Vet.Med.J.,Giza. Vol.42,No.2.(1994):15-19.

DOSE STAPHYLOCOCCUS AUREUS INDUCE EYE LESIONS IN CHICKENS

By

M.M. ZAKI * and M.B. EL-BEGAWEY**

* Dept. Poultry Diseases, Faculty of Veterinary medicine, Cairo University, Giza 12211- Egypt.
** Dept. Pathology, Faculty of Veterinary medicine, Cairo Univeristy, Beni Suef, Egypt.

Recieved: 24/2/1994.

SUMMARY

An outbreak of acute conjunctivitis and seropurulent blepharitis were observed in two different cage-reared laying flocks aged 6 and 7 weeks old. Pathogenic Staph aureus was isolated from both cases with no evidence of any complicating infections. The isolated organism was found sensitive to penicillin and ampicillin, that later was used successfuly in controlling the outbreaks. Experimental conjunctivitis and blepharitis were induced by eye inoculation with isolated Staph. aureus strain in chicks at the same age. The histopathological examination of affected eyes showed marked epithelial hyperplasia and necrosis as well as heterophilic exudation into epithelial covering which were prominant features in all cases. Natural skin microflora, stress, cage-rearing, contamination of vaccination instruments and dust infection were incrimenated as peridisposing factors in such outbreaks. It was concluded that Staph. aureus is one of poultry pathogens that might induce eye lesions in chickens.

INTRODUCTION

Staphylococcus aureus infection is one of the common diseases affecting modern poultry industry. It has been early described by Hole and Purchase (1931) that Staph. aureus induced arthritis and periostitis. Many reports indicated the involvement of Staph. aureus in cases of gangrenous dermatitis (Frazier et al., 1964), osteomyelitis (Mutalib et al., 1983), Omphalitis (Williams and Daines, 1942), granulomas of liver and lungs (Munger and Kelly, 1973) and fatal septicemia (Bickford and Rosenwald, 1975). On the other hand, Staph. aureus has a great public health importance that it produces enterotoxines capable of causing food poisoning in humans (Harvey et al., 1982). The work described in the present paper is a trial to find out the implication of Staph. aureus in outbreaks of conjunctivitis and seropurulent blepharitis recorded in two cage-reared laying flocks aged 6 and 7 weeks.

MATERIAL AND METHODS

Samples

Two outbreaks of conjunctivitis and bilateral swelling of eye lids were observed in two different cage reared growing commercial laying chicken flocks (Fig. 1&2). The first flock was Lohman layer chickens and the clinical signs were detected at age of 6 weeks with 0.8% mortality rate while the second flock was Hisex layer chickens that showed the clinical signs at age of 7 weeks with mortality rate of 0.5%. A caseous-like material was detected at the middle cansus of the eye under the third eye lid, with no pathological changes in cornea or eye ball. Dehydration and emaciation were found to be prominent in dead birds without presence of any pathological lesions in vesceral organs.

Collection of Samples:

Samples were taken from living birds by swabbing the conjunctival sac with sterile cotton gauze swab moistened with sterile peptone water. Swabs were transported to the laboratory in sterile bottles for virological and bacteriological examinations. Conjunctival swabs, liver, spleen and heart were collected aseptically from sacrified

birds while eye lids were kept in 10% formol buffere for histopathological examination.

Bacteriological examination:

Collected samples were cultured on blood agar (bioMerieux), Nutrient agar (Oxoid) and Mackonky agar (bioMrieux). Plates were incubated for 2 days at 37°C then colonies were subjected to identification procedures. Suspected Staph. aureus colonies were picked and cultured into nutrient broth (bioMrieux) and incubated overnight (Thompson et al., 1980) then tested for coagulase activity by the method described by Fisk (1940). Coagulase-Positive staphylococci were purified and maintained on Tryptone Soy Agar (Oxoid) enriched with 5g/L yeast extract (Oxoid). (Thompson et al., 1980).

Culture identification and characterization:

The detection of gelatinase production as well as the crystal violet test and coagulation of bovine plasma were conducted according to the methods stated by Gibbs et al.(1978) while pigment production was detected on glycerol monoacetate agar (Jacobs et al., 1964). The ability of isolates for heamolysis of sheep red cells was detected acccording to the method of Elek and levy (1950). Blood agar base (bioMrieux) containg 12% fresh citrated human plasma was used for detection of finbrinolysis as described by Thampson et al. (1980). Antibiotic senstivity testing of isolates to different antibiotics using oxoid senstivity discos was conducted according to the technique of Thompson et al. (1980).

Pathogenicity test:

Ten fold dilution (1×10^1- 1×10^5) of 24 hours broth cultures was prepared for pathogenicity testing of Staph. aureus strains. Each dilution was inoculated occularly at rate of 50 µl per bird in fifteen 6 week-old male Hisex laying breed (groups 1-5).

A control group birds were inoculated in the same route by 50 µl sterile broth per bird. The inoculated birds were kept for 10 days observation period during which clinical signs, mortalities, reisolation trials and histopathogocial examination were performed.

Virological examination:

Collected samples were subjected to trials for virus isolation in embryonated chicken eggs by different routes. Embryonic deaths or lesions were recorded and exposed to identificantion (Senne, 1989).

Histopathological examination:-

Specimen from eye lids were fixed in 10% formal buffer, embeded in paraffin, sectioned and stained with haematoxylin and eosin and Giemsa stain (Carleton et al., 1967).

RESULTS

Clinical signs and necropsy:-

The naturally infected chicks showed general signs of illness (Fig. 1) with progressively severe conjunctivitis (Fig. 2) accompanied by acute seropurulent blepharitis which resulted in swelling of eye lids and closure of the eyes (Fig. 3). By the time of clinical signs onset there was a sharp decrease in feed and water consumption. Low

Fig.1: Naturally infected chickens showed ruffled feather, recumbency and swallen eye lids resulted in closure of their eyes.

Fig. 2: Naturally affected chicken showed progressive severe conjunctivitis whith acute seropurulet blepharitis.

Fig. 3: Naturally affected chicken showed swelling of eye lids resulted in closure of the eye.

mortality rate (0.5 - 0.8%) was recorded within 3-5 days after appearance of signs. No pathological changes could be detected in visceral organs.

Bacteriology:

Staph. aureus was isolated from the eye of naturally infected chickens with no evidence for ncaltion of any other pathogenic bacteria. The isolated Staph. aureus were found to be coagulase-positive, gelatinase-positive, induced haemolysis, produced pigment and had the ability to produce fibrinolysin. The sensitivity testing for antibiotics revealed that the isolates were found to be sensitive to penicillin and ampicillin, but resistant to tetracyclin, nitrofuranse, flumequine, oxolonic acid, lincomycin and nalidixic acid.

Virology:-

Non of pathogenic viruses were isolated from either samples collected from the affected eyes or internal organs.

Pathogenicity:-

The inoculated organisms in dilutions $1x10^{1}$-$1x10^{5}$ successeded in induction typical lesions in experimentally infected birds similar to that observed in naturally infected birds. The inoculated Staph. aureus was reisolated from all exprimentally infected chicks developed eye lesions. Treatment of affected birds with ampicillin in drinking water at rate of 15 mg per kg living body weight revealed in disappearance of clinical signs with 2-3 days post-treatment.

Histopathology:

The microscopical examination of affected conjunctiva revealed marked epithelial hyperplasia, expanstion and folding. Sloughing of epithelial cells was minimal but vaculation of the cytoplasm was frequent. Necrotic epithelium was seen in some areas. Heterophilic exudation into epithelial covering was prominent feature in all cases. Many spouting capillaries were seen dialated and engorged with erythrocytes in covering epithelium (Fig. 4). Neither intracytoplasmic nor interanuclear inclusion bodies could be detected. The lamina propria was expanded by heavy proteinacaous edema together with a very dialated lymphatics and capillaries. The blood vessels in the propria had a prominent endothelium as well as perivascular cuffing in most of them. Depletion and necrotic changes were seen in the lymphoid follicles in the propria. Lymphocytic and heterophilc infiltration were observed in the lamina prepia (Fig. 4), while in few cases hyperplasia and leucocytic infiltration were obseved in feather follicles (Fig. 5). Examination of Giemsa stained sections proved presence of heavy infiltration with heterophils in both epithelium and propria (Fig. 6).

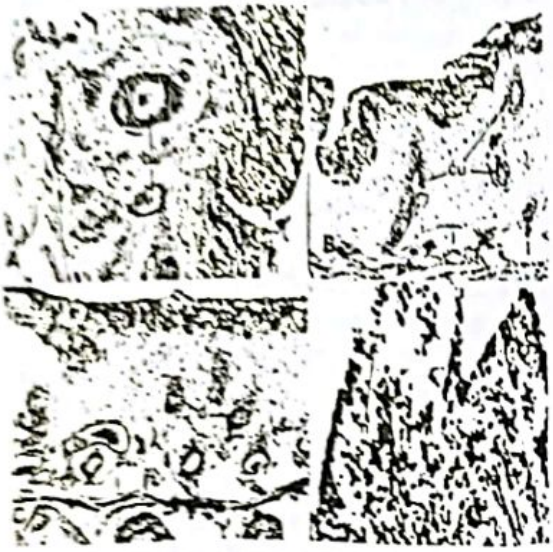

Fig. 4: Histopathology of conjunctiva showed 1. Epithelial hyperplasia (e), folding (f) and expantion (x). 2. Epithelial sloughing and necrosis (n). 3. Heterophilic infiltration (h). 4. Sprouting capillaries in the covering epthelium (p). 5. Prominent endothelium in congested blood vessels (B.V) and dilated lyphatics (L). 6. Depletion and necrosis in lymphoid follicles (d). 7. Perivascular cuffing (cu) and lymphocytic infiltration. (A,B,C. H & E x 100 - D. H & E x 400.

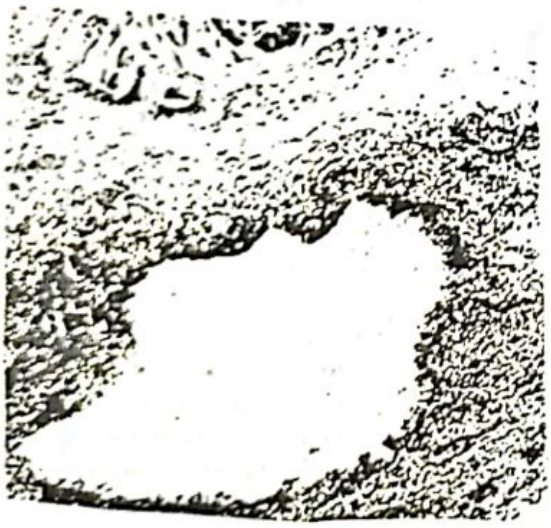

Fig. 5: Histopathology of the eye lid of naturally affected chickens showed hyperplasia and leucocytic infiltration in feather follicles (F.F). H & E x 100.

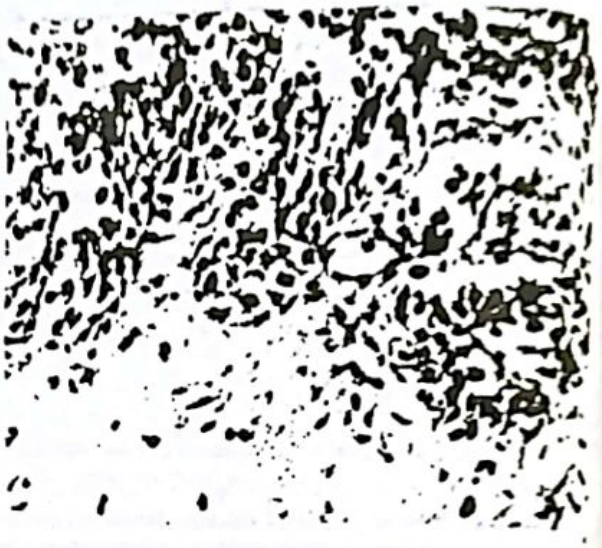

Fig. 6: Histopathology of the eye lid showed heavy infiltration with heterophils (h). Giemisa stain x 400.

DISCUSSION

The clinical signs and the pathologic changes in form of acute bilateral conjunctivitis and seropuruent blepharitis were observed in two different cage-reared laying flocks aged 6 and 7 weeks old. Pathogenic Staph. aureus was isolated from all affected eyes of examined chicks which found sensitive to penicillin and ampicillin. The mortalities recorded in such outbreaks were refered to the uncapability of affected birds to see and to reach the food and water that resulted in emaciation and dehydration.

The eye infection of such chickens with Staph. aureus might be attributed to the extension of contamination from the skin because the Staph. aureus population size increased rapidly on the skin of live chickens until the seventh week of life and thereafter the levels remained high (Devriese et al., 1975). Moreover, Kusch and Gotze (1976) found that staphylococci spread in rearing of a broiler flock which was probably of human origin. On the other hand, the level of the Staph. aureus population of the skin of live hens may be controlled directly or indirectly by changes in the skin chemistry or physiology during the rearing and laying cycle (Thompson et al., 1980). Wos and Jagodzinska (1978) concluded that sharp edges in the environment could predispose the chicks and pullets to staphylococcus infections as a result of traumatical injuries that Roskey and

Hamdy (1975) showed that staphylococci were better able to grow in bruised than normal poultry tissues and that might explain the fact that the cage-reared showed higher incidence of Staph. aureus than the floor-reared flock (Thompson et al., 1980). Furthermore, it had been found that stress may initiate outbreaks in chickens that have a latent staphylococcal infection from an early age (Mutalib et al., 1983). Dust infection and contamination of vaccination instruments were also incremineted in induction of such cases. On the view of histopathological examination of affected eyes, it had been found that neutrophils (heterophils) appeared in the area of irritation very soon after the irritant has been applied specially in bacterial infection and it does not occured in most virus infection unless acute bacterial infection or necrosis occurs (Smith et al., 1972). As a conclusion, our present investigation revealed that Staph. aureus is consedered one of poultry pathogens that induces eye affection in chickens.

REFERENCES

Bickford, A.A. and Rosenwald, A.S. (1975): Staphylococcal infections in chickens. Poult. Dig. (July): 285-287.

Carleton, H. M., Drury, R.A., Willington, E.A. and Coneron, H. (1967): Carleton's Histological Technique, 4th Ed. Oxofd Univ. Press, New York.

Devriese, L.A., Devos, A.H. and Van Damme, L.R. (1975): Quantitative aspects of the staphylococcus aureus flora of poultry. Poult. Sci., 54: 95-101.

Elek, S.D. and levy, E. (1950): Distribution of haemdysins in pathogenic and non-pathogenic staphylococci. J. Pathol. Bacteriol. 62: 541-554.

Fisk, A. (1940): The technique of the coagulase test for staphylococci. British J. Exp. Pathol., 21: 311-314.

Frazier, M.N., Parizek, W.J. and Garner, E. (1964): Gangrenous dermatitis of chikens. Avian. Dis. 8: 269-273.

Gibbs, P.A., Patterson, J.T. and Thompson, J.K. (1978): Characterization of poultry isolates of staphylococcus aureus by a new set of poultry phages. J. Appl. Bacteriol., 44:387-400.

Harvery, J., Patterson, J.T. and Gibbs, P.A. (1982): Enterotoxigenicity of Staphylococcus aureus strains isolated from poultry: Raw poultry carcasses as a potential food-poisoning hazard. J. App. Bacteriol. 52: 251-258.

Hole, N. and Purchase, H.S. (1931): Arthritis and periostitis in pheasants caused by staphylococcus pyogenes aureus. J. Comp. Parthol. Ther. 44: 252-257.

Jacobs, S.I., Willis, A.T. and Goodbwn, G.M. (1964): Pigment production and enzymatic activity of staphylococci: The differentiation of pathogens from commensals. J. Pathol. Bacteriol, 87:151-156.

Kusch, D. and Gotze, U. (1976): Die Ursachen der Staphylokokken kontamination bei der Gewinnung von Brathahnchen. Die Fleischwirtschaft, 56: 707-709.

Munger, L.L., and Kelly, B.L. (1973): Staphylococcal granulomas in a Leghorn hen. Avian Dis. 17: 858-860.

Mutalib, A., Riddell, C. and Osborne, A.D. (1983): Studies on the pathogenesis of staphyhlococcal osteomyelitis in chickens. I. Effect of stress on experimentally induced osteomyelitis. Avian Dis. 27: 141-156.

Roskey, C.T. and Hamdy, M.K. (1975): Persistence of Staphylococcus bruised tissue micro-environment affecting persistence of Staphylococcus aureus. J. Food Sci. 40: 496-499.

Senne, D.A. (1989): Virus propagation in embryonated eggs. In: A Laboratory Manual for the Isolation and Identification Of Avian Pathogens, 3rd Ed., American Association of Avian Pathologists, P. 176.

Smith, H.A., Tones, T.C. and Hunt, R.D. (1972): Veterinary Pathology 4th Ed., Lea & Febiger, Philadelphia.

Thompson, J.K., Patterspm. J.T. and Gibbs, P.A. (1980): The use of a new phage set for typing poultry strains of Staphylococcus aureus obtained from seven countries. British Poult. Sci., 21: 95-102.

Williams, R.B. and Daines, L.L. (1942): The relationship of infectious omphalitis of poults and impetigo staphylogenes in man. J. Am. Vet. Med. Assoc. 101: 26-28.

Wos, Z. and Jagodzinska, H. (1978): Characteristics of staphylococci found on chicken carcasses. Przemysl Spozywczy, 32: 186-187.