100)

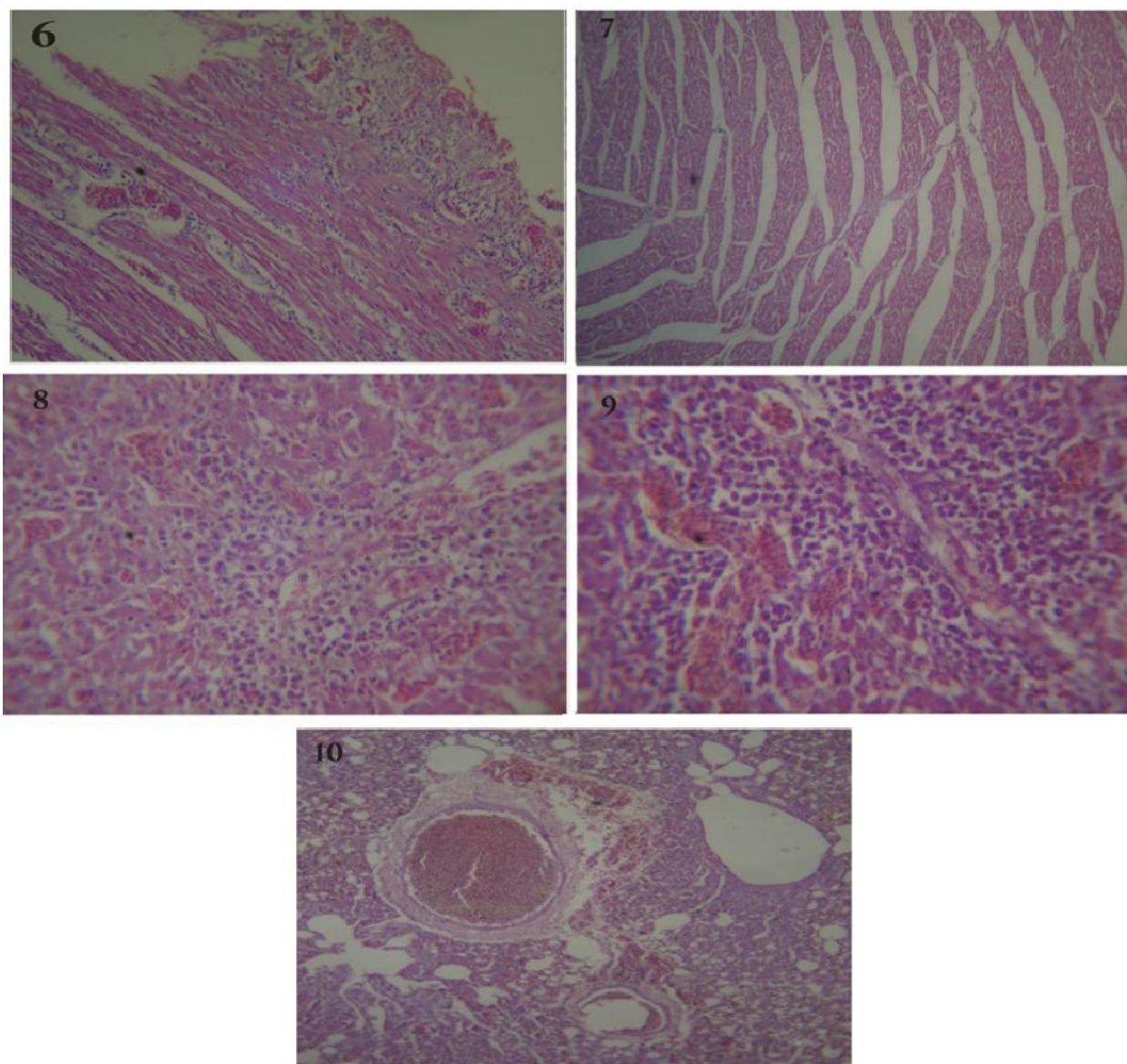


Fig (6): Heart showed myocarditis, the predominant cells are round cells (H&EX100).

Fig (7): Heart showed interstitial oedema (H&Ex100).

Fig (8): Air sac showing airsacculitis, mononuclear cells infiltrating interstitially and around bl. vs. (H&EX 100).

Fig (9): Liver showing area of lymphocytic hepatitis accompanied with congestion (H&Ex100).

Chickens with mixed infection (*E. coli* & *S. aureus*)showing :

Fig (10): Lung showing congestion of pulmonary bl. Vs. accompanied with collaps of air sac and emphysema (H&EX100).

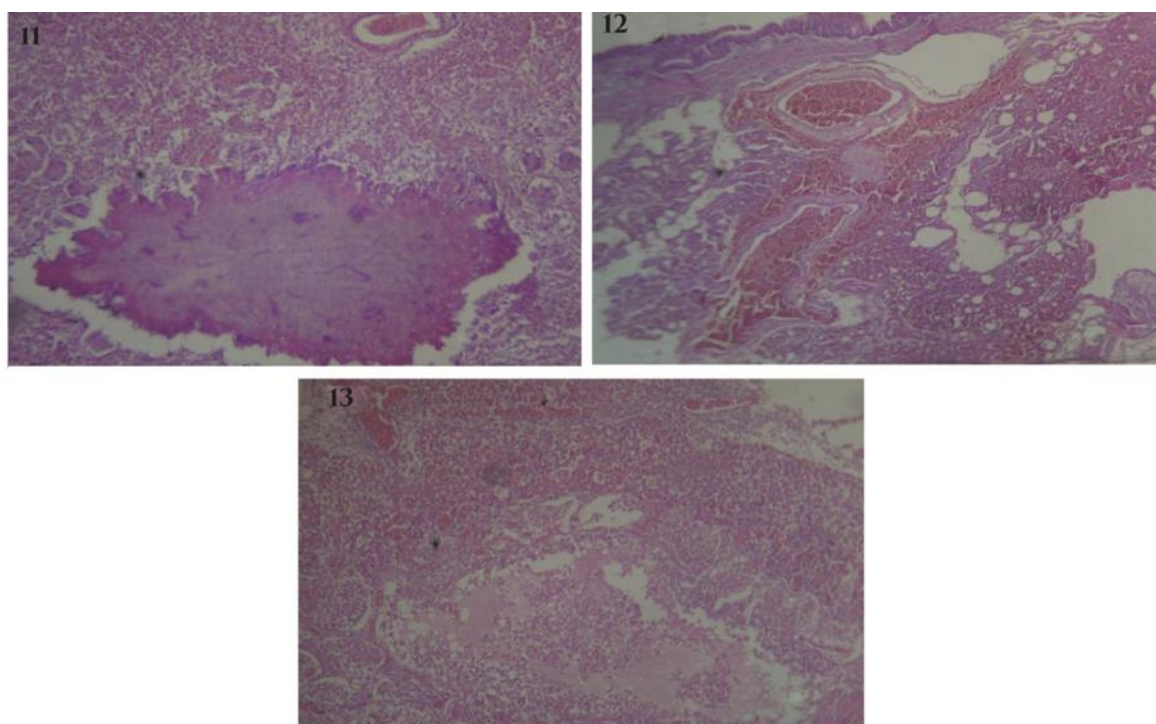


Fig (11): Lung showing focal area of caseous necrosis (H&Ex200).

Fig (12): Lung showing haemorrhage and congestion of pulmonary vasculatures accompanied with collapsing and emphysema of air sac (H&EX100).

Fig (13): Lung showing focal areas of caseous necrosis, the predominant infiltrating cells are polymorphonuclear round cells (H&EX100).

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دراسات باثولوجية وبيوكيميائية عن الامراض التنفسية البكتيرية ببدارى التسمين بمحافظة الشرقية مرفت عبد البديع ايوب* و دينا محمد مهدى* و فائق جابر السيد** و على عبد العال الجمل***

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الملخص العربى

تسبب العديد من الامراض التنفسية التى تصيب دجاج التسمين بمحافظة الشرقية خسائر فادحة للمربين مؤثره على الاقتصاد القومى وخاصة الامراض التنفسية البكتيرية التى يسهل مقاومتها فقط اذا تمكنا من عزلها والتعرف عليها مبكرا وفى خطوه نحو تحقيق هذا الهدف قمنا بعمل بعض الدراسات الباثولوجية والبيوكيميائية وتم تجميع عشر دجاجات من كل مزرعه بمتوسط عشر مزارع منتشرين بارحاء المحافظه . خمس دجاجات بكل مزرعه تعانى من اعراض تنفسية والخمس الاخرى مخالطين لهم بحاله طبيعيه ظاهريا بمتوسط اعمار ٣٠-٤٢ يوما وتم اخذ مسحات انسجه ودم وتم معاملتها ميكروبيولوجيا للعزل والتعرف على الميكروب وعمل اختبارات الحساسيه اللازمه . معزولات الميكروب القولونى تم تصنيفها سيروولوجيا OVA وتم اختيار العشر معزولات من العشر عنابر التى تم اخذ العينات منها كما تم اخذ عينتين دم من كل طائر لعمل الاختبارات البيوكيميائية احدهما لفصل السيرم لقياس معدلات انزيمات الكبد وقياس نسبة الكرياتينين كمؤشر لوظائف الكلى اما العينه الاخرى من الشريان للحصول على دم غير متجلط لقياس نسبة غازات الدم الشرياني كما تم تجميع عينات انسجه من كل من القلب والكبد والرئه والجيوب الهوائية والقصبه الهوائية وذلك بتقنيه خاصه واعدادها للفحص المجهرى . وقد اظهرت النتائج عزل وتصنيف البكتيريا المسببه للمرض من البدارى المريضة حيث وجد أن البكتيريا المسببه هي الإشريشياكولاى و المكورات العنقودية الذهبية، حيث تم فحص عدد ١٤٠ عينة تم تجميعها من بدارى التسمين (مسحات وأنسجة) أظهرت ١٣٥ عينة منها نموا بكتيريا بنسبة (٩٦.٤%)، منها ٨٨ عينة تم عزل الإشريشياكولاى، و ٦٩ عينة بنسبة (٥١.١%) تم عزل المكورات العنقودية الذهبية منها، وبسبب نوع العدوى مجموعتين من الدجاج المصاب بالمرض، مجموعة بها عدوى فردية بميكروب الإشريشياكولاى (٢٨ دجاجة)، والمجموعة الأخرى بها عدوى مختلطة بميكروبى الإشريشياكولاى و المكورات العنقودية الذهبية (٢٢ دجاجة). تم عمل اختبار الحساسيه للمضادات الحيوية بالمعمل باستخدام عشرة انواع منها ودراسة تأثيرها على عشرة معزولات من المكورات العنقودية الذهبية وعشرة معزولات من الإشريشياكولاى، حيث أظهرت النتائج أن ٥٦% من معزولات المكورات العنقودية الذهبية كانت مقاومة لكل المضادات الحيوية المختبرة، بينما ٣٣% منها كانت متوسطة الحساسيه، و ١١% منها كانت حساسة للجنتاميسين والأموكسيسيللين وكلافولينك و سيبروفلوكساسين وكلورامفينيكول. كما أن ٤٩% من معزولات الإشريشياكولاى كانت مقاومة لكل المضادات الحيوية المختبرة، بينما ٢١% منها كانت متوسطة الحساسيه، و ٣٠% منها كانت حساسة للسلفا والجنتاميسين والكلورا والدوكسى سيكلين و سيبروفلوكساسين أظهرت اختبارات وظائف الكبد تغيرات معنوية ملحوظة بين كل المجموعات مقارنة ببعضها، بينما أظهرت اختبارات وظائف الكلى ارتفاع ملحوظ فى معدل الكرياتينين مقارنة بالمجموعة القياسية، غازات الدم الشرياني أظهرت نقص ملحوظ فى PaO_2 , pH, وزيادة مئويه ملحوظة فى PaCO_2 & HCO_3^- . بالفحص تبين شدة الأعراض والعلامات المرضية فى المجموعة التى بها عدوى مختلطة بميكروبى الإشريشياكولاى و المكورات العنقودية الذهبية، و أنزفة نقطيه فى الأعضاء الداخلية، التهاب ليفى بالأوكياس الهوائية وغشاء التامور. ميكروسكوبيا وجد احتقانات بالأوعية الدموية للجيوب الهوائية، تدمير للغشاء المخاطى المبطن للقصبه الهوائية مع تغيرات تنكسية وضمور فى الغدد تحت المخاطية. احتقانات بالأوعية الدموية القلبية مع ارتشاحات للخلايا المناعية أحادية النواة بغشاء التامور وعضلات القلب والأوكياس الهوائية للطيور المصابة، التهابات ليفية بالكبد، كما وجد احتقانات بالرئه وأنزفة حول او عيتها الدموية وتجنبات نخريه مع وجود خلايا ميكروبات المكورات العنقودية الذهبية مرتبة فى شكل عناقيد.