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STAPHYLOCOCCUS AND KLEBSIELLA INFECTION IN BROILER CHICKENS

(With 3 Tables & one Fig.)

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الاصابه بميكروبى المكور العنقودى والكلبسيلا فى بدارى الدجاج

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فى الوباء المتسبب بميكروبى المكور العنقودى والكلبسيلا فى بدارى الدجاج بلغت نسبة الوفيات ٨ر٤٪ خلال فترة المرض وقد لوحظ على الطيور المصابه انكماش وأزرقاق فى منطقة الرأس وكانت أهم الآفات التشريحيه وجود رشح دموى أوديمى تحت الجلد فى منطقة الرأس وكذلك احتقان الأعضاء الداخليه. تم عزل ميكروب المكور العنقودى وميكروب الكلبسيلا المسبب للالتهاب الرئوى من الحالات المريضه وبأجراء العدوى الصناعيه بهذين الميكروبين كانت نسبة الوفيات ٨ ٪ ، ٢ ٪ على التوالى بينما بلغت نسبة الوفيات ١٢ ٪ فى الكتاكيت المحقونه بكلا الميكروبين معاً فى وقت واحد وكانت الاعراض تشبه إلى حد كبير تلك المسجله فى العدوى الطبيعيه وبأجراء أختبار الحساسيه فى المعمل لعترتى المكور العنقودى والكلبسيلا المسببه للالتهاب الرئوى المعزوله أتضح الحساسيه العاليه لكل من البنسلين ، الأمبيسلين ، الانروفلوسين للعترة الأولى وكذلك فلموكين ، انزوفلوسين ، حمض الاكسالينك للعترة الثانيه. فى تجربة حقليه فقد تمت السيطره وعلاج الحالات المصابه بحقن الطيور بخليط من البنسلين – الاستربتومايسين بنتائج ممتازه.

SUMMARY

An outbreak in broiler chickens with 4.8% mortality caused by Staphlococcus aureus (S. aureus) and Klesiella peneumoniae (K. pneumoniae) was encountered by the authors. The clinical signs of the infected broiler ckickens were sligh depression and bluish discolouration of the head region. The macroscopic lesions were oedematous haemorrhagic exudate of the subcutaneous (s/c) tissue in the head region and vascular congestion of the internal organs. Bacteriological cultures of specimens on blood agar and mannitol salt agar as well as biochemical and biological characters revealed S. aureus and K. pneumoniae. 8% and 2% mortalities were noticed in the s/c injected chicks with S. aureus and K. pneumoniae, respectively. On the other hand 12% mortality was reported in chicks injected s/c with both organisms simultaneously. The clinical observations, of experimental infection were similar to a great extent to those of natural infection. In vitro drug sensitivity, S. aureus was highly sensitive to penicillin, ampicillin and enroflocin. K. pneumoniae was highly sensitive to enroflocin, flumequine and oxanilic acid. field trial of simultaneous injection with penicillin streptomycin gave a good results for controlling the outbreak.

INTRODUCTION

especially in Egypt poultry meat is considered one of the most important sources of animal protein. There are many problems facing broilers under the massive production industry causing serious losses. Staphylococcal septicemia in chickens often occurs secondary to or with selenium deficiency (exudative diathesis) and can affect any age resulting to acute death (*BERGMANN et al., 1980; ACHDIJATI, 1983; BITAY et al., 1984; CHEN et al., 1984; SCHWARTZ, 1988 and ABE and KANAI, 1991*). Enterobacteriaceae infection is still causing severe losses especially in young age of birds, Klebsiella infection has been recently shown to be involved (*SARAKBY, 1979; KARAMAN, 1980 and ZAHDEH, 1982*). A field problem occurred suddenly in 2500 of 25-day-old arbor acres chicks manifested discolouration of the head region and deaths, from the field cases a thin film was performed from the oedematous haemorrhagic fluids in the

s/c tissue of the head region as well as from liver on microscopic slides stained by Gram stain for a rapid tentative indentification. Preliminary results indicated Gram positive cocci arranged in clusters and Gram negative bacilli.

The present work was undertaken to study the following points:

-Isolation and identification of the causative organisms.
-Pathogenicity of the incriminated organisms in baby chicks.
-In vitro and in vivo sensitivity testing of the recovered organisms for some antimicrobial agents.

MATERIAL AND METHODS

Specimens:

A total of sixty four freshly dead chicks were subjected to post-morten examination. Cultures were made from bloody exudate of s/c tissue as well as from liver, heart blood and trachea onto nutrient broth followed by subcultivation on sheep blood agar, MacConkey's agar and mannitol salt agar. Plates were incubated at 37° for 24 hours. The isolated organisms were examined for their biological and biochemical properties according to *ELMER* et al., *1991.*

Experimental birds:

One hundred and seventy, one day-old arbor acres chicks were observed for seven days and proved to be free from most pathogenic organisms. These chicks were divided into four groups. The first three groups of 50 ckicks each. Chicks of the first group were injected s/c, each receiving 0.5 ml broth cultures of S.aureus (10^3 viable cells), chicks of the 2nd group were inoculated s/c with K.pneumoniae, each receiving 0.5 ml of broth culture containing (10^3 viable cells). Chicks of the 3rd group receiving a mixture of S.aureus and K.pneumoniae. Furthermore, the fourth group, of 20 chicks was kept as control. The experimental birds were noticed for 2 weeks for recording clinical signs and mortality. The dead birds were necropsied for gross lesions and trials for reisolation of the injected organisms were adopted.

Sensitivity of the isolates to antimicrobial agents:

The paper disc technique was carried out after *FINEGOLD and BARON (1986)* using isolates of S.aureus and K.pneumoniae and 19 chemotherapeutic discs produced by Oxoid Basingstoke, Hampshire, EnGland. The discs included Penicillin G (10 ug), Ampicillin (10 ug), Chlortetracycline (30 ug), Doxycycline hydrochloride (30 ug) Flumequine (30 ug), Enroflocin (5 mcg), Oxytetracycline (30 ug), Neomycin (30 ug), Cephalexin (30 ug), Oxalinic acid (10 ug), Framycetin (100 ug), Gentamicin (10 ug), Apramycin (15 ug), Linco-spectin (5 mcg), Streptomycin (10 ug),

Cloxacillin (5 ug), Colistin sulphate (10 ug), Chloramphenicol (30 ug), and compound Sulphonamide (300 ug). Interpretation of the results was recorded to the recommendation of *CASTLE and ELSTUB (1971).*

RESULTS

The clinical signs of infection that occurred in arbor acres chicks were manifested by discolouration of the head region. The mortalities began with 3, 8, 80, and 20 chicks in the 1st, 2nd, 3rd, and 4th day of infection, respectively but mortalities decreased in the 5th and 6th day that corresponding 24 and 48 hours after drug treatment and reached zero after that, the necropsied dead chicks showed an oedematous haemorrhagic exudate in s/c tissue of the head region with severe congestion of the internal organs. Bacteriological examination of the infected dead chicks revealed small, pigmented, beta hemolytic and another relatively large, non pigmented colonies on blood agar. On mannitol-salt agar the former organism colonies were surrounded by a yellow hallo. Mixed infection of Gram positive cocci and Gram negative bacilli were isolated in pure culture from all samples examined except 14 cases showed only Gram's positive cocci, these organisms were identified biologically and biochemically as S.aureus and K.pneumoniae (Table,1). The clinical signs of chicks experimentally infected with S.aureus were inappetance, severe depression and 8% mortalities was recorded from 3rd to 6th days of infection, the birds that died after short period of infection showed severe congestion of internal organs but s/c haemorrhagic exudate was more clear in birds that remained for longer time. Depression, weakness, diarrhea and 2% mortality was reported in chicks infected with K.pneumoniae. The most prominant lesions were catarrhal exudate in the trachea and congestion of internal organs. 12% mortality was noticed in chicks simultaneously infected with S.aureus and K.pneumoniae (Table, 2). The clinical observations were more or less similar to that seen in natural infection, it is worth to mention that the oedematous haemorrhagic exudate in this group (Fig., 1) was more clear and adequate than that observed in S.aureus infected chicks. specificity of deaths was confirmed by bacterial reisolation from dead carcasses. The results of sensitivity testing of S.aureus and K.pneumoniae isolates to each of 19 antimicrobial agents are given in (Table, 3). Field trial of penicillin-streptomycin administration was highly successful in controlling the natural infection after 48 hours.

DISCUSSION

The clinical observations of naturally infected chicks with Saureus and K.pneumoniae were manifested by bluish discolouration of the head region and oedematous haemorrhagic changes of hypodermis. Severe congestion of the parenchymatous organs was also observed. Similar signs and lesions for S.aureus infection were reported by *KOHLER* et al. *(1980) and BITAY* et al. *(1984).*

Bacteriological examination of the dead chicks revealed mixed infection with S.aureus and K.pneumoniae in most cases and S.aureus only in few ones. Similar findings were recorded by *ACHIJATI (1983).* The mortality rate that reported during the acute course of infection was 4.8%. Similar finding for S.aureus infection was mentioned by *SKEELES* et al. *(1991).*

experimental infection of healthy chicks infected with S.aureus indicated the pathogenic nature of the isolate examined and mortality rate of 8% was recorded. The clinical signs and gross lesions in diseased and dead infected chicks reported were inappetance, depression and s/c pathognomic oedematous haemorrhagic changes resembled to a great extent those observed in natural infection. Our results agree with those reported by *KOHLER* et al. *(1980).*

Also the s/c infected chicks with K.pneumoniae proved the pathogenic nature of the isolate and mortality rate of 2% in this group was noticed. The clinical observations of diseased and dead infected chicks were depression, weakness, diarrhea and tracheal exudate with congestion of the internal organs which resembled to some extent those reported by *ABD-ALLA (1981) and DESSOUKY* et al. *(1982)* and disagreed with those reported by *Sarakby (1979) and ASHGAN (1988)* who proved the non pathogenic nature of the examined isolates for chicks. In chicks simultaneously infected with S.aureus and K.pneumoniae the mortality rate was 12%, the clinical signs and gross lesions were more or less similar to those observed in natural infection.

The in vitro sensitivity testing of S.aureus isolate to each of 19 antimicrobial agents displayed that the isolate examined was highly sensitive to peniciillin, ampicillin and enroflocin, sensitive to flumequine, moderate sensitive to gentamicin, less sensitive to doxycycline hydrochloride and linco-spectin, the isolate proved non sensitive to each of chlortetracycline, oxytetracycline, neomycin, cephalexin, oxalinic acid, framycetin, apramycin, chloramphenicol, streptomycin, cloxacillin, colistin sulphate and compound sulphonamide Our results agree with those reported by *ABE and*

KANAI (1991) and to less extent with those reported by *BHATIA et al. (1980); KAMEL (1982); NABILA (1982); ASHGAN (1988) and AYHAN and AYDIN (1991).*

The sensitivity testing of K.pneumoniae isolate to the previously mentioned antimicrobial agents revealed that the examined isolate was highly sensitive to oxalinic acid, enroflocin and flumequine, sensitive to chlortetracycline and oxytetracycline, moderate sensitive to apramycin, streptomycin, gentamicin and neomycin, less sensitive to penicillin, ampicillin, linco-spectin, colistin sulphate and framycetin. The isolate proved non sensitive to each of doxycycline hydrochloride, cephalexin, chloramphenicol, cloxacillin and compound sulphonamide. Our results agree to some extent with those reported by *ASHGAN (1988).*

This paper showed that S.aureus and to a less extent K.pneumoniae were responsible for considerable losses in chicken farms. Improvement of management, provide nutritionally balanced ration and injection of penicillin-streptomycin in such farms of a great value in minimizing losses from these organisms.

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