EFFICACY OF APRAMYCIN IN CONTROL OF E. COLI INFECTION IN BROILERS

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ABSTRACT

One hundred and two E. coli isolates were isolated from two hundred broiler chicks aged 1-40 day collected from Sharkia Governorate. Seventy one isolates were serotyped into 11 different serogroups, O1, O8, O15, O18, O26, O78, O86, O111, O114, O157 and O169 while 31isolates were un-typed. Antibiogram of isolated E. coli to apramycin and other common antimicrobial agents was performed. Apramycin and fluroquinolones was the most effective antimicrobial against E. coli. The minimal inhibitory concentration (MIC) of Apramycin against tested E. coli was 0.8 ug/ml. The levels of serum AST, ALT, creatinine, uric acid and globulin were significantly increased but albumin level was significantly decreased in chicks infected with E. coli O78. These parameters were improved towards the normal levels in chicks infected with E. coli and treated with apramycin for 5 successive days. A total number of 200 day-old Cobb broiler chicks were divided into 4 equal groups (1, 2, 3 and 4) each containing 50 chicks. Group (1) chicks were remained as negative control (non-infected, non-treated). The remaining groups (2, 3 and 4) were infected with E. coli O78. Group (2) remained as positive control (infected, non-treated). Group (3) was treated with apramycin at 25 mg/kg, b.wt. for 3 successive days, while group (4) was also treated with apramycin at 25mg/kg b.wt. for 5 successive days. The treatment was studied 24 hrs. after infection.

The clinical signs, mortality rate post-mortem lesion, reisolation of E. coli, body weight gain and feed convention rates after 2 weeks of the end of treatment were recorded. The treatment with apramycin at 25 mg/kg b.wt. for 5 successive days was more effective to control E. coli.

INTRODUCTION

In many areas of the world, one of the most cost causes of broiler mortality is the respiratory diseases produced by a mixed infection of Escherichia coli and Mycoplasma. This pathogenic combination produced what is called complicated chronic respiratory disease (C.C.R.D.) causing severe respiratory signs, which represented by an increase of mortalities, reduced weight gain and condemnation of birds at the time of slaughter **(Stipkovits, 1988 and Kaul et al., 1992)**. The species of *E. coli* are serologically divided in serogroups and serotypes on basis of its antigenic composition (somatic "O" antigens for serogroups and flagella "H" antigens

for serotypes). Many strains express a third class of antigens (capsular "K" antigens); **(Compos et al., 2004)**.

Apramycin is an aminoglycoside antibiotic (shown to be potent inhibitory of translocation step of protein synthesis in bacteria in vitro and vivo) being used in the treatment of colibacellosis and salmonellosis in poultry. It is also used for rabbits. Apramycin is not authorized for use in laying birds **(Walton, 1978; Perzynski et al., 1979 and Ryden and Moore, 1997)**.

The aim of this study was planned to isolate and identify *E. coli* causing respiratory manifestations in broiler chicken at Sharkia Governorate and to determine the efficacy of apramycin for controlling experimental *E. coli* infection in chicken.

MATERIAL AND METHODS

Samples for bacteriological examination:

Heart blood, liver, spleen, unabsorbed yolk sac and air sacs specimens were collected from two hundred either freshly dead or diseased chicken aging one day up to 40 day old. The examined chicks were suffered from severe respiratory manifestations and brought to clinic of Animal Health Research Laboratory, Zagazig City from different localities of Sharkia Governorate.

Bacteriological examination for detection of E. coli:

Specimens from heart blood, liver, spleen, unabsorbed yolk sac and air sacs were cultured on to MacConkey's and blood agar media and incubated at 37°C for 24 hrs. The suspected colonies were identified morphologically as well as biochemically according to **Quinn et al., (2002)**.

Serological identification:

Antisera of *E. coli* were used for serological identification of somatic antigen "O" using slide agglutination test according to **Edward and Ewing, (1972)**. Antisera of E. coli were obtained from Denka Sicken Co. Ltd Tokyo, Japan.

Experimental chicks:

Two hundreds, one day old Cobb broiler chicks obtained from local commercial hatchery and were fed a balanced ration free from any medication.

Drug:

Apramycin (Apracin) powder, each gram contain 0.33 mega units of apramycin produced by Unipgarma Company, Egypt.

Antimicrobial susceptibility test: The antibiotic activity test of the isolated *E. coli* against some antimicrobial agents using disc-diffusion method was carried out **(Quinn et al., 1994)** using Muller-Hinton agar plates. The results were interpreted according to Oxoid Manual Company **(Oxoid, 2000)**.

The minimal inhibitory concentration value (MIC) of apramycin and other antimicrobial agents were determined against *E. coli* isolates using serial broth dilution method **(Chessbrough, 1985)**.

Experimental design:

Two hundred day-old Cobb chicks were divided into four groups (1, 2, 3, 4) each containing 50 chicks were reared in a separate unit and fed on ration without any medication. Water and ration were provided ad-libitum. Group (1) chicks were kept as a negative control (non-infected and non-treated). At 15-day old Groups 2, 3 and 4 were infected intrathoracic with pathogenic *E. coli* O78 (0-25 ml of 2 x 10^6 CFU/ml) according to **Stipkovits (1988)**.

Chicks of the second group were served as positive control (infected, non-treated), while the third group was treated with a dose of 25mg/kg. b.wt apramycin via drinking water for 3 successive days. The fourth group was given apramycin at 25mg/kg. b.wt via drinking water for 5 successive days. The treatment starts 24 hrs. post-infection. The clinical signs, mortality, postmortem and reisolation trials of *E. coli* were recorded during experimental period.

Blood samples for biochemical examination:

At the end of treatment, two weeks post-treatment, blood and serum samples were collected and analyzed for total protein (Doumas, 1975); AST and ALT (Ritman and Frank 1957); creatinine (Husdan and Report, 1968) and uric acid **(Caraway 1963)**. All remained chicken were sacrificed for PM examination.

Statistical analysis:

Data were analyzed using ANOVA test a significance level of $p>0.05$ **(Snedecor and Cochran, 1980)**.

RESULTS

(1) Bacteriological and serological identification:

The results of bacteriological examination revealed that the prevalence rate of E. coli infection among 200 naturally infected birds were 51%. The bacterial isolates were 102 in number, 71 isolates were serotyptd into 11 different serogroups, O_1, O_8, O_{15}, O_{18}, O_{26}, O_{78}, O_{86}, O_{111}, O_{114}, O_{157} and O_{169} while 31 isolates were un-typed (Table1).

(2) Results of antibiogram study:

The field isolates exhibited, in vitro high susceptibility to aparmycin in addition to enrofloxacin, ciprofloxacin, kanamycin and pefloxacin and less sensitivity to gentamicin, ampicillin and colistin were recorded (Table 2). The MIC of apramycin against *E. coli* was 8 ug/ml.

(3) Clinical signs, mortality rate and lesion score:

Infected, non-treated birds showed signs of depression, off food, diarrhoea, rales and difficult breathing with severe PM lesions (air saculitis, pericarditis and perihepatitis) and high mortality rate (44%). These symptoms were disappeared after treatment with aparamycin and the mortality rate reduced to 9 % with mild lesion scores. All chicks of group (1) control were negative in culture for *E. coli* (O78). However, a higher frequency of reisolation of the pathogen from heart blood and liver in group (2) chicks when compared with that in group 4 was observed (Table 3).

(4) Influence of infection and treatment on body weight, feed consumption (F.C) and feed conversion rate (FCR):

The mean body gain of infected, untreated chicks was significantly ($p<0.05$) decreased than those infected and treated chicks (Table 4).

After cessation of drugs by 2 weeks, the highest mean body gain and lowest feed conversion rate in infected groups were recorded in the group treated with apramycin for 5 successive days.

(5) Influence of infection and treatment on some biochemical parameters:

Alteration of some liver and kidney functions in all infected groups were detected. The measured parameters were improved toward its normal level after 2 week post treatment (Table 5).

DISCUSSION

Colibacillosis refers to any localized or systemic infection caused entirely or partly by *E. coli* including colisepticemia, granuloma, chronic respiratory disease (CRD), peritonitis, swollen head syndrome, arthritis, synovitis panophthalmitis, perihepatitis and pericarditis **(Gross, 1991)**. About 43% of broiler carcasses condemned for disease at processing had lesion consistent with coli septicemia **(Kaul et al., 1992)**. Rate of isolation of *E. coli* was 102 isolates from 200 birds suffered from respiratory manifestation with ratio 51%, these result nearly coincided with those reported by **Youssef and Azzam (1985) and Yun et al., (1997)**. The *E. coli* serogroups isolated in the present work were reported previously by **Youssef et al., (1983) and Heba, (2008)**. The most prevalent serogroup was O78 (27.45%). Similar observations were previously recorded by **Gomis et al., (2000); El-Sukhon et al., (2002) and Heba, (2008)**.

Antibiogram of isolated *E. coli* to apramycin and other commonly used antimicrobials was performed. The obtained results indicated that, the isolated *E. coli* was highly sensitive to apramycin. Similar findings were cited in previous studies by **Rolinski et al., (2002)** who recorded that *E. coli* showed 100% sensitivity to apramycin. In addition, MIC of apramycin was determined (0.8 ug/ml) and was similar to the MIC that reported by **Freidlin et al., (1985) and Musangu et al., (2002)**. Our results were also nearly identical to **Ziv et al., (1985)** who reported that apramycin had potent antimicrobial activity at low concentration when compared with other classes of antimicrobial agents.

Experimentally infected chicks with the isolated *E. coli* O78 showed clinical signs in the form of severe respiratory signs with air saculitis, pericarditis and perihepatitis on postmortem examination. Experimental infected chicks showed a high mortality rate reached 44%. The mortality rate of infected chicks was reduced to 18% and 9% after treatment with apramycin for 3 and 5 successive days.

Oral administration of apramycin for 5 successive days were more effective than treatment with apramycin for 3 days for controlling the infection with *E. coli* and decreasing mortality from 44% (in infected non-treated) to 9%, this may be due to apramycin activity against Gram negative baeteria **(Hunter et al., 1999)**. Same result was demonstrated in buffalo-calves by **Alexander, (1985)** who reported that administration of apramycin to pneumonic calves improved the cure rate. Likewise, apramycin improved lesions of all infected chicks, these results added further support to those previously reported by **Cranckel et al., (1986); Leithner et al., (2001) and Mnsangu et al., (2002)**.

The obtained results revealed that the administration of apramycin (25mg/kg b.wt.) for 5 successive days to infected chicks as well as healthy non-infected chicks (control group) showed no significant variation in body gain. On contrary, infected non-treated chicks had less body weight gain when compared with both the control and infected treated groups. Treatment with apramycin at 25mg/kg b.wt. for 3 successive days partly improved the performance of chicks infected with *E. coli*.

The decrease in the body weight post infection may be attributed to the deleterious effect of the microorganism which invade the host

and retard its metabolic activity **(Abd Allah, Amany 1993)**. On other hand, the body gain of chicks infected with *E. coli* increased after treatment with apramycin compared with infected non-treated chicks (Donos, 1982). The improvement of body gain in infected and treated chicks might be attributed to bactericidal effect of drug on the infection and consequently improved the general health condition **(Oleas, 2000)**. These results provide a further reason for effieacy to apramycin in control of *E. coli*.

Biochemieal analysis of serum from chicks infected with *E. coli* showed that serum AST, ALT and globulins were significantly ($p<0.05$) increased these parameters, were improve towards the normal levels as a result of treatment of infected chicks with apramycin.

The increase in serum AST and ALT activity after infection suggests a hepatoeellular damage **(Doxey, 1971)**. The treatment with apramycin at a dose level of 25mg/kg. b.wt. for 5 successive days displayed non-significant change in serum AST and ALT as well as total protein and albumin. Similar findings were cited in previous studies by **Pashov et al., (1987) and Harrsion and Harrison (1986)**.

Creatinine and uric acid levels were significantly increased in infected non-treated chicks with *E. coli*. This finding was disagreed with the result obtained by Abdalah, **Amany (1993)**. Infected chicks with *E. coli* and treated with apramycin for 5 suceessive days, showed significant decrease in ereatinine and urie acid level, these results coineide with **Ziv et al., (1985)**. The treatment with apramycin for 3 suceessive days resulted in a significant decrease in ereatinine and urie acid in comparison with infeeted, non-treated chicks but failed in returning to the normal level.

Finally, it could be concluded that the medication of *E. coli* infected broilers by apramycin at dose 25mg/kg b.wt. for 5 successive days has superior activity and efficacy than administration for 3 succcssive days.

Table (1): Serological identification of isolated E. coli (102 strains) from broiler chickens suffering from respiratory manifestations

Serogroup	1	8	15	18	26	78	86	111	114	157	169	Un-typed Strains
Number	5	4	5	4	3	28	4	5	8	3	2	31
%	4.9	3.92	4.9	3.92	2.94	27.45	3.92	4.9	7.84	2.94	1.96	30.39

Table (2): In vitro susceptibility of E. coli to apramycin and some commonly used antimicrobials

Chemotherapy	*Disc potency (ug)*	*Mean Zone of inhibition (m.m.)*	*MIC (ug)/ml*
Apramycin	(15)	22	8
Enrofloxacin	(10)	24	0.15
Ciprofloxacin	(5)	22	0.1
Kanamycin	(30)	19	2.5
Pefloxacin	(5)	22	0.3
Gentamicin	(10)	16	0.6
Ampicillin	(30)	14	32
Colestin	(25)	15	16

Table (3): Mortality rate, lesion scores and reisolation of E. coli (O78) following infection and treatment with apramycin (25 mg/kg b.wt.) for 3 and 5 successive days (n =50)

Parameter / Group	Mortality rate %	Lesion sores			Frequency of reisolation of E. coli %
		Air saculitis %	Pericarditis %	Perihepatitis %	
(1) Non infected, Non-treated (control)	0	0	0	0	0
(2) Infected, Non-treated	44	44	68	72	75
(3) Infected and treated with Apramycin 3 Succ. days	18	14	19	22	34
(4) Infected and treated with Apramycin 5 succ. days	9	4	5	12	13

Table (4): Effect of oral administration of apramycin (25 mg/kg b.wt.) for 3 and 5 consecutive days on body weight, feed consumption (FC), feed conversion rate (F.C.R) of broiler chicken experimentally infected with E. coli

Parameter / Group	Before treatment at age of 15 days			Two week post treatment at the age 33day		
	Body weight	FC gm	FCR	Body weight gain	FC gm	FCR
(1) Control	230.8 ± 9.9^{a}	356.5	1.56	1135.9 ± 30.18^{a}	905	1.71
(2) Infected, Non-treated	242.8 ± 8.1^{a}	370.4	1.54	850.0 ± 58.2^{c}	607	2.41
(3) Infected and treated with Apramycin 3 succ. days	223.4 ± 11.2^{ab}	360	1.61	1031 ± 15^{b}	799	1.86
(4) Infected and treated with Apramycin 5 succ. days	235.4 ± 6.6^{a}	360.6	1.58	1090 ± 24^{ab}	866	1.73

Different letter in the same column denote significant changes at ($p<0.05$)

Table (5): Effect of oral administration of apramycin (25 mg/kg b.wt.) for 3 and 5 successive days on some serum biochemical parameters of chicks after experimentally infected with E. coli (O78)

Parameter / Group	AST IU/L	ALT IU/L	Total proteins gm/dl	Uric acid mg/dl	Creatinine mg/dl
(1) Control	86 ± 5^{b}	9 ± 0.6	3.35 ± 0.12^{bc}	3.4 ± 0.08^{bc}	1.11 ± 0.09^{cd}
(2) Infected, Non-treated	118 ± 9^{a}	10 ± 0.5	3.82 ± 0.11^{a}	6.3 ± 0.6^{a}	3.01 ± 41^{a}
(3) Infected and treated with Apramycin 3 succ. days	98 ± 8^{b}	9 ± 0.7	3.46^{b}	4.2 ± 0.1^{b}	1.3 ± 0.1^{cd}
(4) Infected and treated with Apramycin 5 succ. days	82 ± 6^{bc}	8 ± 0.8	3.33^{b}	3.9 ± 0.08^{bc}	1.32 ± 0.11^{cd}

Different letter in the same column denote significant changes at ($p<0.05$).

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الملخص العربى

كفاءة الأبراميسين فى الوقاية من العدوى بالإيشيريشيا كولاى

السيد مسعود حلمى إبراهيم البنا إيلين منقريوس حسام حسن علام

معهد بحوث صحة الحيوان - فرع الزقازيق

تم عزل ١٠٢ معزولات من الإيشيريشياكولاى من كتاكيت تسمين من عمر ١-٤٠ يوم من بعض المزارع بمحافظة الشرقية.

تم تصنيف هذه المعزولات إلى ١١ نوع وهم :

O_1، O_8، O_{15}، O_{18}، O_{26}، O_{78}، O_{86}، O_{111}، O_{114}، O_{157}، O_{169}،

ووجد أن ٣١ معزولة لم تصنف. بعمل إختبار الحساسية لأكثر المعزولات من الإيشيريشيا كولاى وجد أنها حساسة للأبراميسين وكانت MIC للأبراميسين بالنسبة للإيشيريشيا كولاى المعزولة هى ٨ر٠ مليجرام / مللى.

وجد زيادة معنوية بعمل إختبارات AST، ALT والكيرياتينين وحمض اليوريك والجلوبيولين بينما وجد نقص معنوى فى نسبة الألبيومين فى الدجاج الذى أصيب بالإيشيريشيا كولاى وحدث تحسن فى المجاميع التى أصيبت بالايشيريشيا كولاى وتم علاجها بالأبراميسين وخصوصاً التى عولجت بجرعة ٢٥مجم/كيلو من وزن الطائر الحى لمدة خمسة أيام.

تمت هذه التجربة على ٢٠٠ طائر قسمت على أربعة مجاميع، المجموعة الأولى كمجموعة ضابطة أما المجاميع الثانية والثالثة والرابعة فقد أعديت بالإيشيريشيا كولاى 078.

فى المجموعة الثالثة تم إعطاء الأبراميسين بجرعة ٢٥مجم/ك وزن حى لمدة ثلاثة أيام أما المجموعة الرابعة فقد أعطيت نفس الجرعة لمدة خمسة أيام.

وتم تسجيل الأعراض والصفة التشريحية وإعادة العزل لمدة إسبوعين، فوجد أن العلاج بالأبراميسين بجرعة ٢٥ مجم وزن كيلو حى فعال فى الوقاية ضد الإيشيريشيا كولاى.