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STUDIES ON PROBLEMS OF *KLEBSIELLA SPECIES* INFECTION IN BROILER CHICKENS IN ASSIUT GOVERNORATE

(With 2 Tables and One Figure)

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**دراسات عن مشاكل الإصابة بميكروب الكلبسيلا في بدارى الدجاج
بمحافظة أسيوط**

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فى هذا البحث تم فحص عدد ٧٥ (٣-٤٠ يوم) من بدارى دجاج التسمين النافقة حديثاً من مزارع مختلفة بمحافظة أسيوط وقد لوحظ على الطيور المصابة ضعف وإنكماش وتأخر فى النمو بينما كانت أهم الأفات التشريحية هى إحتقان فى الأعضاء الداخلية ووجود نزف دموى وتورم تحت الجلد فى منطقة الرأس والبطن وقد تم عزل ٥ حالات إيجابية لميكروب الكلبسيلا نيمنى بنسبة ٦,٧% وبإجراء العدوى الصناعية بهذا الميكروب فى كتاكيت عمر ٣ أيام عن طريق الحقن تحت الجلد وعن طريق الفم وُجد أن الحقن تحت الجلد كان أكثر تأثيراً حيث وصلت نسبة النفوق إلى ٤٠% فى حين كانت ٢٠% فى الكتاكيت التى حقنت عن طريق الفم وكانت الأعراض والصفة التشريحية تشبه إلى حد كبير تلك التى لوحظت فى حالات العدوى الطبيعية وقدم إعادة عزل الميكروب مرة أخرى من الكتاكيت المعدية صناعياً وبإجراء إختبار الحساسية للعترات المعزولة وجد إنها جميعاً عالية الحساسية لكل من الجنتاميسين والدانوفلوكساسين والإنروفلوكساسين والإستربتوميسين.

SUMMARY

A total of 75 freshly dead, growing broiler chickens (3-40 days) collected from different farms of Assiut Governorate. The clinical signs of the infected broiler chickens were severe weakness, depression and retardation of growth, while the most postmortem lesions were severe congestion of the paranchymatous organs and oedema of the subcutaneous tissue in the head region and abdomen. Five positive isolates of *klebsiella pneumoniae* (*K. pneumoniae*) were recovered with an incidence of 6.7%. The experimental infection of 3-day-old chicks by isolated organism through subcutaneous (s/c) route and orally revealed

that (s/c) route was highly effective with mortality rate 40% while oral route of infection gave 20% mortality. The clinical observation and postmortem lesions were similar to a great extent to those of natural infection. Reisolation of the inoculated organism from dead chicks were conducted. In vitro antibiotics sensitivity test showed that the examined isolates were highly sensitive to Gentamycin, Danofloxacin, Enrofloxacin and Streptomycin.

Key words: *Klebsiella, Broiler chickens*

INTRODUCTION

In Egypt, poultry meat is considered one of the most important source of animal protein. There are many problems facing broilers under the massive production and industry causing serious losses. Enterobacteriaceae infection is still causing severe losses specially in young age of birds. However, regarding the available literatures, it seems that, detailed and complete study on klebsiella infection in poultry is still lacking. Moreover, klebsiella infection in poultry has been recently shown to be involved (Sarakby, 1979, Kaxaman 1980, Zahden 1982, Abd - El- Motelib and El- Zanaty 1993).

Sekariah and Seth (1957) stated that, adult birds acted as a carriers for the klebsiella and the infection is conveyed to the developing chicks, as well as, to very young chicks after hatching. Sandra and Duarte Carreira (1998) suggested that the upper respiratory tract of healthy bird can harbour klebsiella microorganism which can behave as opportunistic pathogens. Shivaprasad (1998) recorded that *K. pneumoniae* can cause localized or systemic infection in poultry and other birds. Comis *et al*; (2001) concluded that *K. pneumoniae* organisms can cause cellulitis lesions in turkey could be associated with primary contact dermatitis and skin abrasions.

K. pneumoniae infections in growing broiler chickens did not receive much care in our country in spite of considerable importance which are found to be implicated in poultry diseases resulting in significant losses, therefore the work reported in this paper was undertaken to give an idea about the following:

- Isolation and identification of *K. pneumoniae* organism from growing broiler chickens at Assiut Governorate
- Experimental infections using the isolated organism in – 3 – day old chicks by different routes of inoculation.

- In vitro sensitivity test of the isolated organism against different antibiotics.

MATERIALS and METHODS

Materials:

1- Samples:

A total of 75 freshly dead growing broiler chickens were subjected to postmortem examination. Tissue samples from liver, spleen, kidney, heart blood, tracheal exudate, lungs, bloody exudate of subcutaneous tissue in the head region were collected from these cases and subjected to bacteriological examination.

2- Media:

- Liquid: Peptone water, nutrient broth, glucose phosphate broth, semisolid agar, sugars (glucose, lactose, dulcitol, sucrose, mannitol and arabinose).
- Solid: MacConkey's agar, Nutrient agar, Simmon's citrate agar, T.S.I agar and urea agar base.
- Reagents, Chemicals and stain: Kovac's, urea, methyl red, andrade's indicator, Gram's stain.

3- Experimental birds:

55, one – day old chicks (balady) obtained from baby chicks production farm, Assiut were used for Pathogenicity test.

4- Antimicrobial sensitivity discs:

were produced by oxoid- laboratories including:

Gentamycin (10 µg), Streptomycin (10 µg), Chloramphenicol (30 µg), Ampicillin (10 µg), Colistine (10 µg), Erythromycin (15 µg), Tetracycline (30µg), Compound sulphonamide (300 µg), Danofloxacin (30 µg), Trimethobrim (25 µg), Penicillin (10 µg), Cloxacillin (5 µg), Rifampein (30 µg), Enrofloxacin (5 µg), Unasyn (30 µg), Linco – Spectin (15 µg), Cefobid (75 µg), Doxacycline (30 µg).

Methods:

1- Isolation and identification of K. Species:

Samples from individual bird including liver, heart, spleen, kidney, lungs, tracheal and subcutaneous exudate of the head region 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 MacConkey's agar plates at 37 °C for 24 – 48 h., suspected colonies were picked up and subjected to further

identifications based on colonial morphology, sugar fermentation and other biochemical test (Cruickshank *et al.*; 1975, Wilson and Miles 1984).

2- Pathogenicity test:

Fifty five, one day - old chicks (balady) were 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, postmortem examination as well as bacteriological examination which proved to be healthy and free from any infection.

3- Reisolation of inoculated organism (as in the same methods of isolation and identification of *K. species*).

4- Sensitivity test:

The paper discs technique was carried out after fingold and Baron (1986) using identified *K. pneumoniae* isolated and 18 chemotherapeutic discs produced by oxoid Basingstake Hampshire, England in order to determine their antibiogram.

RESULTS

I- Isolation and identification of *K. Species*:

According to the morphological and biochemical studies specially (hydrolysis of urea and indole production) of the suspected *K. organisms*, 5 isolates were identified to be *K. species* in percentage of 6.7%, and according to biochemical testes reported by Buchanan and Gibbons (1674) the isolates were identified as *K. pneumoniae*.

II- Results of experimental infection in baby chicks:

Fifty chick, 3 – day old age were classified into 3 groups:

- chicks of group I (20) were inoculated subcutaneously by 0.1 ml peptone water culture of *K. pneumoniae* (10^3 viable cells / bird).
- 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 15 days (period of observation) with daily examination for clinical signs. Dead and sacrificed chicks survived till the end of the experiment were subjected to postmortem and bacteriological examination for lesions and trials of reisolation were conducted.

The clinical signs observed in infected chicks were depression, weakness diarrhea, cough, ruffled feathers, coma, ataxia, congestion and oedema of the head and abdomen. Some birds showed signs of

lameness. The gross lesions were severe congestion of the internal organs and intestine, catarrhal exudate in the trachea, haemorrhagic exudate in (s/c) tissue of the head and abdomen, Unabsorbed yolk sac may be observed in some chicks and ascities (Fig.I). Survived chicks till the end of the observation period showed retardation of growth with haemorrhagic spots on the surface of the lungs, kidneys and liver.

- No symptoms or lesions were observed in control group.

The results of pathogenicity test in baby chicks are given in table (1) and it is clear that s/c inoculation was more effective in birds than oral inoculation.

III- Reisolation trials were positive from internal organs of dead and sacrificed birds.

IV- The effect of different antibiotics on the isolated *K. pneumoniae* isolates are illustrated in table (2).

DISCUSSION

Klebsiella has been regarded as a potential pathogen for man and animals (Cruickshank *et al.*; (1975), Wilson and Miles (1975), and Niazi (1976). In Egypt only few investigators have been mentioned the role of *K.* in inducing infection in poultry (Sarakby (1979), Abd-Alla (1981), Dessouky *et al.*; (1982), Ashgan (1988), Abd – El Motelib and El-Zanaty (1993).

Bacteriological examination of freshly dead growing broiler chickens revealed that the *K. pneumoniae* organism was recovered from 6.7% of examined birds, this percent is somewhat higher than that reported by Sarakby (2.9%) and Abd– El– Motelib and El-Zanaty 4.8%.

In the present study our findings of the clinical observation and the most prominent gross lesions of dead broiler chickens from which *K. pneumoniae* was isolated exactly recorded by Abd El- Motelib and El-Zanaty (1993) and more or less with Shivaprasad (1998).

The experimental infection of 3-day old chicks by s/c and oral route with both cultures of *K. pneumoniae* revealed that s/c route of inoculation was effective than oral route producing mortality rate about 40% while 20% mortality in oral route. The clinical observation of diseased and dead infected chicks were resembled to some extent those recorded by Dessouky *et al.*; (1982). Abd El- Motelib and El- Zanaty (1993) but we disagreed with those reported by Sarakby (1979) and Ashgan (1988) who proved that neither clinical signs nor gross lesions could be observed in the infected chicks during the period of observation.

In this present study our results of experimental infection in baby chicks was much higher than the results which reported by Abd El-Motelib and El- Zanaty (1993) who proved that the pathogenic nature of *K. pneumoniae* isolate with mortality rate of 2%, on the other side Picoff (1966) reported high mortalities (84%) among 3 and 6 day old chickens within 24 hours after s/c inoculation with 10^3 viable cells. As contrast Sarakby (1979) and Ashgan (1988) reported that *K. pneumoniae* was non lethal to baby chicks infected s/c or orally with the some dose (10^3 viable cells).

In vitro sensitivity testing of the isolate to 18 different antimicrobial agents revealed that the isolates examined were highly sensitive to Gentamycin, Danofloxacin, Enrofloxacin, Streptomycin and moderately sensitive to Tetracycline, Erythromycin, while less sensitive to Linco-spectin, Colistine, Ampicillin, Penicillin, Rifampein, but Unasyn, Cefobid, Doxacycline, Chloramphenicol, Trimethoprim, Cloxacillin and Copound sulphonamide. Our results are agree to some extent to those reported by Niazi (1976), Ashgan (1988), Abd El-Motelib and El- Zanaty (1993) and AAVLD (2004).

Finally it may be concluded that *K. pneumoniae* is responsible for a less considerable losses among the growing broiler chickens and further studies are needed to complete these investigations specially for prevention and control.

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Table 1: Showing the result of Pathogenicity test in chicks

Group No.	No. of infected chicks	Route of infection	Dose of inoculum K. pneumoniae	Daily deaths postinfection											Total No. of deaths	No. of survivors	Mortality rate
				2	3	4	5	6	7	8	9	15				
1	20	S/c	10 ⁵	4	2				2						8	12	40%
2	20	Oral	10 ⁵	4											4	16	20%
3	10	control	0	0	0	0	0	0	0	0	0	0	0	0	10	0%

Table 2: showing the result of antimicrobial discs.

Antimicrobial agents	Sensitivity of K. pneumoniae isolates
Gentamycin	+++
Danofloxacin	+++
Enrofloxacin	+++
Streptomycin	+++
Tetracycline	++
Erythromycin	++
Linco-Spectin	+
Colistine	+
Ampicillin	+
Penicillin	+
Rifampein	+
Unasyn	-
Cefobid	-
Doxacycline	-
Chloramphenicol	-
Trimethoprim	-
Cloxacillin	-
Compound sulphonamide	-

+++ = Highly sensitive
 + = Less sensitive

++ = Moderate sensitive
 - = Resistant

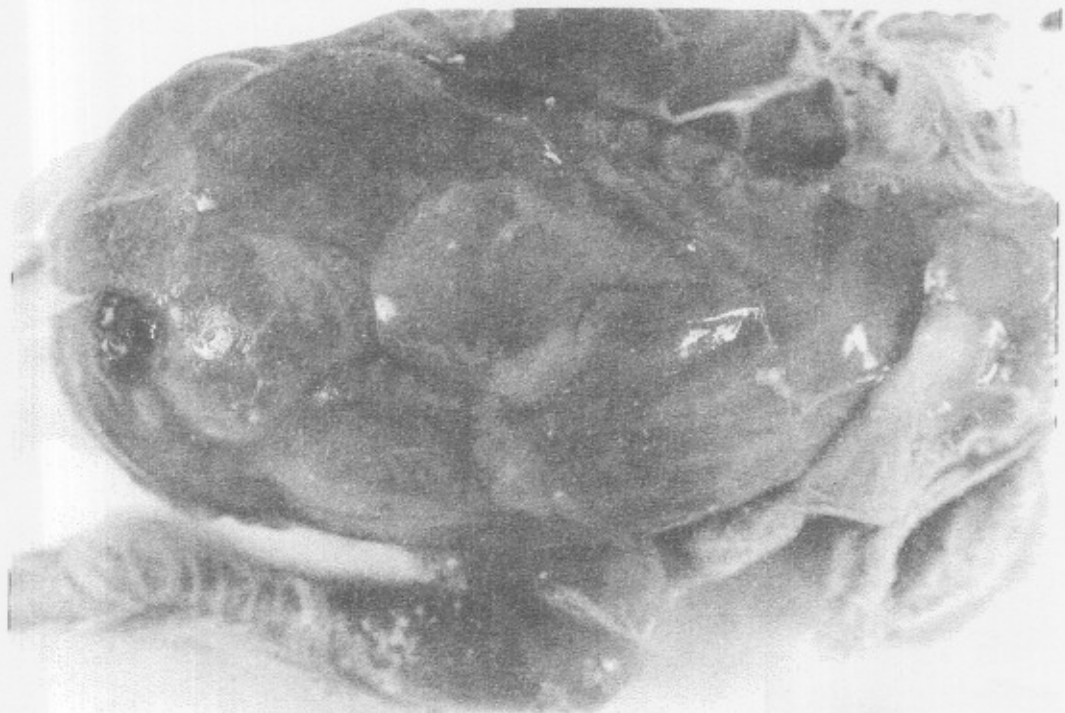


Fig. 1: Showing ascitis and oedematous haemorrhagic exudate in s/c tissue in the abdomen region