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SOME STUDIES ON PSEUDOMONAS SPECIES IN CHICKEN EMBRYOS AND BROILERS IN ASSIUT GOVERNORATE

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ABSTRACT :

One hundred, dead-In- shell embryos from balady hatcheries, eighty-five, (2-15 days baby chicks) and sixty broilers 5–8 weeks old (diseased and freshly dead) were collected from different location in Assiut Governorate. pseudomonas aeruginosa (Ps. aeruginosa) was isolated from these sample at rates of 21%, 17.6 %, and 3.3% respectively. Experimental infection with isolated Ps. aeruginosa revealed deaths of all embryos inoculated via yolk sac route, 80% mortality of 3-days old chicks inoculated subcutaneouslly. No mortality recorded between 3-days old chicks and 9-weeks old broilers inoculated orally and intramuscullary respectively but mild swollen of head was observed in 3-days chicks. Antibiotics sensitivity test showed that the isolated ps. aeruginosa was highly sensitive to the norfloxacin, chloramphenicol and streptomycin.

INTRODUCTION:

-Ps. is widely distributed in nature and it is common inhabitant in the soil. Septicaemic infection in poultry has been reported by Ray and Banerji (1969) and Narula and Kuppuswamy (1969).

-Ps. aeruginosa is the most common avian pathogens and it produce a variety of toxins and enzymes that may contribute to pathogenicity and Ps. stutzeri has been isolated from chickens with respiratory disease and produced only low mortality in experimentally inoculated chickens, but Ps. fluorescence can cause turkey embryo mortality and it has been associated with multicausal respiratory disease of chickens and turkeys (Hinz *et al*, 1992 and lin *et al*., 1993).

-The disease (Pseudomoniasis) may be localized in the infraorbital sinuses, air sacs or cellulitis or it is a systemic septicaemic disease affecting many organs and tissues. Morbidity and mortality varies from 2 to 100%, but more commonly about 2-12% with greatest losses in very young birds. The infection may occur through skin wounds or contaminated vaccines, egg dipping or egg inoculation or through contamination of needles used for injection, infection can also spread from infected to susceptible flocks on the same premises under conditions of inadequate hygiene. (John Barnes, 1997).

-Ps. aeruginosa is an opportunistic pathogen that can invade fertile eggs causing death of embryos and virulent strains can cause diarrhea, dehydration, dyspnea, septicemia

and death to newly hatched chicks. (Walker *et al*., 2002).

The aim of this study :

-Isolation and identification of Ps. species from dead-in shell chicken embryos, baby chicks and broilers.

-Experimentally demonstrate the disease in fertile chicken eggs, young chicks and broilers, using isolated organism

-Sensitivity test to show the most effective drugs against isolated Ps.

MATERIAL AND METHODS:

Material:

1-Speciemens:

-A total of 100 dead in shell chicken embryos, 85 diseased and freshly dead baby chicks and 60 diseased and freshly dead broilers were collected from Assiut governorate.

2- Media:

Solid:

-Nutrient agar plates and slopes.
-MacConkey's agar plates.
-Milk agar plates.
-Blood agar plates.
-Urea agar base.

Liquid:

-Nutrient broth.
-Sugars (glucose, sucrose and maltose)
-Semi solid agar tubes (for motility test).

Reagents:

-Methyl red. **-Kovac's.**
-Urea. **-Oxidase.**

Stain:

-Gram's stain.

3- Pathogenicity test :

We used :

-Twenty five, 3-days – old chicks.
-Fifteen 9-weeks-old chickens.
-Thirty –7 days-old fertile chicken eggs (balady), they obtained from the faculty of Agriculture farm in Assiut.

4- Antibiotic sensitivity discs:

Include: Streptomycin (10µg), Chloramphenicol (30µg) Gentamycin (10µg). Trimethobrim (5µg), Neomycin (30µg), Ampicillin (10µg), Oxytetracyclin (30µg), Norfloxacin (10µg) and lincomycin (2 µg).

Methods:

1-Speciemens from liver, spleen, heart blood, intestinal content and gall-bladder content (from freshly dead and diseased chicks, broilers and dead embryos) were cultured in nutrient broth tubes and incubated for 24 hour at 37ºC, then loopfull from broth was subcultured on to nutrient agar, MacConkey's agar, blood agar and milk agar plates and incubated for 24-48 hour at 37ºC, suspected colonies to be Ps. were kept onto slope agar for further identification for the character of the colony, production of pigment, biochemichal reactions (urease, catalase, oxidase, methyl red, voges-proskauer, indol and fermentation of glucose, sucrose and maltose).

2-Pathogenicity test:

a-Chicken embryos: Thirty, 7-days-old were used, five from them were taken at random and examined bacteriologically to ensure that they were Ps. free the remaining twenty five eggs were divided into 2 groups: the first group consisting of twenty eggs were inoculated via yolk sac route by 0.1 ml of 24 hour broth culture contain $14x10^7$ viable cell of Ps./ml

(Saad *et al*., 1981). The second group consisting of five eggs were kept as control.

b-Chicks: Twenty five, 3- days-old chicks were divided into 3 groups as follow:

Group 1: Ten chicks were inoculated subcutaneously with 24 hour broth culture of the isolated organism (Awaad *et al*., 1981).

Group 2: Ten chicks were infected orally with 10^8 viable microorganisms (Awaad *et al*., 1981).

Group 3: Five chicks were kept as control.

c-Broilers: Fifteen, 9-weeks-old chickens were used, Ten from them infected subcutaneously with 0.5 ml of 18-hour broth culture of isolated organism (Bapat *et al*; 1985) and the remaining five chickens left as control.

3- Sensitivity test:

The determination of sensitivity of the isolated organism against different antibiotic discs were done according to Sadasivan *et al*., (1977).

RESULTS:

-Bacteriological examination revealed that the suspected colony was large, irregular, transluscent and produced a greenish diffusable pigment and characterized by its ability to grow on 42ºC and by its fruity smell. On blood agar the colony produced beta heamolysis. The organism is gram-negative motile rod.

-Biochemichal reactions revealed that suspected isolate of Ps. was oxidase, catalase and urea positive, ferment glucose while methyl red, voges-proskauer and indol tests were negative.

These Characters were conformity with those for Ps. aeruginosa (Buxton and Fraser 1977).

The frequency of the isolated Ps. aeruginosa is shown in table (1).

Table (1)

Samples	No. of samples	Isolated Ps. aeruginosa	
		No.	%
1- Dead in-shell chicken embryos	100	21	21
2- Baby chicks	85	15	17.6
3- Broilers	60	2	3.3

Pathogenicity test:

a-Chicken embryos : Chicken embryos inoculation showing 100% mortality to the embryos between day 2 and 3 postinoculation (p-i) with formation of caseated material.

b-Chicks: Group I developed signs 24 hour p-i, they showed sleepy appearance, closed eyes, sitting on hocks (Fig.1) and diarrhea with mortality reached 80% within 36 – 72 hour p-i. Gross lesions revealed congestion of the carcasses (Fig. 2), peticheal haemorrhages on liver and spleen with congestion of them and pericarditis (Fig. 3), lungs were pneumonic, congestion and swollen of kidneys with deposition of ureats in the urters (Fig.4), also enteritis, enlargement of the gall-bladder and unabsorbed congested yolk sacs were present. Beside this some birds laid on one side and exhibited convulsions in the legs. Group 2 showing the same signs of group 1, 8 days p-i without death with appearance of mild swollen head (Fig. 5) 2 weeks p-i. Gross lesions of sacrificed birds showed congestion of the liver, spleen and kidneys, sinusitis and unabsorbed yolk sacs. Group 3 showed no any clinical signs or lesions.

c-Broilers: No clinical signs were observed in infected group and no deaths but gross lesions showed mild congestion of the liver and kidneys

and enlargement of the gall bladder. Control group was clinically healthy without any signs or lesions.

Fig. (1) : Chick is sitting on hocks

Fig. (2) : Congestion of the carcasses

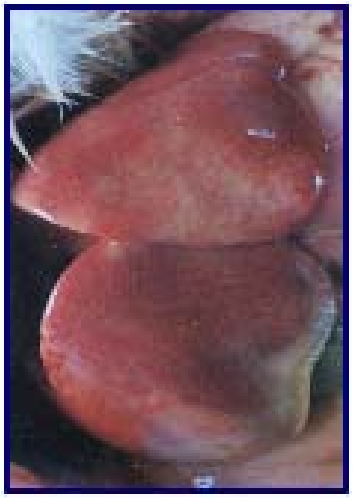

Fig. (3) : Peticheal haemorrhages of the liver and Pericarditis

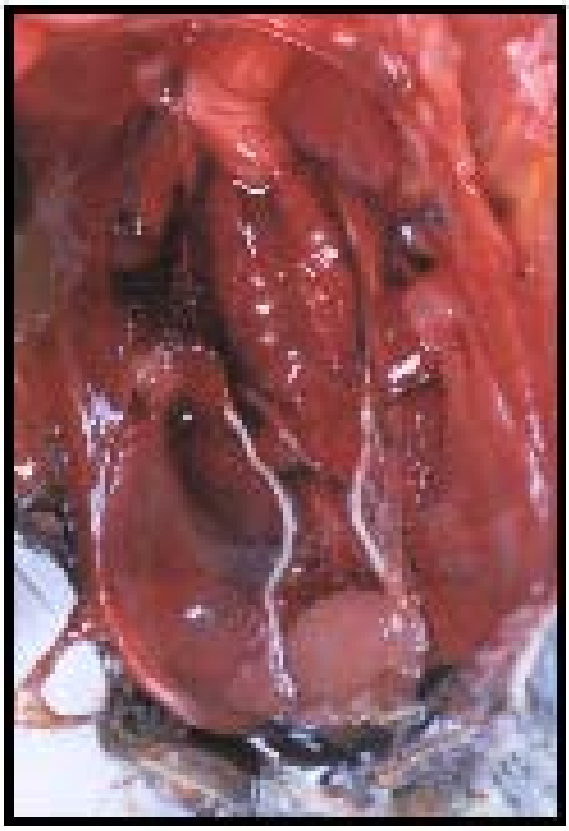

Fig. (4) : Congestion and swollen of Kidneys with deposition of ureats in the urters

Fig. (5) : Mild swollen of the head in the orally infected chick

Reisolation of Ps. aeruginosa from inoculated chicks and dead chicken embryos was succeeded but not from broilers.

Sensitivity test:

The effect of the different antibiotics on the isolated Ps. aeruginosa are illustrated in table (2).

Table (2)

Antibiotic discs	Sensitivity of Ps. aeruginosa isolates
Norfloxacin	+ + +
Chloramphenicol	+ + +
Streptomycin	+ + +
Gentamycin	+ +
Oxytetracyclin	+
Neomycin	+
Lincomycin	+
Ampicillin	-
Trimethobrim	-

+ + + sensitive
+ + moderate sensitive
+ week sensitive - resistant

DISCUSSION:

Ps. can cause localized or systemic diseases in young and growing poultry and invade fertile eggs causing death of embryos and newly hatched chicks, this suggest a possible egg borne infection (John Barnes 1997).

In this study, bacteriological examination of dead in shell chicken embryos revealed that Ps. aeruginosa was recovered from 21% of examined eggs, this percentage is higher than reported by Muschin and ziv (1973) and Nashed (1981) who recovered Ps. aeruginosa in percentage of 15% and 14.1% respectively from unhatched chicken eggs, but our result was nearly similar to that reported by Saif – Edin (1983) who isolated the organism in percentage of 18.8%.

In baby chicks Ps. aeruginosa was isolated in percentage of 17.6%, this result is somewhat less than that reported by Saif-Edin (1983) who isolated the organism at the rate of 20%. Also we recorded Ps. aeruginosa from 3.3% of examined broilers, our result is nearly similar to that reported by Mrden *et al*., (1988), Shahata *et al*; (1988) and Younes *et al*; (1990) who recovered Ps. aeruginosa with an incidence of 3.6%, 4.76% and 4.6% respectively, but our percentage is much lower than that observed by Saif–Edin (1983) who isolated the same organism with an incidence of 21.6% at kena Governorate.

Experimental infection of isolated organism to chicken embryos revealed 100% mortality to embryos, this result is inagreement with that reported by Saad *et al*; (1981).

Subcutaneous inoculation of baby chicks with isolated organism revealed that Ps. aeruginosa was pathogenic to chicks and leading to the appeance of many clinical signs and lesions with mortality rate reached to 80%, our result is similar to that reported by Awaad *et al*; (1981), but they recorded 100% mortality to the inoculated chicks.

Infection of baby chicks orally showed signs and lesions similar to that reported by Awaad *et al*; (1981), but we differ with them that they recorded mortality rat to chicks reached 6.60%, but in this study no death was occur to the chicks.

Intramuscular inoculation of broilers with the isolated Ps. aeruginose revealed no death and organism was not pathogenic for them, our result is similar to that observed by Bapat *et al*; (1985).

Senstivity test revealed that the isolated organism was highly sensitive to Norfloxacin, Chloramphenical and Streptomycin, our result is somewhat similar to that reported by Sadasivan *et al*; (1977) who observed that Ps. aeruginosa is sensitive to Chloramphenicol, Streptomycin and Gentamycin. Our study is differed with Walker *et al*; (2002) who found that Gentamycin was most effective for the organism but in our study the isolated organism was moderately sensitive to Gentamycin.

Therefore, it is concluded that the Ps. aeruginosa is pathogenic for chicken embryos and baby chicks, but has less effect on broilers, so good hygiene especially in hatcheries is fundamental to Ps. control also the use of suitable antibiotic in the day – old chicks could have helped reduce flock mortality.

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بعض الدراسات على ميكروب السودوموناس فى أجنة البيض وبدارى الدجاج فى محافظة أسيوط

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تم جمع ١٠٠ بيضة كابسة من مفرخات بلدية بمحافظة أسيوط و ٨٥ كتكوت مريض ونافق حديثاً (٢-١٥ يوماً) و ٦٠ دجاجة من كتاكيت بدارى التسمين (نافقة حديثاً) عمر ٥-٨ أسبوع، وقد أمكن عزل ميكروب السودوموناس أرجينوزا منها بالنسب التالية ٢١% ، ١٧,٦% ، ٣,٣% على التوالى، وتم إجراء عدوى صناعية بالميكروب المعزول على أجنة بيض عمر ٧ أيام عن طريق الحقن فى كيس المح، والذى أدى إلى نفوق جميع الأجنة. أيضاً تم إجراء عدوى صناعية على كتاكيت عمر ٣ أيام عن طريق الحقن تحت الجلد والذى أدى إلى نسبة نفوق وصلت إلى ٨٠%، أما العدوى عن طريق الفم لم تؤدى إلى أى نفوق ولكن أظهرت تورم خفيف فى الرأس. وبعمل عدوى صناعية بالميكروب المعزول على بدارى تسمين عمر ٩ أسابيع عن طريق الحقن فى العضل لم يحدث نفوق فى الدجاج. وبإجراء اختبار الحساسية لميكروب السودوموناس أرجينوزا المعزول. وجد أن النورفلوكساسين والكلورمفينيكول والإستربتوميسين هم الأدوية الأكثر تأثيراً.