

Animal Health Research Institute,
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GRAM POSITIVE COCCI CAUSING SEPTICAEMIA IN CHICKENS IN ASSIUT GOVERNORATE

(With 5 Tables and 3 Figures)

By

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**المكورات موجبة الجرام المسببة لحالات التسمم الدموى الجرثومي
في الدجاج في محافظة أسيوط**

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أجريت هذه الدراسة كمحاولة لعزل ميكروب المكور العنقودي و الميكروب المكور السبجى والتي تسبب التسمم الدموى فى الدجاج. وقد تم عزل ٢٤ عترة من الميكروب العنقودى وكذلك ٤٤ عترة من الميكروب السبجى . تم اختبار حساسية هذه العترات لانواع مختلفة من المضادات الحيوية وأظهرت النتائج أن عترات الميكروب العنقودى كانت حساسة لكل من الانروفلوكسين والكيتاموكس بينما كان الجاراميسين والكلورامفينيكول والانروفلوكسين والامبيسلين أكثرهم تأثيرا على الميكروب السبجى. وقد تمت دراسة العدوى الصناعية للميكروب العنقودى فى كتاكيت عمر ثلاثة أيام.

SUMMARY

A trial was made in Assiut to isolate Gram positive cocci (Staph and strept) from some poultry farms suffering from septicaemia. Twenty four isolates of staphylococci and forty streptococci isolates were recovered from diseased birds. In vitro sensitivity tests of staphylococcus and streptococcus isolates were carried out. Staphylococcus isolates were sensitive to Enrofloxacin and Kitamox while streptococcus isolates were sensitive to Garamycin, Chloramphenicol, Enrofloxacin and Ampicillin. The pathogenesis of Staph. aureus for 3 days chicks caused very virulent pictures and early deaths for chicks.

Key words: Gram positive cocci causing septicaemia in chickens in Assiut.

INTRODUCTION

Streptococcus infections of poultry are world wide and occasionally responsible for serious and attractive problems in poultry farms. Most of the staphylococcal diseases in poultry are caused by *Staph. aureus* infections which considered pathogenic for poultry and are isolated from comb necrosis (Nakamura, 1997), material and exudate of swollen head (Litjens and Van-Willigen, 1989), internal organs (Nabila, 1982), beaks (Witte, 1967). Streptococcal infections of poultry although not common, they cause acute or chronic infections with losses up to 50% (Argimi, 1956). The organism was isolated from internal organs (Nabila, 1982). The infection was associated with caseous material in skin (Messiers *et al.*, 1993). Lesions of the eye lides and ulceration (Cheville *et al.*, 1988).

Therefore the present study was designed to cover the following points:

- ◆ Isolation and identification of the organisms from broilers 3-9 W.
- ◆ Sensitivity of the isolated strains to antimicrobial agents.
- ◆ Pathogenicity of *Staph. aureus* to 3-day old chicks

MATERIAL and METHODS

1-Specimens:

About 100 samples were collected from broilers 3-9 weeks with clinical signs of vesicular dermatitis, skin lesions, swollen joints and bumble foot as well as dead birds showing post-mortem lesions of caseous or purulent exudate in the swollen joints, congestion and enlargement of the liver and spleen and petichial haemorrhages on the coronary fat which were suggestive staphylococcosis. But the chickens of diarrhoea, respiratory difficulties, emaciation, joint abscesses and lameness as well as dead birds with lesions of congestion of subcutaneous tissues, trachea, lungs, heart, spleen, liver and intestine, unabsorbed yolk sac and joint abscesses, all these symptoms suggestive for streptococcosis.

The collected samples were cultured for isolating staphylococcosis onto nutrient broth (Biolife V. le Monza 272 Italy) incubated at 37°C for 24 hours followed by plating onto selective media for staphylococci (*Staphylococcus* medium No. 115, Oxoid CM 145

England). The selective plates were incubated at 37°C for 24-48 hours under aerobic atmosphere. Suspected colonies were examined microscopically for the appearance of gram positive coccus occurring in pairs and irregular clusters.

In case of isolation of streptococcosis, samples were inoculated onto nutrient broth, incubated at 37°C for 24 hours followed by plating onto crystal violet blood agar medium (Cruickshank *et al.*, 1975). The selective media for isolation of streptococci were incubated at 37°C for 24 hours under aerobic atmosphere and examined for appearance of gram positive spheres in pairs or chains.

Identification of isolates:

Smears from isolates were stained with Gram's method and examined microscopically. Pure colonies of staphylococci were identified using the following tests coagulase production as well as sugar fermentation tests including manitol and arabinose (Buchanan and Gibbons 1974). For streptococci, pure colonies were adopted for identification by reaction on litmus milk, sugar fermentation tests including manitol and arabinose (Sherman, 1937 and Buchanan and Gibbons 1974).

2- The in vitro sensitivity of the isolated staphylococci and streptococci to different antibacterial agents:

Mono discs including: Enrofloxacin (Enro 10), Kitassamycin+Amoxycillin (Kitamox 70), Garamycin (CN 10), Oxytetracycline (OT 30), Lincocin (Li 30), Chloramphenicol (C 30), Fucidin (Fu 10), Streptomycin (S 10), Ampicillin (Amp 10) were used.

Sensitivity test: 5 isolates of each staphylococcus and streptococcus were used to study their sensitivity against "9" antibacterial agents by using disc diffusion technique. Each isolate was cultured on broth media, incubated at 37°C overnight. The broth culture was flooded over the surface of sensitivity test agar medium, excess inoculum was removed with a Pasteur pipette and the surface was allowed to dry before discs were applied with a sterile forceps. The petri dishes were incubated at 37°C for 24-48 hours before recording the results of sensitivity test.

3-Pathogenicity of isolated *Staph. aureus* to young chicks:

Thirty, 3 day old Lohman chicks were used for pathogenicity studies of *Staphylococcus aureus* isolates using different routes of infection. The chicks were divided into 3 equal groups (A, B, C). Chicks group "A" was inoculated subcutaneously (S/C) with 10^5 viable

microorganisms of *Staph. aureus*. Chicks of Group "B" were inoculated orally with 5×10^6 viable microorganisms. Group "C" control chicks was injected with broth and served as uninfected control. Infected and control birds were kept separately and received a strater ration. They were observed daily for 3 weeks and clinical signs, P/M lesions, deaths and reisolation were recorded.

RESULTS

The obtained results are tabulated in Tables 1-5

Results of Pathogenicity test:

Chicks of group "A" showed 100% mortality 24-48 hours post inoculation while birds of group "B" revealed 100% mortality within 2-6 days post inoculation. Experimentally chicks showed clinical signs in the form of dropping wings, depression, dullness and ruffled feathers, in addition to diarrhoea and pasty vent in orally inoculated chicks. PM lesions observed were septicaemic picture including different sites, congestion of the subcutaneous blood vessels, breast and thigh muscles, lungs, spleen and liver. Enlargement of the gall bladder, unabsorbed yolk sac and enteritis were noticed in orally infected chicks. Reisolation of the organism was made from all organs. Control birds remained apparently healthy throughout the experimental period, showed no clinical signs or gross lesions.

DISCUSSION

Staphylococcus and streptococcus infections have been considered of importance in the causation of certain problems in intensive poultry farms. So the presence of staphylococci among chickens was investigated. The clinical signs observed in chickens from which staphylococcus could be isolated were in the form of ruffling of feathers, depression, skin infection, bumble foot, conjunctivitis, swollen joints, swollen head and ulceration of comb. Similar findings were described by Fielder (1949); Guarda *et al.* (1979), Bergman *et al.* (1980); Litjens and Van-Willigen (1989); Mark and Douglas (1992); Shirai *et al.*, (1993) and Nakamura (1997). The present work revealed that 24 staphylococcal

isolates were recovered from 100 chickens with clinical signs suggesting staphylococcosis and *Staph. aureus* was the most dominant species recovered "15 strains" (62.5%). This result agreed with the view of Thompson et al (1980) and Bhatia et al, (1980) who were successful in isolating *Staph. aureus* in an incidence of more than 14.02%.

Post mortem examination of birds infected with staphylococci revealed congestion of muscles of the breast and thigh, unabsorbed yolk sac, sometimes abscesses in the liver and spleen and peticial haemorrhages on the coronary fat. Our findings appeared quite similar to these of Bhatia et al. (1980).

In case of streptococcal infection in chickens, 44 streptococcal isolates were detected from naturally diseased chickens (3-9 week old) which gave a clinical signs like diarrhoea, respiratory difficulties, emaciation, blood stained tissues, severe fibrinopurulent inflammation of eyelids and skin lesions. Similar symptoms were explained by Sato et al. (1960); Cheville et al. (1988) and Messier et al. (1993). Post-mortem picture of streptococcosis among naturally infected birds was in the form of congestion of subcutaneous blood vessels, trachea, lungs, heart, spleen, liver, unabsorbed yolk sacs and joint abscesses. Similar PM findings were observed by Buxton (1952) and Newton et al. (1962).

Concerning the in-vitro sensitivity of staph isolates to antibacterial agents Enrofloxacin and Kitamox were the most effective ones. On the other hand the in-vitro susceptibility of strept isolates to antibacterial agents indicated that the isolates were highly sensitive to Garamycin, Chloramphenicol, Enrofloxacin and Ampicillin. These results more or less agree with Linkh (1981) and Kostakev (1963).

Pathogenicity test revealed that 100% of experimentally infected chicks with *Staph. aureus* died using S/C rout while oral route gave 80%, similar findings were reported by Kohler et al. (1980). Chick showed depression, dullness, ruffled feathers, diarrhoea and pasty vent, all these were described Kuramasu et al. (1968) and Kohler et al. (1980).

Post-mortem lesions recorded were in the form of septicaemia including haemorrhages and congestion of subcutaneous blood vessels. Congestion of lungs, liver, spleen, intestine and was also observed unabsorbed yolk sac. These lesions were similar to those observed by Sato et al. (1961); Issar (1966) and Ginzburg (1975). *Staph. aureus* was reisolated from yolk sac, lungs, intestinal content, heart blood and liver. Similar finding was reported by Kohler et al. (1980).

The present work indicated that staphylococcal and streptococcal infections are common among chickens in the area of Assiut Province.

REFERENCES

- Agrimi, P. (1956):* Experimental study of streptococcal infection in fowls. *Zooprofilassi*, 11: 491.
- Bergman, V.; Köhler, B. and Vogel, K. (1980):* Staphylococcus aureus infection of fowls on industrialized poultry units 1- Types of infection. *Archiv. Fur Experim. Veterinarmedizin*, 34 (6): 891.
- Bhatia, K.C.; Kalra, D.S. and Kulshreshtha, R.C. (1980):* Staphylococcosis in turkeys. *Haryana Agricultural J.*, 10 (3): 448.
- Buchanan, R.E. and Gibbons, N.E. (1974):* Bergey's Manual of Determinative Bacteriology. 8th. Edt., Williams and Wilkins Company.
- Buxton, J.C. (1952):* Disease in Poultry associated with *Streptococcus zooepidemicus*. *Vet. Rec.* 64: 221.
- Cheville, J.; Tappe, M. Achernann and Jensen, A. (1988):* Acute fibrinopurulent blepharitis and conjunctivitis associated with *Staphylococcus hyicus*, *Escherichia coli* and streptococcus sp. in chickens and Turkeys. *Vet. Pathol.* Sep. 25 (5): 369-375.
- Cooper, J.E. and Needham, J.R. (1976):* An investigation into the prevalence of *Staphylococcus aureus* on avian feet. *Vet. Rec.*, 98: 172-174.
- Cruickshank, R.; Duguid, J.R.; Marmion, B.P. and Swain, R.H.A. (1975):* Medical Microbiology. 12 Edt. Churchill livingstone, Edinburgh and New York.
- Fielder, F. G. (1949):* A note on streptococcosis in chickens. *Aust. Vet. J.*, 25: 230.
- Ginzburg, V. V. (1975):* Differentiation of staphylococci isolated from fowls. *Veterinariya Moscow*, 8: 32.
- Guarda, F.; Cortellezzi, G.C. and Cucco, C. (1979):* Blindness due to *Staphylococcus aureus* in turkeys. *Clinica Vet.*, 102: 315.
- Harry, E.G. (1967a):* the characteristics of *Staphylococcus aureus* isolated from cases of staphylococcosis in poultry *Res. Vet. Sci.*, 8: 479-489.

- Harry, E.G. (1967b):* Some characteristics of *Staphylococcus aureus* isolated from the skin and upper respiratory tract of domesticated and wild (feral) birds. *Res. Vet. Sci.*, 8: 490:499.
- Issar, S.L. (1966):* The influence of prednisolone on the morbid anatomy of induced *Staphylococcus aureus* and *Staph.epidermidis* in chickens. *Diss. Abst.* , 26: 7265.
- Kohler, B.; Nattermann, H.; Witte, W.; Friedrichs, F. and Kunter, E. (1980):* *Staphylococcus aureus* infection of fowls on industrialized poultry units. II- microbiological tests for *staph. aureus* and other pathogens. *Archv Exper. Veterinärmedizin*, 34 (6): 905.
- Kostakev, A. (1963):* Streptococcal septicaemia in newly hatched chicks . *Parazit. Bolesti. sofia* , 9: 137.
- Kuramasu, S.; Imamura, Y. and Tajima, Y. (1968):* Studies on staphylococcosis in chickens. II- Pathogenicity to various experimental animals. *Zentbl. Vet. Med.*, 15 (B): 640.
- Linckh, E. (1981):* Characterization of staphylococci of chickens with reference to their resistance to antibiotics. *Inaugural Diss.*, Ludwigmaximilians Universität, München (1980) .
- Litjens-JB and Van-Willigen (1989):* A case of swollen head syndrome in a flock of guinea fowl. *Tijdsch-Diergeneeskd* . Jul. 1., 114 (13): 719-720.
- Mark, A. and Douglas, W. (1992):* Clinical and pathological findings in young Georgia broiler chickens with oculofacial respiratory Disease ("So-Called swollen heads ") *Avian Dis.* 38: 376-378.
- Messiers, S.; Quessy, Y.; Robinson, L. A.; Devriese, J. Homnez and Fairbrother, J.M. (1993):* Focal dermatitis and cellulitis in broiler chickens, bacteriological and pathological findings . *Avian Dis.* 37 (3): 839-844.
- Nabila, M.D. (1982):* Studies on staphylococcosis and streptococcosis in poultry. *M.V.Sc. Thesis Faculty of Vet. Med.*, Cairo University.
- Nakamura, K. (1997):* Out break of comb necrosis in layer breeder chickens. *Avian Dis.* 41 (1): 252-256.
- Newton, L.G.; Connole, M.D. and Ranby, P. (1962):* Avian streptococcosis, *Proc. 12 th World's poult. Congress Sydney*, 355.

- Sato, G.; Miura, S. and Uschijma, J. (1960):* An outbreak of haemolytic streptococcal infection among chickens of a flock . Jap. J. Vet. Res., 8: 285.
- Sato, G.; Miura, S.; Nakagawa, M. and Ito, A. (1961):* Characters of staphylococci isolated from dead chick embryos and from pathological conditions in chickens . Jap. J. Vet. Res., 9: 10.
- Sherman, J. M. (1937):* The streptococci and related enterococci .Bact. Rev.,1 : 3.
- Shirai, M.; Maeda, M.; Fujii, S. and Kuniyashi (1993):* Swollen head syndrome is not associated with turkey Rhinotracheitis virus. Vet. Res. 132, 41-42.
- Thompson, J.K.; Gibbs, P.A. and Patterson, J.T. (1980):* Staphylococcus aureus in commercial flocks, incidence and characteristics of strains isolated from chicks and pullets . Brit. Poul. Sci., 21 (4): 315.
- Witte, W. (1977):* Ecology of Staphylococcus aureus : characterization of strains from chickens, Z. Allg. Mikrobiol., 17: 639-649.

Table (1): Results of isolated staphylococcus and streptococcus from different collected samples

Number of specimens	Staph isolates				Strept. isolates			
	S.H	internal organs isolates	total	%	S.H	internal organs isolates	total	%
100	9	15	24	24	12	32	44	44

S.H = Swollen head

Table (2) Results of biochemical identification of isolated staphylococcus strains

Test	Staph. species		
	<i>Staph. aureus</i> No. of isolates (15)	<i>Staph. epidermidis</i> No. of isolates (5)	<i>Staph. saprophyticus</i> No. of isolates (4)
Coagulase production test	+	-	-
<u>Sugar fermentation</u>			
Manitol	AG	-	A or -
Arabinose	-	-	-

negative = - positive = + Acid and gas = Ag Acid only = A

Table (3) Results of biochemical identification of isolated streptococcus strains

Test	Strept. Species		
	<i>Strept. pyogenes</i> No. of isolates (10)	<i>Strept. zooepidemicus</i> No. of isolates (11)	<i>Strept. faecalis</i> No. of isolates (23)
Haemolysis	Beta	Beta	Alfa or Beta
Litmus milk	Acid & reduction	Acid	Acid
<u>Sugar fermentation</u>			
Manitol	±	-	+
Arabinose	-	-	-

negative = - positive = + suspect = ±

Table (4) Results of in-vitro sensitivity testing 5 of staph. isolates

Antimicrobial	No. of tested isolates	Sensitive		Resistant	
		No.	%	No.	%
Ernofloxacin	5	5	100	-	0
Kitamox	5	5	100	-	0
Garamycin	5	4	80	1	20
Oxytetracycline	5	4	80	1	20
lincocin	5	3	60	2	40
Chloramphenicol	5	4	80	1	20
Fucidin	5	3	60	2	40
Streptomycin	5	2	40	3	60
Ampicillin	5	2	40	3	60

Table (5) Results of in-vitro sensitivity testing 5 of strept. isolates

Antimicrobial	No. of tested isolates	Sensitive		Resistant	
		No.	%	No.	%
Ernofloxacin	5	5	100	-	0
Kitamox	5	2	40	3	60
Garamycin	5	5	100	-	0
Oxytetracycline	5	-	0	5	100
lincocin	5	1	20	4	80
Chloramphenicol	5	5	100	-	0
Fucidin	5	4	80	1	20
Streptomycin	5	2	40	3	60
Ampicillin	5	5	100	-	0

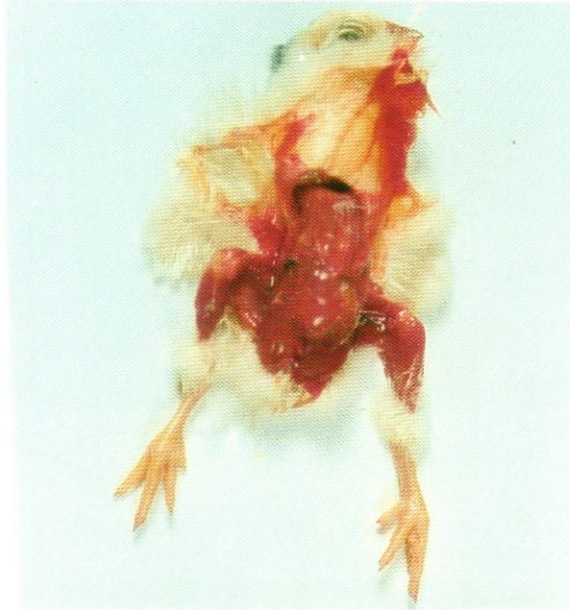


Fig. (1) Chicks inoculated S/C with Staph. Aureus showing congestion of the subcutaneous tissues, muscles of thigh and breast .



Fig. (2) Chick inoculated orally with Staph.aureus showing congestion , enlargement of liver and gall bladder .



Fig. (3) : Chicks inoculated orally with Staph. aureus showing
• **Congestion of intestine and unabsorbed yolk sac**