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STUDIES ON *PROTEUS MIRABILIS* INFECTION IN BROILER CHICKS IN ASSIUT FARMS

(With 3 Tables)

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دراسات عن ميكروب البروتيس ميرابيلس في كتاكيت التسمين في مزارع اسيوط

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في هذا البحث تم فحص ٢١٠ من بدارى دجاج التسمين النافقة حديثا من مـــزارع حكوميـــة وخاصة بمحافظة أسيوط. ولقد تم عزل ١٢ حالة ايجابية لميكروب البروتيس ميرابيلس بنسبة ٥.٧١% وبإجراء العدوى الصناعية بهذا الميكروب في الكتكوت عمر يوم ثبت ان الحقن في كيس المح كان اكثر تأثيرا حيث وصلت نسبة النفوق إلى ٥٥% في حين كانت ٢٠% فـــي الكتاكيت التي حقنت عن طريق الفم , وقد كانت الأعراض والصفة التشريحية تشبه إلى حـــد كبير تلك المسجلة في العدوى الطبيعية . هذا وقد تم عزل الميكـــروب مـــرة أخـــرى مـــن الكتاكيت النافقة . وبإجراء اختبار الحساسية في المعمل للعترات المعزولة وجد أنها جميعـــا عالية الحساسية لكل من الجنتاميسين والكاناميسن والاستربتومايسين والكلورامفنيكول وحمض النالديكسيك.

SUMMARY

A total of 210 freshly dead broiler chicks collected from different governmental and private farms at Assiut Governorate. Only 12 positive cases of *Proteus (Pr) mirabilis* were isolated with an incidence of 5.71%. The experimental infection in one day old chicks by different route of inoculation revealed that yolk sac route was highly effective with mortality rate 55% while oral route of infection was almost less (20% mortality). The clinical observation and the post-mortem lesions of experimental infection were similar to a great extent to those of natural infection. Reisolation of inoculated organism from dead chicks were conducted. In vitro antibiotics sensitivity tests showed that the examined

isolates were highly sensitive to gentamycin, Kanamycin, Streptomycin, Chloramephnicol and Naldixic acid.

Key words: *Proteus, Broiler chicks mirabilis.*

INTRODUCTION

Several microbial infections are responsible for the losses of poultry industry, from an economic point of view *Pr.mirabilis* infection is not only responsible for embryonic mortality during hatching but also for mortality in chicks and of broilers (Dhawedkar and Dhaneser 1960; Pathak et al., 1960 recovered Pr. organism from 3% of examined chicks and Bahatia et al., 1972 who studied the occurrence of pathogenic lesions in the form of haemorrhages and degenerative changes in the liver and oedema of yolk sac in experimentally infected day-old chickens with *Proteus mirabilis* by intra-peritoneal injection or navel swabing.

In Egypt. The effect of Proteus organisms on eggs, embryos and chicks was studied by Rokia-Karaman (1980); Zaki (1985), Ashgan (1988) and Abd El-Gwad (1989). Zaki (1985) recovered 8 isolates from dead chicks (4.23%); Ashgan (1988) detected *Pr. mirabilis* from dead in shell piped embryos and fresh eggs collected from different balady hatcheries in Assiut Governorate and she proved that the organism was highly pathogenic to embryos with 100% mortality, and Abd El-Gwad (1989) had isolated 33 isolates of *Pr.mirabilis* out of 310 dead baby chicks with a ratio of 10.6% also in Assiut Governorate.

Lin et al. (1993) recovered one isolate of *Pr.morganii* from respiratory illness and from the bone marrow of dead birds distributed in the southern part of Taiwan and found that it caused 50 % mortality when inoculated intraperitonial in 4-week-old white male leghorn chicks. Bahrani and Mobley (1993) concluded that *Pr.mirabilis* [mannose resistant proteus-like (MR/P) Fimbriae] a cause of serious urinary tract infection and acute pyelonephritis, produce several patative virulence determinats among Fimbriae in chicken. JianG et al. (1996) found that *Pr. mirabilis* infection in chickens characterized by paralysis of limbs and diarrhoea. Young chicks up to 4 weeks of age were more susceptable, birds above 4 weeks of age may developed suppurative osteomyelities with 4% mortality. Omayma and Ahlam (1997) isolated 10 isolates of *Pr.mirabilis* from sick and dead chicks with a percentage of 6%. Hokama et al. (2000) studied whether a pH changes induced by *Pr.mirabilis* contributes to ascorbate conversion to oxalate in vitro and

concluded that urinary ascorbate, if present at a high concentration in association with *Pr. mirabilis* infection appears to be locally degraded to oxalate, potentially leading to calcium oxalate deposition on infection stones.

The present work was designed to cover the following items:

- Isolation and identification of *Pr.mirabilis* organism from broiler chicks at Assiut Governorate
- Experimental infections using the isolated organism in one-day old chicks by different routes
- In vitro sensitivity test of the isolated organism against different antibiotics.

MATERIAL and METHODS

Material:

1- samples:

A total of 210, freshly dead broiler chicks (age from 2-6 weeks) were obtained from governmental and private farms at Assiut Governorate (Diroute, El-Kosseia, Assiut Agriculture Farm, Bany-Mor and El-Balaiza). Birds in these farms suffer from slight depression, reluctant to move, dropping of wings with extension of head and neck with high mortality. Freshly dead birds were subjected to post-mortem examination. Tissue samples 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 phosphate broth, semisolid agar, sugars (glucose, sucrose, dulcitol, galactose and maltose)

B- solid: Nutrient agar, Mac Conkey's, Simmon's citrate agar, triple sugars iron agar, Urea agar base.

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

4- experimental animals: 55, one day-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: Gentamycin (10 µg), Kanamycin (30 µg), Streptomycin (10 µg), Chloramphenicol (30 µg), Nalidixic acid (30

µg), Amoxacillin (25 µg), Neomycin (30 µg), Ampecillin (10 µg), Colistine (10 µg), Furazalidone (300 µg), Spectinomycin (10 µg), Erythromycin (15 µg), Tetracycline (30 µg), Nitrofuraration (200 µg), Compound sulphonamide (300 µg).

Methods:

1- Isolation and identification of *Pr.strains*:

Samples from individual bird including liver, heart, spleen, kidney and lungs were collected aseptically. Loopfuls from these organs were inoculated into broth tubes and incubated 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, detection of smell (Fishy smell and swarming appearance on the surface of nutrient agar), oxidase test, sugar fermentation and other biochemical tests (Cruickshank et al., 1975, Wilson and Miles 1984)

2- Pathogenecity test:

Fifty five, one day-old chicks (balady) used in this study. Chicks were observed for 3 days and proved to be free from most pathogenic organisms by taking a random sample of 5 chicks, subjected to clinical, post mortem as well as bacteriological examination which proved to be healthy and free from any infection. because *Pr.mirabilis* isolates differ in their pathogenicity, so we selected one of the highly pathogenic isolate in our experimental work.

3- Reisolation of inoculated organism.

4- Sensitivity test:

The paper disc technique was carried out after Finegold and Baron (1986) using identified Pr.mirabilis isolate and 15 chemotherapeutic discs produced by Oxoid Basingstake, Hampshire, England. in order to determine their antibiogram.

RESULTS

I- Isolation and identification of *Pr.species*:

According to the morphological and biochemical studies especially (Fishy smell, swarming appearance and hydrolysis of

urea) of the suspectd Pr. organisms, 12 isolates were identified to be *Pr.mirabilis*, frequency and percentage of infection are summarized as presented in Table (1).

II- Results of experimental infection in baby chicks:

Fifty chicks one-day old were calssified into 3 groups.

- Chicks of group 1 (20) were inocualed via yolk sac route with (50-300 C.F.U.) concentration peptone water culture of the identified Pr.mirabilis isolate.
- Chicks of group 2 (20) were inoculated orally with the same previous organism and dose.
- Birds of group 3 (10) were kept without inoculation as control.

All chicks were kept for 21 days (period of observation) with daily examination for clinical signs. Dead and sacrificed chicks survived till the end of the observation period were subjected to P.M. as well as bacteriological examination for lesions and trials of reisolation were conducted.

- The clinical signs observed in infected chicks wer severe depression, ruffled feathers, dropping of wings, coma, ataxia, extension of head and neck. Signs of lameness and sitting on hocks were observed in some birds.
- The P.M. lesions recorded were congestion of liver, heart, lungs, spleen and subcutaneous blood vesseles, unabsorbed yolk sac and omphalitis, enlargement of gall bladder, enlargement and congestion of kidneys were recorded in some cases. Preciptation of urates on pericardium with severe nephritis recorded in some cases died after 10 days of inoculation.
- Survived chicks till the end of the observation period showed emaciation, retardation of growth with paleness and degeneration of kidneys and haemorrhagic spots on the surface of kidneys, liver.
- No symptoms were observed in control group.

The results of pathogenecity test in baby chicks are given in Table (2) and it is clear that it is high in birds infected via yolk sac than those infected orally.

III- Reisolation trials were positive from internal organs especially heart blood, liver, lungs, kidney, spleen of dead and scarified birds.

IV- The effect of different antibiotics on the isolated *Pr.mirabilis* isolates are illustrated in Table (3).

DISCUSSION

The process of poultry production faces a great losses mainly due to microbial contamination. By the present work we tried to throw some light on the role played by *Pr.mirabilis* which may be incriminated in broiler deaths.

The present work aimed to study the role played by *Pr.mirabilis* in broiler chicks. As evident from our results the bacteriological examination of dead broiler chicks revealed that the organism was recovered from 5.71% of the examined birds. A nearly similar finding was mentioned by Zahdah (1982) 6.42% in Egypt, Omayma and Ahlam (1997) who isolated *Pr.mirabilis* with a percentage of 6% at Sharkia Governorate. A lower percentage was reported by Watts and Rae (1958) 3%, Pathak et al. (1960) 3%, Zaki (1985) 4.23%, JianG et al. (1996) 4% mortality. A much higher percentage was reported by Abd El-Gwad (1989) who isolated the same organism with an incidence of 10.6% at Assiut Governorate.

The post mortem lesions of dead broiler chicks from which *Pr.mirabilis* was isolated showed liver, lungs and kidney congestion. Unabsorbed yolk sac, abnormal yolk appearance, enteritis and omphalitis were observed in dead chicks. Exactly the same observations were recorded by Zaki (1985), Abd El-Gwad (1989), JianG et al. (1996), Omayma and Ahlam (1997).

The experimental infection of one-day-old chicks by yolk sac and oral route with both cultures of *Pr.mirabilis* revealed that yolk sac route of inoculation was effective than oral route producing mortality rate about 55% while 20% mortality in oral route. the pathogenic effect of Pr.mirabilis in chicks was previously reported by Bhatia et al. (1972) and Zaki (1985) who recorded that mortality was varied from 10% to 45% in 7-day-old chicks inoculated orally or via intraperitoneal routes.

In this present study our results of experimental infection in baby chicks was higher to some extent to the results were reported by Abd El-Gwad (1989) who found that the *Pr.mirabilis* caused 35% mortality when inoculated via yolk sac and 10% mortality when inoculated orally in one-day-old chicks with the same dose (50:300 C.F.U.). On the other side, Rokia-Karaman (1980) and Ashgan (1988) reported that *Pr.mirabilis* was non lethal to baby chicks infected subcutaneously, orally and via yolk sac routes.

Reisolation of the organism from dead and sacrified chicks were conducted and this proved that the inoculated isolates were responsible for the pathogenic effect mentioned before.

In vitro sensitivity testing of the isolate to 15 antimicrobial agents revealed that the isolates examined were highly sensitive to Gentamycin, Kanamycin, streptomycin, Chloramephenicol, Nalidixic acid and moderately sensitive to Neomycin, Furazalidone, Spectinomycin, Nitrofuran, while weekly sensitive to Ampcillin, Colistine, but Amoxacillin, Erythromycin, Tetracyclin, compound Sulfonamide had no effect at all. In this respect our results agree but to some extent to those reported by Zaki (1985), Ashgan (1988), Abd El-Gwad (1989), but disagreed with those reported by ORsini and Spencer (1997) who found that *Pr.species* were susceptable to Amikacin than Gentamycin and Amikacin had an overall susceptability level of 94.3% compared with a level of 77.7% for Gentamycin.

Finally it may be concluded from the present investigation that *Pr.mirabilis* infection in broiler chicks is of especial significance and that causes economic losses among baby and broiler chicks, so a strict sanitation during egg collection, transportation, storage and incubation to avoid contamination and penetration of the motile organism causing infection and deaths after hatching of chicks.

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Table 1: Frequency and prcentage of isolated *Pr. mirabilis.*

Source	No. of examined birds	+ve cases	% of positive cases
Dairoute	50	4	8
El-Kosseia	50	3	6
Assiut Agri. Farm	30	1	3.33
Bany-Mor	45	3	6.66
El-Balaiza	35	1	2.85
Total	210	12	5.71

Table 2: Showing the results of pathogenecity in chicks

Group No.	No. of infected chicks	Route Of infection	Dose of inoculum *Pr. Mirabilis*	Daily deaths post infection 1 2 3 4 5 6 7 8 9 10 11 12 13 21	Total No. of deaths	No. of survivors	mortality rate
1	20	yolk sac	50-300 C.F.U	6 3 1 1	11	9	55%
2	20	oral	50-300 C.F.U	2 1 1	4	16	20%
3	10	control	0	0 0 0 0 0 0 0 0 0 0	0	10	0.0%

Table 3: Results of sensitivity of *Pr. mirabilis isolates.*

Antimicrobial agents	Sensitivity of *Pr.mirabilis* isolates
Gentamycin	+++
Kanamycin	+++
Streptomycin	+++
Chloramephenicol	+++
Nalidixic acid	+++
Neomycin	++
Furazalidone	++
Spectinomycin	++
Nitrofuran	++
Ampcillin	+
Colistine	+
Amoxacillin	-
Erythromycin	-
Tetracycline	-
Compound sulfonamide	-

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