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SOME STUDIES ON PSEUDOMONAS INFECTIONS IN GROWING CHICKENS IN ASSIUT FARMS

(With 3 Tables)

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بعض الدراسات عن ميكروب السودوموناس فى بدارى الدجاج فى مزارع اسيوط

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فى هذا البحث تم فحص عدد ٢٠٠ من بدارى دجاج التسمين النافقة حديثا من مزارع حكومية مختلفة بمحافظة اسيوط. ولقدتم عزل١٦ حالة ايجابية لميكروب السودوموناس ارجينوزا بنسبة ٨٪. و باجراء العدوى الصناعية بهذا الميكروب فى الكتكوت عمر ثلاثة ايام ثبت أن الحقن تحت الجلد كان اكثر تاثيرا حيث وصلت نسبة النفوق الى ٩٥٪ فى حين كانت ٤٠٪ فى الكتاكيت التى حقنت عن طريق الفم. هذا وقد تم عزل الميكروب مرة أخرى من الكتاكيت النافقة. وباجراء اختبار الحساسية فى المعمل للعترات المعزولة وجد أنها جميعا عالية الحساسية لكل من الجينتاميسين والنيومايسين والدانوفلوكساسين والكلوستين والاميكين والاستربتومايسين.

SUMMARY

Atotal of 200 freshly dead growing chickens collected from different governmental farms at Assiut Governorate. Only 16 positive cases of *Pseudomonas (Ps) aeruginosa* were isolated with an incidence of 8%. The experimental infection in 3-day old chicks by different routes of inoculation revealed that subcutaneous route was highly effective with mortality rate 95%, while oral route of infection was almost less (40% mortality). Reisolation of inoculated organism from dead chicks were conducted. In vitro antibiotics sensitivity tests showed that the examined isolates were

highly sensitive to gentamycin, neomycin, danofloxacin, colistin, amikin and streptomycin.

Key words: Pseudomonas - Growing Chickens

INTRODUCTION

The poultry industry in A.R.E. has been greatly developed in last few years as a trial to full fill the increased demands of animal protein consumption .

Several microbial infections are responsible in the losses of poultry industry, from an economic point of view *Ps . aeruginosa* infection is not only responsible for embryonic mortality but also for mortality in chicks and heavy losses of broilers (Valadae, 1961; Saad et el 1981; Andreev et al 1982 and Bapat et al., 1985) .

Ps. aeruginosa was isolated from liver of 38 (3.6%) of 1067 dead broilers by Mrden et al (1988). Younes et al (1990) examined 406 dead chickens in Assiut Governorate, *Ps. aeruginosa* was isolated from 20 (4.6%). Lin et al. (1993) recovered 10 isolates of *Ps.aeroginosa*, 2 isolates of Ps. fluorescens and one isolate of *Ps. stutzeri* from respiratory illness or from the bone marrow of dead birds.

Ps. infection in growing chickens did not receive much care in our country in spite of routine work of laboratory diagnosis of most chicken problems at Assiut Governorate revealed that *Ps. aeruginosa* were usually isolated, there fore the work reported in this paper was undertaken to give an idea about the following:

*The incidence of Ps. species from growing chickens at Assiut Governorate.

*Experimental infections using the isolated organisms in 3-day old chicks by different routes and doses .

*In vitro sensitivity test of the isolated *Ps. species* against different antibiotics.

MATERIAL and METHODS

Material

1- Samples :

A total of 200, freshly dead growing chickens were obtained from Governmental farms at Assiut Governorate . Tissue sample from liver , spleen, kidney, heart blood and lungs were collected from these cases and subjected to bacteriological examination .

2-Media:

A- Liquid: peptone water, nutrient broth, glucose phpsphate broth 1%, peptone broth, semisolid agar, sugars (glucose, sucrose, dulcitol, galactose and maltose).

B- Solid: MacConkey's, Simmon's citrate agar, Triple sugar iron agar, urea agar base.

3- Reagents ,chemicals and stains used were, Kovac's, urea, methyle red, oxidase, andrade's indicator, chloroform, Gram's stain.

4- Experimental animals. 50, three days- old chicks (balady) obtained from baby chicks production farm, Assiut, were used for pathogenecity tests .

5- Antimicrobial sensitivity discs. were produced by Oxoid- Laboratories including: Garamycin (10μg), Neomycin (10 μg), Danofloxacin (30 μg), Colistin (10μg), Amikin (30μg), Steptomycin (10μg), Chloramephenicol (30μg), Lincomycin (2μg), Nitrofuran (300μg), Trimethobrim (25μg), Cefoxin (30μg), Ampecillin (10μg), Erythrocin (15μg), Oxytetracyclin (30μg).

Methods:

1- Isolation and identification of Ps. strains:

Samples from individual bird including liver, heart, spleen, kidney and lungs were collected a septically. Loopfuls from these organs were inoculated into neutrient broth tubes and icubated at 37 °c for 18-24 h. followed by subculturing on neutrient agar, Mac-Conkey's agar plates at 37 ° c for 24-48 h. Suspected colonies were picked up and subjected to further identifications based on colonial and cellular morphology, pigment production, detection of musty smell, oxidase test, solubility of pigment in chloroform, sugar ferementation and other biochemical tests (Buchanan and Gibbons, 1975) and Wilson and Miles (1975).

2- Pathogencity test:

Baby chicks used in this study were proved healthy and free from *Ps. infection* by post mortem (P.M.) and bacteriological examination .

3- Sensitivity test:

In vitro antibiotic sensitivity testing of identified *Ps. spp.* was performed by the disc plate technique described by Blair et al. (1970) in order to determine their antibiogramme.

RESULTS

I- Isolation and identification of Ps. species :

According to the morphological and biochemical studies especially oxidase test of the suspected *Ps. organisms*, 16 isolates were identified to be

Ps. aeruginosa, while the other isolates were negative, "Saif-Edin (1983) and Shahata et al. (1988)" frequency and percntage of infection are summarized as presented in table (1) .

II- Results of experimental infection in baby chicks :

(50) 3-day old chicks were classified into 3groups.

-Chicks of group 1 (20) were inoculated subcutaneously by 0.1ml peptone water culture of *Ps. aeruginosa* .(10^8 viable cells) / bird (Saif-Edin, 1983 and Shahata et al., 1988).

- Chicks of group 2(20) were inoculated orally by 0.1 ml peptone water culture of the same of the previous organism (10^8 viable cells) / bird (Saif-Edin, 1983 and Shahata et al., 1988).

- Birds of group 3 (10) were kept without inoculation as control .

During the observation period (one month) clinical signs, P.M. lesions were recorded and trials for reisolation of Ps. from dead bird.

* The clinical signs of chicks experimentaly infected subcutaneously appeared after 18h . of infection. Birds showed sleepy appearance, closed eyes, pasty vent and dyspnea. Signs of lamness and sitting on hocks were observed in this group infected subcutaneously. 19 out of 20 chicks inoculated with organism died within 1-3 days after infection.

* The clinical signs of chicks experimentaly infected orally appeared after 24 h. of infection. Birds showed depression, ruffled feathers, dropping of wings, coughing, sneezing, anoroxia, diarrhoea and finally death . 8 out of 20 chicks inoculated with the organism died within 2-7 days after infection.

* No symptoms were observed in control group.

The results of pathogencity test in baby chicks are given in table (2) and it is clear that it is high in birds infected subcutaneously than those infected orally.

* The P.M. lesions of subcutaneously infection with *Ps. aeruginosa* on baby chicks include catarrhal enteritis, liver, heart, kidneys are pale in colour and friable, spleen, brain were congested and thickned, while the P.M. lesions of oral infections of dead chicks were restricted to the alimentary tract which was congested and covered with mucous exudate, liver enlarged, congested and shows necrotic foci, heart, kidney appeared flabby. The control group of 10 chicks did not show any signs of illness and survived till the end of 30 days. Reisolation trials were positive from internal organs especially heart blood , liver , lungs and spleen of dead chicks.

III- The effect of different antibiotics on the isolated *Ps.aeruginosa* isolates are illustrated in Table 3.

DISCUSSION

In Egypt, poultry is considered as important source of good quality and economic animal protein. Recently Ps. infection appeared to be of high significance as a cause of losses among the poultry flocks.

Bacteriological examination of dead growing chickens revealed that the organism was recovered from 8% of the examined birds . A much lower percentage was reported by Shahata et al (1988), Mrden et al (1988) and Younes et al (1990) who recovered *Ps.aeurginosa* from dead growing chickens with an incidence of 4.76%, 3.6% and 4.6% respectively. Moreover, a much higher percentage was reported by Saif-Edin (1983)who isolated the same organism with an incidence of 21.6% at Kena Governorate.

The experimental infections in baby chicks by subcutaneous and oral route with both cultures of *Ps. aeruginosa* revealed that the subcutaneous route of inoculation was the most effective once producing mortality rate about 95% ,but oral route was milder with mortality rate about 40%. The pathogenic effect of *Ps. aeruginosa* in chicks was reported by Ray and Banerji (1969), Markaryan (1975) and Mrden (1988) who reported that the *Ps. aeruginosa* isolates were pathogenic to chicks leading to similar signs to those described by our results .

In this present study , the experimental infection in baby chicks closely nearly similar to the results were reported by Saif-Edin (1983), Shahata et al (1988) and Yoyness et al (1990) who found that the *Ps. aeruginosa* caused 100% mortality when inoculated subcutaneously in 3 or 7-day old chicks with the same dose (10^8 organism). Reisolations of the organism from dead and sacrified chicks were conducted and this disagreed with the work of El-Nasaan et al (1975) who failed in reisolation of the organism from dead chicks .

In vitro sensitivity testing of the isolate to 14 antimicrobial agents revealed that the isolates examined were highly sensitive to gentamycin, neomycin, danofloxacin, colistin, amikin, streptomycin and moderately sensitive to chloramephenicol, lincomycin, nitrofuran, trimethobrim, cefoxitin while, ampcillin, erythromycin, oxytetracycline had no effect at all. In this respect our results agree but to some extent to those reported by Awaad et

al (1981), Saif-edin (1983), Bapat et al (1985), Shahata et al (1988), Siohu et al (1989), Utomo and Poernomo (1990) and Khalil (1992).

Finally it may be concluded that Ps. infections are resposible for high losses among the growing chickens and further studies are needed especially for prevention and control.

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Table (1) : Frequency and percentage of isolated *Ps . aeruginosa*

Examined specimens	No. of samples	*Ps. aeruginosa*	
		No.	%
Growing dead chickens	200	I6	8

Table (2) : Showing the results of pathogencity in chicks

Group No.	No. of infected chicks	Route of infection	Dose of inoculum *Ps.aeruginosa*	Daily deaths post infection 1 2 3 4 5 6 7 8 30	Total No of deaths.	No. of survivors	Mortality rate
1	20	s/c	10^8	9 7 3	19	1	95%
2	20	oral	10^8	2 1 1 2 1 1	8	12	40%
3	10	Control	0	0 0 0 0 0 0 0.....0	0	10	0.0%

Table (3) : Results of sensitivity of *Ps . aeruginosa* isolates

Antimicobial agents	Sensitivity of *Ps . aeruginosa* isolates
Garamycin	+++
Neomycin	+++
Danofloxacin	+++
Colistin	+++
Amikin	+++
Streptomycin	+++
Chloramephenicol	++
Lincomycin	++
Nitrofuron	++
Trimethobrim	++
Cefoxitin	++
Ampecillin	-
Erthromycin	-
Oxytetracycline	-

+++ = Highly sensitive
++ = Moderate sensitive
- = Resistant