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SOME STUDIES ON STAPHYLOCOCCUS INFECTION IN CHICKEN IN ASSIUT GOVERNORATE

(With 2 Table and 16 Figures)

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بعض الدراسات عن عدوى الميكروب المكور العنقودي في الدجاج بمحافظة أسيوط

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تم جمع ١٠٥ عينة (كبد-مفصل-عرف-أنسجة حول العين) من دجاج نافق حديثا (عمر ٤-١٨ شهراً) وبه احتقانات بالكبد مع تنكرز وحالات أخرى كان بها عرج وأخرى بها تنكرز بالعرف أو التهابات بالعين وذلك من مزارع مختلفة بمحافظة أسيوط وتم زرع تلك العينات علي مستنبتات غذائية مختلفة خاصة بميكروب المكور العنقودي وبعد عمل الاختبارات البيوكيميائية تم التعرف علي Staph.aureus بعد عزلها من الكبد والمفصل والأحشاء الداخلية للطائر والأنسجة حول العين كما تم التعرف علي Staph.aureus بعد عزله من العرف. وتم إجراء عدوى صناعية علي دجاج عمر ٤ أسابيع بميكروب Staph.aureus المعزول عن طريق الحقن بالوريد والذي أدي إلي أعراض تسمم ونسبة نفوق وصلت إلي ٣٠ % وذلك خلال يومين من بداية العدوى مع وجود احتقان وتضخم في جميع الأعضاء الداخلية للطائر وفي اليوم الخامس كانت هناك مشاكل في حركة الطائر حيث كانت هناك التهابات بمفصل الركبة مع ضعف عام وانكماش بالطيور أما اليوم السابع من بداية الحقن كانت ٨٠ % من الطيور تعاني من عرج (بعض الطيور بها ساق واحدة مصابة والبعض به الساقان) وفي نهاية التجربة (بعد ٣ أسابيع) كانت هناك إفرازات صديدية بيضاء إلي صفراء اللون في المفصل مع احتقان بالأوعية الدموية أيضاً كان هناك احتقان وتنكرز بالكبد واحتقان مع ترسيب حمض اليوريك بالحالبين بالإضافة إلي انخفاض في وزن الطائر. أما العدوى عن طريق خدش الأنسجة حول العين بمحلول يحتوى علي ميكروب Staph.hyicus نتج عنه التهابات بالعين بدأت من اليوم الثاني من بداية العدوى وفي اليوم الرابع كانت العين مغلقة تماماً وقد تم عمل الصورة الباثولوجية للعدوى الطبيعية والعدوى الصناعية والتي كانت متماثلة وأوضحت وجود تنكرز في أنسجة العرف خاصة في الأدمه وانتشار الخلايا الليمفاوية وخلايا متعددة الصبغات مع التهابات في الأوعية الدموية خاصة الشرايين وعند فحص المفصل وجدت التهابات صديدية فيبرينيه في مفصل الركبة مع وجود التهابات وتنكرز في الغضاريف النامية الموجودة في نهايات العظام الطويلة للساق

وفي الكبد لوحظ وجود بقع تنكرز وتغيرات باثولوجية في خلايا الكبد وانتشار خلايا متعادلة الصبغة بين خلايا الكبد ووجود جلطات في الأوعية الدموية أما العين فكانت الإصابة مرتكزة في جفن العين حيث ظهر التنكرز واضحاً في طبقات الجفن المختلفة وانتشرت الخلايا الليمفاوية والخلايا متعادلة الصبغات حول بصيلات الريش. وبإجراء اختبار الحساسية لميكروب المكور العنقودي المعزول وجد أن الانروفلوكساسين والاستربتوميسين والاميكاسين هم الأدوية الأكثر تأثيراً.

SUMMARY

One hundred and five tissue specimens (liver, joint, comb and tissues around the eye) were collected from freshly dead chickens (4–18 month age) in Assiut Governorate farms. These cases were suffering from lamness, necrosis of comb, inflammation of eye and congestion with necrosis of liver. These specimens were cultured on different media which specific for Staph. Biochemical reactions revealed Staph. aureus which isolated from comb, liver and joint, also revealed. Staph. hyicus which isolated from inflammed eye. Experimental infection of 4–weeks old chickens with isolated Staph. aureus intravenously revealed septicemia and mortality rate reached to 30% within 2 days postinoculation with congestion and enlargement in all internal organs of the bird. On the 5th day, there were problems in the movement of the bird where the knee joint was inflammed and warm when palpated with depression and ruffling of feather of the bird. On the 7th day postionculation, 80% of the birds were suffering from lamness (some birds had one leg affected and others had the two legs). At the end of the experiment (3 weeks), there were white to yellow purulent exudate in the joint accompanied by congestion of the blood vessels. Also there were congestion and necrosis of the liver, and congestion with precipitation of urates in the ureters beside the decrease of body weight of the bird. Scratching of the tissues around the eye with saline containing Staph. hyicus revealed inflammation of the eye 2nd day post inoculation and at the 4th day the eye was closed completely. Histopathological picture was done which revealed that the natural and experimental infections were similar in the lesions. The comb tissue revealed necrosis in the dermis with infiltration of lymphocytes and heterophil cells with vasculitis. In case of joint, there was arthritis associated with necrosis in the epiphyseal and physeal cartilage at the bony ends. The liver showed focal necrobiotic changes in the hepatocytes with heterophilic cells infiltration, The portal vein was thrombosed. The lesion in the eye was concentrated in the eyelid with inflammatory cellular infiltration (Lymphocytes and heterophils) around the feather follicle and in all

dermis. In vitro sensitivity test for isolated Staph. showed that Enrofloxacin, Streptomycin and Amikacin were the most effective drugs. This work was done to give an idea about the causative agent (Staph. species) of comb necrosis, eye affection, lamness by the following points: Isolation and identification of Staph. species. Experimental infection of broilers with isolated Staph. organism. The main pathological lesions in the comb, eye, liver and joint of naturally and experimentally infected bird. In vitro sensitivity test of the isolated Staph. against different antimicrobial discs.

Key words: *Comb necrosis, eye affection, synovitis, osteomyelitis, Staph. aureus, Staph. hyicus*

INTRODUCTION

Avian Staphylococcosis occurs in several syndromes: Omphalitis in one day old csshicks, severe dermatitis of adult birds and septicemia associated with chronic disseminated Staphylococcosis I.e., localization of Staphylococcosis in tissues with major inflammatory lesions in joints "synovitis", tendon sheaths "tendonitis", heart valves "endocarditis", and bone marrow "osteomyelitis". (Cheville *et al.,* 1988). Also these authors stated that inoculation of Staphylococcus (Staph. hyicus) into the eyelid causes lesion consisted of small foci of necrosis and ulceration contained inflammatory exudates and large cocci and at the most severe stage of disease, eyelid had diffuse necrosis with loss of epithelium, massive accumulation of fibrino purulent exudate and marked inflammation of underlying cutaneous tissue.

Kikuyasu *et al.,* (1997) could isolated Staphylococcus aureus (Staph. aureus) from comb necrosis in 41–day layer breeder chickens and characterized by eosinophilic liquefactive necrosis of epidermal cells with degeneration of the keratocytes.

Genus Staphylococcus contains approximately 20 species, it is the most important genus in the family Micrococcaceae. The term Staphylococcus refers to the morphology of the microorganisms in staind smears which resemble grape like clusters. Staph which has been isolated frequently from poultey include Staph. aureus and Staph. epidermidis. Another species are Staph. gallinarum which isolated from processed poultry and Staph. hyicus which associated with fibrinoheterophilic blepharitis in chickens (kirk Skeeles 1997), He also stated that the lesions of septicemic Staphylococcal infection consist of necrosis of the hepatocytes and vascular congestion of liver.

The pathogenesis of Staph. aureus is complex and probably involves the synthesis of surface–associated protein along with the secretion of exotoxins resulting in damaging effect on the host cell. (Takeuchi *et al.*, 2001)

Abdel-Ghaffar *et al.*, (2004) found that inoculation of 4–7 weeks old broiler with Staph. aureus resulting in subacute fibrinopurulent arthritis.

MATERIALS and METHODS

Materials

Specimens:

Specimens from comb, eye, liver and joint were collected from 105 freshly dead chickens (4–12 month of age)

Media:

Baird parker agar, mannitol salt ager, nutrient ager, blood ager, nutrient broth, glucose, mannitol, sucrose, lactose, fructose and gelatin.

Reagent and solution:

Hydrogen peroxide, voges-proskauer.

Rabbit plasma.

Polymyxin B sensitivity disc (300 unite).

Stain:

Gram's stain

Experimental birds:

Thirty–4 weeks old balady chickens were obtained from Faculty of Agriculture Farm, Assiut University for pathogenicity test.

In vitro sensitivity discs:

Include: Streptomycin (10 μg), Amoxycillin (25 μg), Ampicillin (10 μg), Enrofloxacin (5 μg), Amikacin (30 μg), Gentamycin (10 μg), Neomycin (30 μg), Lincospectin (15 μg) Tetracycline (30 μg), Penicillin (10 μg) and Kanamycin (30 μg)

Methods

Specimens:

One hundred and five freshly dead chickens were subjected to postmortem examination. Speciemens from liver, comb, joint and tissues around the eye were cultured in nutrient broth and incubated at 37°C for 18–24 h followed by subculturing on selective media for Staph. (Baird–parker agar media) at 37 for 48 h. Also a loophful from inoculated broth subcultured on blood agar, mannitol salt agar, MacConkey's agar media and incubated at 37°C for 24 h. Suspected colonies to be Staph. were kept onto slope tubes for further identification.

Identification of the isolated organism:

The suspected colony was examined for its morphology (shape– colour– size–odour) and films from suspected colony was stained by Gram's stain.

Biochemical reactions: The most important biochemical reactions of Staph. species were done according to Ellen *et al.*, (1994), and Kirk Skeeles (1997).

Pathogenicity test:

Thirty–4 weeks old balady chickens S.P.F were used in this study, 5 chickens were slaughtered to be sure free from Staph. and the others were divided as follow:

1st group: Ten birds were inoculated intravenously with 0.5 ml of 24 h trypticase soy broth containing 1×10^5 Staph. aureus/chick (Griffiths *et al,* 1984)

2nd group: Five birds were left as control

3rd group: Ten birds were scratching into the junction of the epidermis and the mucosa of the eyelid with culture fluid containing Staph. hyicus.

4th group: Five birds were scratching with saline into the eyelid.

Histopathological study:

The hock joint, comb, eyelid and the liver were taken from the naturally and experimentally infected cases then fixed in 10% phosphate buffer formalin. The bony tissue decalcified in nitric acid /formalin solution, then dehydrated in ethanol embeded in paraffin sectioned and stained with hematoxylin and eosin (according to Carlton 1967)

In vitro sensitivity test:

The determination of sensitivity of the isolated organism against different antibiotic discs were done according to Finegold and Baron (1986).

RESULTS

Postmortem examination of naturally infected freshly dead chickens showed swelling and necrosis of the whole comb in some birds, the skin of the upper and lower eyelids was adhered in other birds. Another birds had necrosis and congestion of liver, spleen, kidneys and lungs, other birds had affected joints (swollen and filled with exudate).

Bacteriological examination revealed that the suspected Staph. colony was black surrounded by faint yellow zone on Baird – Parker agar media, and give beta haemolysis on blood agar media and was pigmented white to orange on nutrient agar while other colony was white. On mannitol salt agar the colony appears yellow in colour.

Gram's stain revealed gram – positive cocci found in clusters. The result of biochemical reactions are showen in Table I. Staph. hyicus was isolated from necrotic comb.

Pathogenicity test:

Chickens in group I which inoculated intravenously with isolated Staph. aureus showing 30% mortality within 24 h postinoculation (P I) with symptoms of septicemia, congestion and enlargement of all internal organs. On the 5th day P I there were problems in movement of the birds (the knee joint was warm and swollen compared with the control (Fig. 11). Beside this, there were weakness and ruffling feather of the affected birds (Fig. 10). On the 7th day P I, 80 % of infected birds had lamness (some birds had only one leg affected, the others were bilaterally affected) and at the end of the experiment (3 weeks) when opened the joint, we noticed white to yellow purulent exudates and congestion of blood vessels (Fig. 12). Also there was congestion and focal area of necrosis in the liver (Fig. 7), congestion of kidneys with precipitation of urates in the ureters with decreased in the body weight. Chickens on the 3rd group which scratched their eyelids with culture fluid containing Staph. hyicus reveled eye affection 3rd day P I (Fig. 3) and showing blindness on the 5th day P I (Fig. 4). There were absence of signs, lesions or death in any bird in group 2 and 4.

Reisolation of Staph. aureus from liver, spleen, joint and Staph. hyicus from tissue around eye of experimentally infected chickens was succeeded.

Microscopic appearance:

The pathological examination of the affected joints, bones, comb, liver and eye revealed similar lesions in both the experimental and field cases.

The Comb: Showed predominantly lymphocytic and heterophilic inflammatory cell reaction in the dermis, subcutis and the core of the comb. The blood vessels in the dermis mainly veins were congested and filled with inflammatory cells and its wall was degenerated which refer to vasculitis. (Fig. 1, 2).

The eyelid: Showed necrobiotic changes in all layers of the eyelid with heterophilic and lymphocytic infiltration in the dermis. (Fig. 5, 6).

The Liver: Showed area of necrobiotic changes in the hepatocytes focally indistribution with heterophilic infiltration. The hepatocytes showed coagulative necrosis. The portal vein was thrombosed with degenerative changes in their walls (Fig. 8, 9).

Joint: The knee joint showed severe pathological changes. The synovium was severely thickned due to synovial hypertrophy associated with fibrinopurulent reaction, these indicate fibrinopurulent arthritis. The blood vessels in the synovium were thrombosed. The epiphyseal cartilage from the head of the femur show necrobiotic changes which demonstrated by shrunken eosinophilic cytoplasm of the chondrocytes and the presence of a few pyknotic nuclei, in some area the cartilagenous tissue was destructed. The vascular canal at the epiphyseal cartilage of the bone was occluded with fibrinopurulent exudates. The epiphyseal blood vessels was thrombosed and their wall showed necrobiotic changes, proliferation of fibrous tissue around the blood vessels was seen else where in the cartilage, this tissue infiltrated with inflammatory cells. (Fig. 13, 14, 15, 16).

In vitro sensitivity test: The effect of different antibiotics on the isolated Staph. is illustrated in Table II.

Table I: showing biochemical tests:

Biochemical tests	Staph . aureus	Staph . hyicus
• Colony pigment	+	-
• Hemolysis	+	-
• Tube coagulase	+	-
• Voges - proskauer	+	-
• Catalase	+	+
• Gelatine liquefaction	+	-
• Fermentation of sugar :		
Sucrose	+	+
Glucose	+	+
Lactose	+	+
Fructose	+	+
Mannitol	+	-
Maltose	+	-
• Zone of inhibition for polymyxin B < 10 mm	+	+

Table II: Illustrated the results of in virto sensitivity test:

Antimicrobial disks	Sensitivity of isolated Staph.
Enrofloxacin	+++
Streptomycin	+++
Amikacin	+++
Ampicillin	++
Tetracycline	++
Amoxycillin	++
Gentamycin	+
Lincospectin	+
Kanamycin	-
Neomycin	-
Penicillin	-

+++ sensitive ++ moderate sensitive + weak sensitive - negative

LEGEND OF FIGURES

Fig. 1: Comb of naturally affected bird showing necrobiotic changes of the dermis with heterophilic infiltration (H. & E.).

Fig. 2: Comb of naturally affected bird showing inflammatory cell infiltration in the vein. (H. & E.).

Fig. 3, 4: Experimentally infected chicken showing swelling of eyelid, conjunctivitis and completely closed eye.

Fig. 5: Eyelid of infected bird (naturally and experimentally) showing heterophilic and lymphocytic infiltration. (H. & E.).

Fig. 6: Eyelid of experimentally and naturally infected chicken showing hair corpusle surrounded by plasma cell and lymphcytic infiltration. (H. & E.).

Fig. 7: Liver of experimentally infected chicken showing congestion and focal area of necrosis.

Fig. 8: Liver of naturally and experimentally infected chicken showing focal area of necrobiotic changes of hepatocyte with lymphocyte and heterophil cell infiltration. (H. & E.).

Fig. 9: Liver of naturally and experimentally infected chicken showing portal vein with thrombus and degeneration of the vein wall. (H. & E.).

Fig. 10: Experimentally infected chicken showing depression, ruffling feather, lamness and the bird could not stand.

Fig. 11: Swelling and reddness of knee joint of experimentally infected chicken.

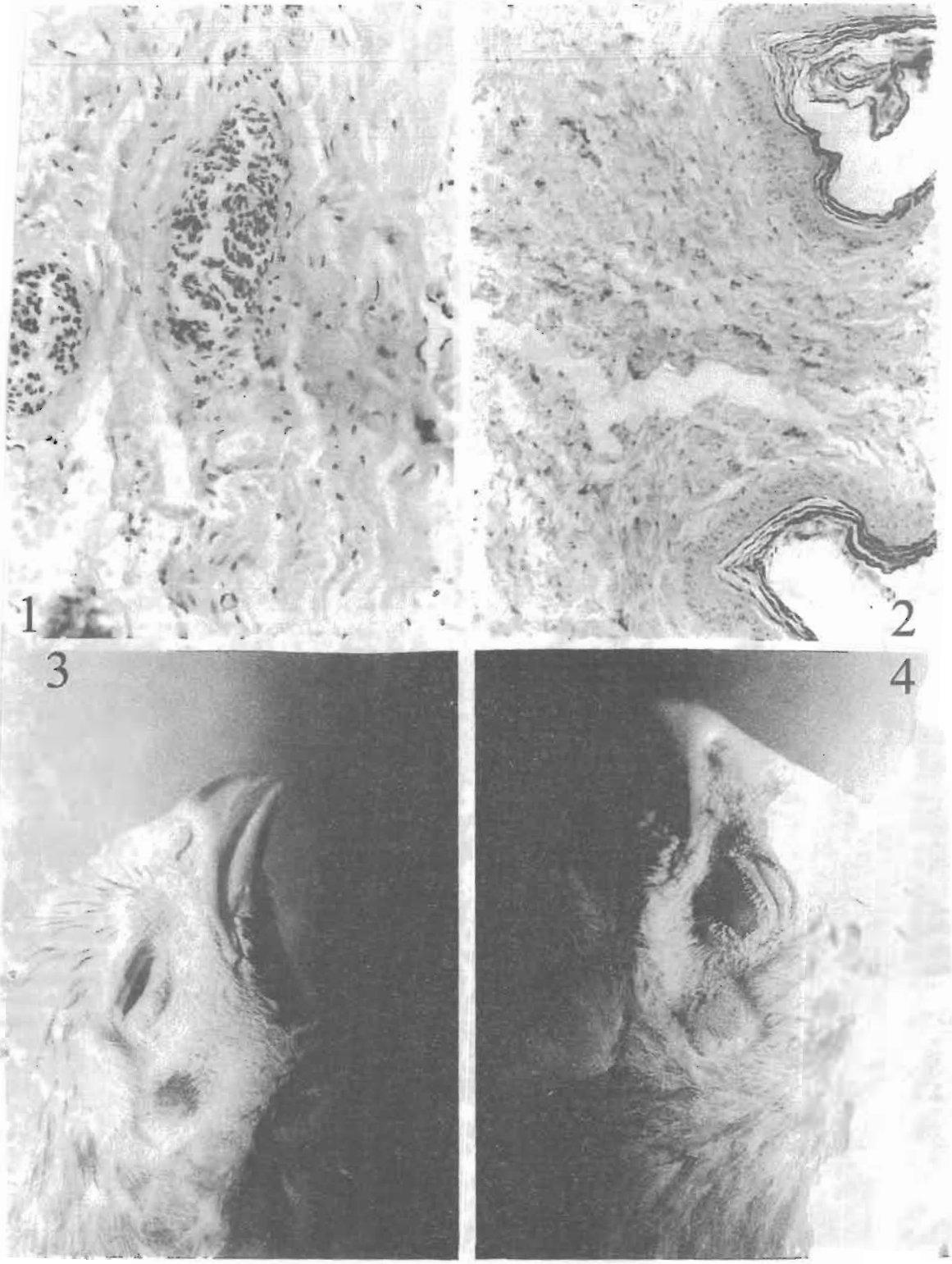
Fig. 12: knee joint of experimentally infected bird showing white to yellow purulent exudates and congestion of blood vessels.

Fig. 13: Knee joint of naturally and experimentally infected bird showing epiphyseal blood vessels surrounded by proliferating fibrous tissue with thrombus formation and degeneration of cartilaginous structure (H. & E.).

Fig. 14: Knee joint of naturally and experimentally infected chicken showing thrombus formation of epiphyseal venules (H. & E.).

Fig. 15: Knee joint with synovium proliferation (H. & E.).

Fig. 16: Heterophilic infiltration with fibrous proliferation of synovium.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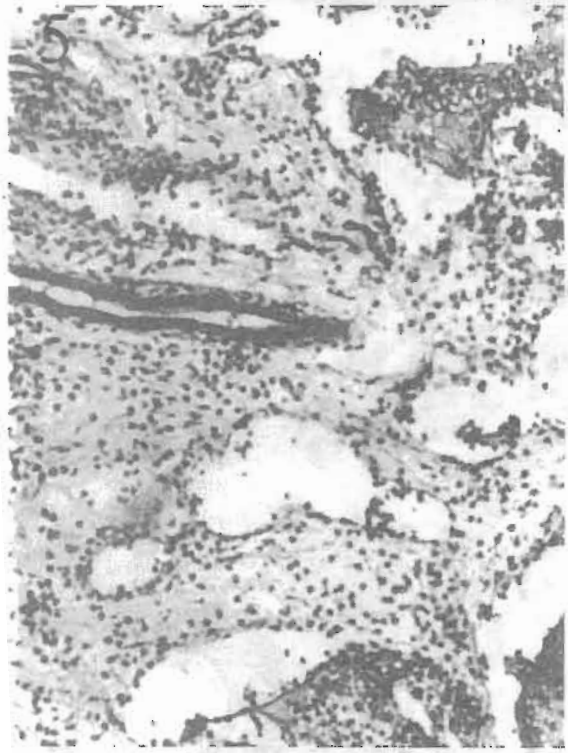

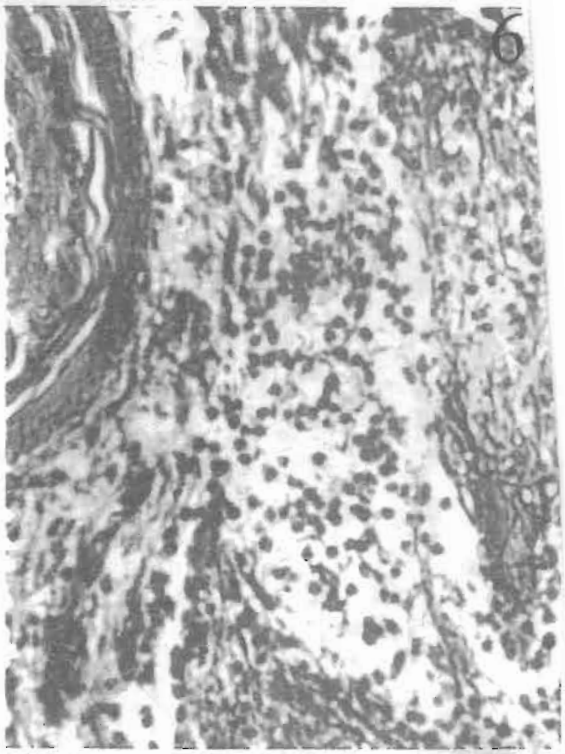

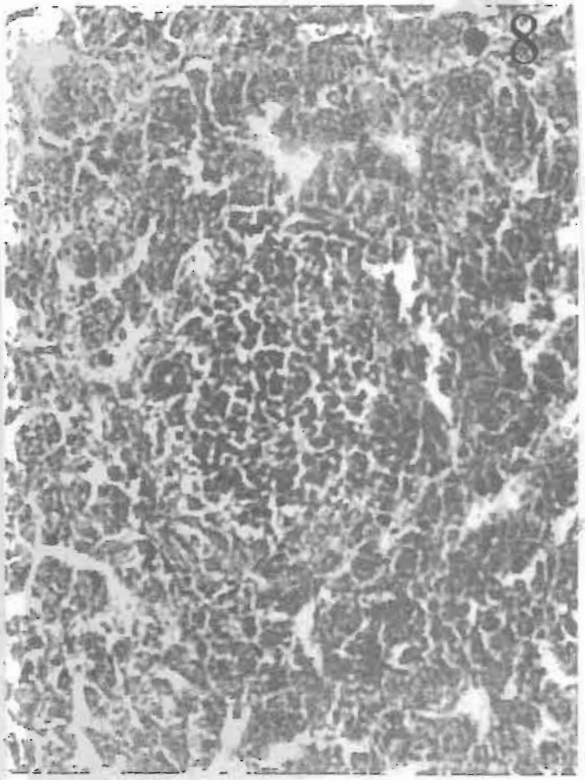

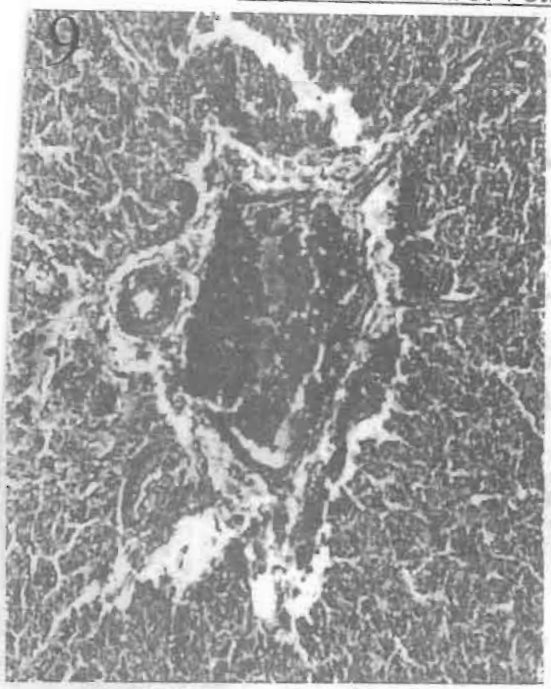
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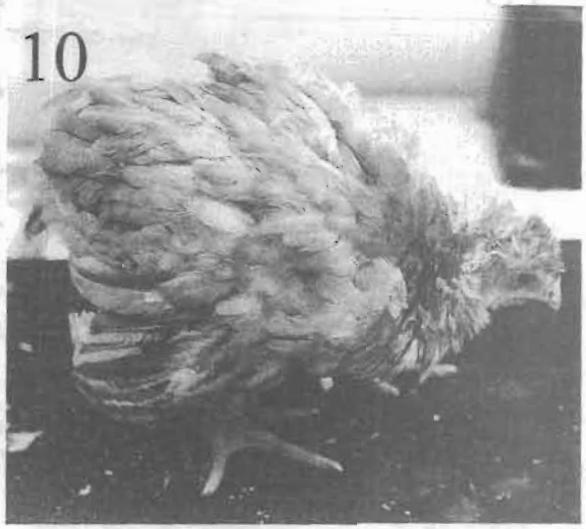
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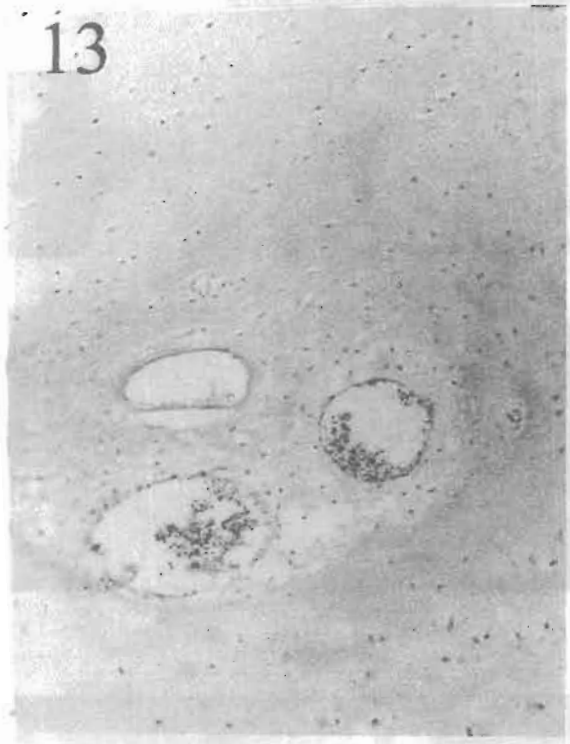
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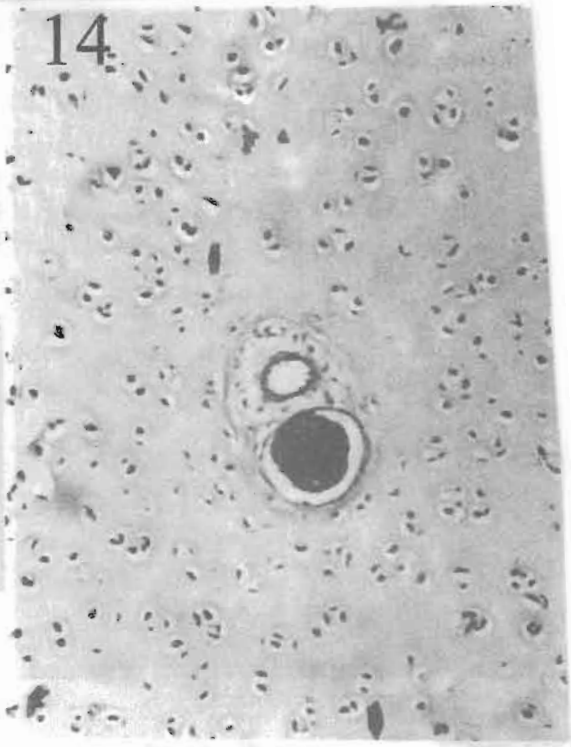
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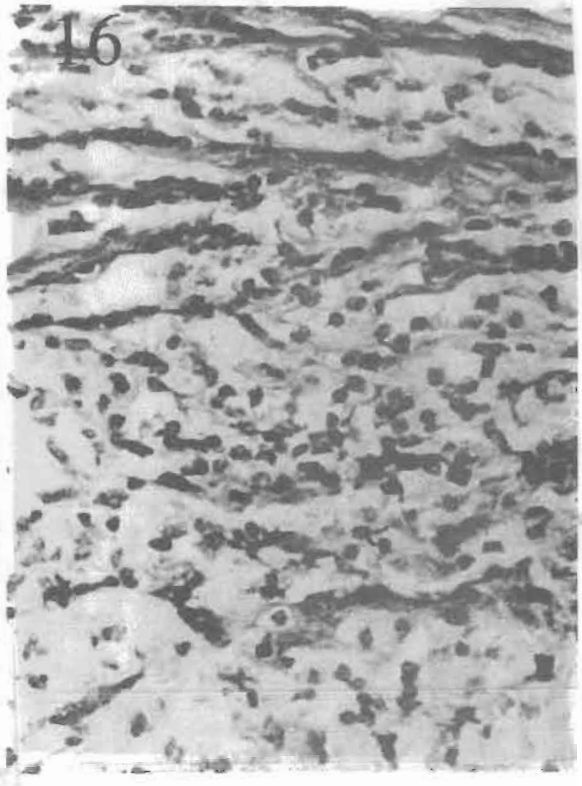
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DISCUSSION

The economic significance of Staph. aureus infection to the poultry industry is difficult to determine because of the prolonged mortality seen over time with poultry flocks, this mortality results not only in the economic loss of the breeder but also the potential loss of broilers (Amita *et al,* 2002). While Staph. hyicus was identified as the cause of skin lesions and conjunctivitis in chickens.

Bacteriological examination of freshly dead layer revealed isolation of Staph. aureus from swelling and necrotic comb, this finding is in agreement with that observed by Kikuyasu *et al* (1997). Also we could isolated Staph. aureus from birds suffering from joint affection and lamness, congestion and necrosis of liver, spleen, kidney and lung and from the skin of the upper and lower eyelids, this result is also reported by Alderson *et al.,* (1986), Cheville *et al.* (1988) and Kirk Skeeles
(1997).

In this study we found that the pathology of field cases which suffering from lamness, eye affection and liver lesions was similar to experimental cases which inoculated with Staph. aureus and Staph. hyicus, this observation was also noticed by Thorp *et al.,* (1993) and Abdel-Ghaffar *et al* ., (2004).

The lesion in the comb of naturally infected chickens showing necrosis mainly liquefactive necrosis of epidermal cells and the basal layer of the epidermis has not been degenerated in the necrotic area, this notice is similar to that noticed by Kikuyasu *et al.,* (1997), they found that the skin lesions caused by Staph. aureus mainly localized in the epidermis and this healed without scars.

Experimental infection of the isolated Staph. aureus to 4 – weeks old chickens intravenously revealed septicemia, congestion and enlargement of all internal organs. The liver was congested and had focal area of necrosis and manifested by hepatocellular degeneration and thrombus formation in the hepatic vasculature, this result is inagreement with that noticed by Kikuyasu *et al.,* (1997) and Kirk Skeels (1997) who found that Staphylococcal septicemic infection cause necrosis and vascular congestion in the liver and explain the formation of the thrombi in hepatic sinusoids is due to the enterotoxin of the Staph. which has effect on blood vessels and blood composition.

Also intravenous inoculation of chickens with isolated Staph. aureus revealed lamness and joint affection, where bacterial arthritis in

the knee joint and osteomylitis in the proximal and distal ends of the femur were the predominant and important causes of lamness in broilers with clinical evidence of joint problem (Thorp *et al.,* 1993). The pathogenesis of these lesions depend mainly on the ability of the organism to reach the circulatory system, once the bacteria reached the blood through wound infection due to trauma or mosquito bites in naturally infected cases (Kirk Skeels 1997) or through intravenous incoculation in experimental study (Daum *et al,* 1990) the Staph. has, a high affinity for collagen – rich surface such as the articular surface of the joints and synovial sheaths located around joints and tendons, also the organism tend to localize in the growth plate to actively growing bones (Mutalib and Maslin 1996), and KirkSkeels (1997), this explains the higher incidence of femoral head necrosis and osteomylitis in young chickens cartilage vascular canal enables the bacteria to spread from the metaphysis to physis and epiphysis, this leads to their necrosis (Alderson *et al,* 1986 and Abdel - Ghaffar *et al.,* 2004). The degeneration in the walls of the blood vessels in the vascular canal of growing cartilage leads to their thrombosis and subsequently degeneration and necrosis of the epiphyseal and physeal cartilages and articular surface (Mutalib and Maslim 1996). So the lesions began with arthritis followed by extenstion of infection down word to the articular surfaces and the growing cartilage of the long bones leading to osteomylitis and necrosis.

Also in this study we noticed eye affection after scratching of the eye lids of the birds with culture fluid containing Staph. hyicus, the lesions consisted of necrobiotic changes in the all layer of the eyelid with heterophilic and lymphocytic infiltration, vasculitis was also observed, this result is inagreement with Cheville *et al.,* (1988) as they say that inoculation of Staph. hyicus into the eyelid cause small foci of necrosis and ulceration contained inflammatory exudates and large cocci. The blood vessels showed necrosis of their walls with thrombus formation, they explain these vascular lesion that its distantce from the site of bacterial colonization suggest that a toxin might have been released into the tissues, even though no systemic lesions attributable to a bacterial toxin were present.

We could reisolated staph. organism from experimentally infected birds, this result is resemble to that observed by Kirk Skeels (1997).

In vitro sensitivity test revealed that Enrofloxacin, Streptomycin, Amikacin and Ampicillin are the most effective drugs, this result is in agreement with that observed by Cheville *et al.,* (1988).

Our conclusion in this study proved that Staph. aureus is a pathogen with a tropism for growing bone and liver in the laboratory and field cases, affected birds with S. were stunted compared to that unaffected ones. Staphylococcosis in chickens is considered a problem in the field. Where Staph. aureus is probably enter the blood stream from trauma of skin and mucous membranes causing arthritis and possible secondary complications of tenosynovitis. While Staph. hyicus enter the blood stream from conjunctival lesions but are destroyed before producing systemic lesions.

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