EFFICACY OF KANAMYCIN AND SPIRAMYCIN FOR CONTROLLING RESPIRATORY DISEASE IN BROILER CHICKS

ABD EL-FATAH, T.[1], H.R. EL-BANNA[1]., E.A. MASOAD[1] AND H.H. ALAM[2]

1- Faculty of Vet. Medicine, Moneffia University, Egypt.
2- Animal Health Institute, Zagazig Provincial Lab.

Abstract

One hundred E. coli isolates were isolated from one hundred and fifty broiler chicks aged 1 – 45 days collected from broiler farms. These isolates were serotyped into O1, O8, O12, O18, O26, O78, O86, O111, O114, O169 and untyped (35 serotypes). 200 one day old Cubb broiler chickens were divided into 5 equal groups (1, 2, 3, 4 and 5) each containing 40 chicks. Group (1) chicks were remained a negative control (non infected and non treated). Groups (2, 3, 4 and 5) chicks were infected with the pericardium E. coli isolated (O78) and a standard Mycoplasma gallisepticum strain. Group (2) chicks were remained as positive control (infected non-treated). Group (3) chicks were injected I/M with Spiramycin (1ml/10 kg b.wt.); group (4) chicks were injected I/M with Kanamycin (1ml/10 kg b.wt.), while group (5) chicks were injected with combination of the two drugs at the same dose. The injection was performed 24hrs after infection for 3 successive days. The clinical signs, mortality rate, post-mortem lesions, reisolation of E. coli, detection of positive cases of Mycoplasma, body weight gain and feed conversion rate after two weeks post-treatment were recorded. The combinations of two drugs were more effective for controlling mixed infections than each drug alone. Moreover, infected chicks were suffered from elevations of some liver and kidneys enzymes. These parameters were improved towards the normal levels in chicks injected with combination of two drugs. However, infected chicks and treated with Spiramycin as alone therapy was the lowest values for controlling infection compared with treated with Kanamycin. Finally, it could be concluded that the combination of Spiramycin and Kanamycin has superior activity and efficacy than use of each drug alone in the treatment of mixed infections of broiler chicks with E. coli and Mycoplasma.

INTRODUCTION

In many areas of the world, one of the most common and most of cost causes of broiler mortality is respiratory diseases produced by a mixed infection of E. coli and mycoplasma. This pathogenic combination produced what is called complicated chronic respiratory disease (C.C.R.D) causing severe respiratory signs, an increase of mortalities and reduction of feed efficiency (Stipkovits, 1988).

The trail of treatment of C.C.R.D. with combination of more than one drug was performed by several veterinarians in Egyptian field. This combination was carried out

to their synergistic effect, treat mixed infection, prevent drug resistance from developing and treat sever infection where the organisms were unknown (Thomas and Jarold, 1981 and Hamed, 2002).

Administration of simultaneous drug in poultry is adopted via three routes, (water, feed and injection). However, the injectable route provides therapeutic blood levels faster than any other methods of drug administration (Baggot, 1984 and Ahmed, 1994).

Spiramycin is a macrolide antibiotic approved for the control of G +ve and G –ve and Mycoplasmosis in poultry (Kleven and Anderson 1972 and Kempf *et. al.,* 1989). Meanwhile, kanamycin is aminoglycoside antibiotic active against G –ve bacteria used in poultry for controlling Collibacillosis (Ries *et. al.,* 1973 and Kstman *et. al.,* 1996).

The aim of this study was planned to isolate and identify E. coli causing respiratory manifestation in broiler chickens at Sharkia Governorate and evaluate the efficacy of both single and/or simultaneous injection of spiramycin and kanamycin for controlling CCRD in broiler chicks.

MATERIAL AND METHODS

Samples:

One hundred and fifty either freshly dead or diseased broiler chickens aging one-day up to 45 days were used in this investigation. The examined chicks were suffering from sever respiratory manifestation and collected from different broiler farms at Sharkia Governorate.

Bacteriological examination:

Specimens from heart blood, liver, spleen, bone-marrows, unabsorbed youlk sac and air sacs were cultured on MacConkey and blood agar, incubated at 37oC for 24h for detection of E. coli. The suspected colonies were identified morphologically as well as biochemically according to Kstman *et. al.,* (1996).

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).

N.B.: Antisera of E. coli were obtained from Denka Sciken Co. Ltd., Tokyo, Japan.

Mycoplasma gallisepticum were obtained from Department of Bacteriology, Faculty of Veterinary Medicine, Sadat University.

Experimental chicks:

Two hundreds, one day-old Cubb broiler chicks were obtained from local commercial hatchery and fed on balanced ration free from medication.

Drug:

(1) Spiramycin (Mycospira)®: an aqueous injectable solution each ml containing 540.000 IU of spiramycin produced by Laboratories Mayed Barcelona, Spain. The recommended dose: 1ml/10kg b.wt. injected I/M.

(2) Kanamycin (Kanacin)®: each 100 ml contain 10 gm kanamycin produced by Unipharma, Elobour City, Cairo, Egypt, used in a dose of 10mg/kg. b.wt. I/M.

Experimental design:

Two hundred, day one-old Cubb chicks were divided into five equal groups (1, 2, 3, 4 and 5) of 40 chicks each were reared in a separate unite and fed on ration without any medication. Group (1) chickens were kept as a negative control (non infected and non treated). At 15 day-old, groups 2, 3, 4, and 5 chicks were injected with both Mycoplasma strain and E. coli O78 in a dose of 107 CFU/0.1ml and 0.25ml of 2 x 106 CFU/ml via eye- installation and intra-tracheal injection respectively according to Stipkovits, (1988). Group (2) chickens were remained as positive control (infected, non-treated), group (3) chickens were treated with a dose of 1ml/10kg. b.wt. spiramycin via I/M route.

Group (4) chickens were treated with a dose of 10mg/kg b.wt. via I/M. Group (5) chicken were injected I/M with a combination of spiramycin and kanamycin with the same dose. The treatment carried out 24 hours post infection for 3 successive days. The clinical signs, mortality, postmortem and reisolation of inoculated E. coli was carried out during experimental period.

Blood sampling:

Two weeks post treatment (32 day-old) blood and serum samples were collected. Serum samples were examined by serum plate agglutination test (SPA) according to Adler and Yamamato, (1965) for detection of mycoplasma antibodies and analysied for total protein (Doumas, 1975), AST and ALT according to Reitman and Frankel, (1957), creatinine according to Husdan and Rapoport (1968) and uric acid according to Caraway, (1963). All remained chickens were scarified for P.M. examination.

Statistical analysis:

Data were analysed using ANOVA test at a significance level ($P<0.05$) according to Snedicor and Cochran, (1980).

RESULTS

(1) Bacteriological and serological identification:

The results of bacteriological examination of 150 diseased chickens for detection of E. coli showed that 100 strains of E. coli were isolated. Serogrouping of these isolates indicates presence of 10 different serogroups and 35 serotypes were untyped as illustrated in table (1).

(2) Clinical signs, mortality rate and lesions score:

Infected, non-treated birds showed signs of depression, off food, diarrhea, rales and difficult breathing with sever P/M lesion (air sacculitis, pericarditis and perihepatitis) and high mortality rate (50%). These findings were subsided post-treatment with combination of two drugs, were mortality rate was reduced to 7% with mild lesion score.

All chicks of group 1 (control) were negative on culture for E. coli (O78). However, a higher frequency of reisolation of the pathogen from liver and heart of group 2 chickens compared with those of group 5 for CRD symptoms were observed. Moreover, high positive cases of mycoplasma antibodies were detected in infected untreated chicks (68%) compared with those of infected treated with spiramycin (18%) as well as combination of two drugs (10%) as shown in table (2).

(3) The influence of infection with E. coli and Mycoplasma gallisepticum 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, treated chicks (Table 3).

Two weeks post treatment with combination of kanamycin and spiramycin, the highest mean body gain and lowest feed conversion rate were recorded.

(4) Biochemical parameters of both infected and treated chickens:

Alteration of some liver and kidney functions in all groups were detected in treated birds. The measured parameters improved towards its normal level after 2 weeks post-treatment (Table 4).

Table 1. Serological identification of pathogenic avian E. coli (100 strains) isolated from broiler chicks suffering from respiratory manifestations

Serogroup	O1	O8	O18	O26	O78	O86	O111	O157	O169	Untyped strains
Number of isolates	7	2	3	3	26	3	4	9	8	35
%	7	2	3	3	26	3	4	9	8	35

Table 2. Mortality rate, lesion scores reisolation of E. coli (O_{78}) and positive cases of antibodies of Mycoplasma isolates following infections and treatment with I/M injection of sipramycin (1ml/10kg b.wt.) or and kanamycin (10mg/kg b.wt. for 3 successive days (n = 30).

Parameter / *Group*	*Mortality rate*	*Lesion scores*			*Frequency of reisolation E. coli*	*Positive cases of mycoplasma %*
		Air saculitis	Pericarditis	Perihepatitis		
(1) Non-infected , non treated (control)	0	0	0	0	0	0
(2) Infected, not treated	50	50	70	75	75	68
(3) Infected and treated with spiramycin	30	35	50	35	55	18
(4) Infected and treated with kanamycin	22	18	25	22	35	26
(5) Infected and treated with combination	7	3	10	12	13	10

Table 3. The influence of spiramycin (1ml/10kg b.wt.), kanamycin (10mg/kg b.wt.) and simultaneous injection of both spiramycin and kanamycin on experimental chicken performance (n = 10)

Parameter	*Before treatment and infection of 15 days old*				*Two weeks post treatment of 32 days old*			
Group	Body weight (gm)	Body weight gain	F.C. (gm)	FCR	Body weight (gm)	Body weight gain	F.C. (gm)	FCR
(1) Non-infected , non treated (control)	240.7 ± 8^{ab}	200 ± 5	360.5	1.54	1140 ± 30^{a}	900 ± 20	1560	1.73
(2) Infected, not treated	246.6 ± 4^{a}	201 ± 4	371.6	1.54	845 ± 60^{c}	599 ± 22	1470	2.55
(3) Infected and treated with spiramycin	251 ± 11.4^{a}	207 ± 2	390.5	1.56	960 ± 42^{c}	709 ± 12	1440	2.1
(4) Infected and treated with kanamycin	225.2 ± 8^{ab}	190 ± 4	356.2	1.55	1010 ± 31^{b}	785 ± 14	1490	1.9
(5) Infected and treated with combination	236 ± 2.9^{ab}	198 ± 3	362.5	1.54	1100 ± 35^{ab}	879 ± 30	1480	1.72

Different letters in the same column denote significant difference ($P \leq 0.05$)

Table 4. The influence of spiramycin (1ml/10kg b.wt.), kanamycin (10mg/kg b.wt.) and simultaneous injection of both spiramycin and kanamycin on some biochemical parameters of experimental infected chickens (n = 10)

Parameter / *Group*	*AST IU/L)*	*ALT (IU/L)*	*Total proteins*	*Uric acid (mg/dI)*	*Creatinine (mg/dL)*
(1) Non-infected , non treated (control)	86 ± 5^{a}	9 ± 0.6	3.4 ± 0.12^{bc}	3.41 ± 0.08^{bc}	1.12 ± 0.08^{cd}
(2) Infected, not treated	118 ± 9^{a}	10 ± 0.4	3.84 ± 0.11^{a}	6.2 ± 0.62^{a}	3.0 ± 0.41^{a}
(3) Infected and treated with spiramycin	102 ± 8^{ab}	$9 \pm .9$	3.51 ± 0.08^{b}	4.5 ± 0.1^{b}	1.45 ± 0.4^{bc}
(4) Infected and treated with kanamycin	98 ± 12^{b}	$9 \pm .7$	3.25 ± 0.11^{c}	3.8 ± 0.12^{c}	1.37 ± 0.5^{cd}
(5) Infected and treated with combination	82 ± 6^{bc}	8 ± 0.7	3.5 ± 0.56^{bc}	4.1 ± 0.08^{bc}	1.33 ± 0.11^{cd}

Different letters in the same column denote significant difference ($P \leq 0.05$)

DISCUSSIONS

The rate of isolation of E. coli was 100 isolates from 150 birds suffered from respiratory manifestations with a ratio of 66.6%. This results nearly coincide with those reported by Youssef and Azzam, (1985). The E. coli serogroups isolated in the present work were reported previously by Youssef *et. al.,* (1983).

Experimentally, infected chicks with isolated E. coli O78 and Mycoplasma gallisepticum (mixed infection) were showed clinical signs in the form of severe respiratory signs with air saculitis, pericarditis and perihepatitis on post-mortem examination. Experimentally infected chickens showed high mortality rate (50%). The mortality rate of infected chickens was reduced to 30% and 22% after treatment with spiramycin and kanamycin respectively. Meanwhile, simultaneous administration of the two drugs resulted in subsided clinical signs, reducing of lesion scores and mortality rate (7%).

Treatment with kanamycin was more effective than spiramycin for controlling mixed infection with E. coli supporting it is still effective leads to decreasing mortality from 50% (infected and untreated) to 22%, this may be due to kanamycin as a broad spectrum antibiotic active against both G-ve, G +ve and Mycoplasma bacteria (Yousef *et. al.,* 1983 and Abdalla and Adayiel, 2006). The same result was demonstrated in buffalo- calves, (21), where I/M injection of kanamycin to pneumonic calves improved the cure rate. This may be attributed to the potent effect of kanamycin against all isolated E. coli (22 serotypes).

Nearly similar results were obtained by Karney *et. al.,* (1973) and Ries *et. al.,* (1973). They found that kanamycin has a greater activity than other aminoglycosides against many pathogenic strains of family enterobacteriaceae. In addition, Ahmed, (1994) recommended the use of kanamycin for treatment of some bacterial infection as it is concentrated in lung tissue at high level, while Ali and Youssef, (2003) mentioned that isolated E. coli was the least resistant organisms to kanamycin. On contrary, it was reported that the isolated E. coli from chicken was resistant to kanamycin.

The intramuscular injection of spiramycin alone has low efficacy for controlling of mixed infections where the highest lesion scores with high mortality rate of infected and not treated chicks were reported, this may be due to the fact that spiramycin is a macrolides active mainly against G +ve and mycoplasma (Kleven and Anderson, 1972 and Kempf *et. al.,* 1989). Moreover,

several studies showed that spiramycin has a good activity against Mycoplasmosis in poultry (Baggot, 1984).

Simultaneous administration of both spiramycin and kanamycin showed their effectiveness in controlling mixed infections, however, no literature dealing with this point was available. Our results suggested that the effectiveness of the combination was better than therapy with each drug alone (Kempf *et. al.,* 1989). The latter author mentioned that combination of sipramycin (aminoglycoside) with lincomycin (macroloides) has a good activity against mixed infection.

Serological test (SPA) for mycoplasma revealed that chicks treated with combination of two drugs and spiramycin alone were the lowest cases (10%) and (8%) respectively compared infective non-treated chicks. This indicated good efficacy of spiramycin against Mycoplasma beside this Kempf *et. al.,* (1989) found that the use of spiramycin in birds infected with Mycoplasma gallispticum prevent Mycoplasma isolation from trachea of all treated infected chicks. Badr, (2003) stated that antibiotic injection of infected breeding stock with Mycoplasma was able to produce reduction in rate of mycoplasma infection. Also, improvement of general condition, body weight gain and feed conversion rate caused by mixed infection were recorded after injection of both drug in this study. Ferends *et. al.,* (1998) stated that the improvement of body gain in infected and treated chicks might attribute to bacteriological effect of antibiotics on the infection and consequently improvement of general health condition. The body weight gain of chicks infected with mixed infection was increased after injection of drugs compared with infected non-treated one. These results provide a further support for efficacy of combination of two drugs in control of mixed infection.

Mixed infection of E. coli and Mycoplasma resulted in elevation of the estimated AST and ALT enzymes (Amani, 1993). Injection of two drugs to infected chicks improved these enzymes towards the normal levels indicating the potent antimicrobial effect of the drugs for preventing deleterious effect of pathogens. These results were supported by Ahmed, (1994), who mentioned that adverse effect of kanamycin is reversible and re-against their normality one week post the end of I/M injection for 5 days.

Based on obtained results simultaneous medication with combination of spiramycin and kanamycin by I/M route rapidly reduced respiratory signs of disease decreased mortality % and reduced severity of lesion compared to therapy with each drug alone.

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كفاءة الكاناميسين والاسبيراميسين في الوقاية من الأمراض التنفسية في قطعان التسمين

طه عبد الفتاح[1] ، حلمي إبراهيم البنا [1]، السيد مسعود[1]، حسام حسن علام[2]

١- كلية طب بيطرى جامعة المنوفية

٢ - معهد بحوث صحة الحيوان بالزقازيق

يهدف هذا البحث لتقييم العلاج بحقن الكاناميسين والاسبيراميسين لدجاج التسمين المريضة بمرض التنفس المزمن المعقد لهذا تم عزل ١٠٠ عترة من الميكروب القولوني من عدد ١٥٠ كتكوت تسمين مريضة بمشاكل تنفسية، أعمارها تتراوح من ١ – ٤٥ يوم بمزارع التسمين المختلفة وبإجراء الاختبارات السيرولوجية اتضح أنه يوجد ١١ عترة من هذا الميكروب تم التعرف عليها وأخري لم يتم التعرف عليها.

ولمعرفة فاعلية الأدوية تم تقسيم ٢٠٠ كتكوت تسمين كب عمر ١٥ يوم إلي خمسة مجاميع متساوية وكانت المجموعة الأولي ضابطة (غير معالجة وغير معدية) وتم عمل عدوي معملية بالميكروب القولوني (O78) والميكوبلازما المعزولة في المجاميع الأربعة الباقية وتركت المجموعة الثانية بغير علاج وتم علاج المجموعة الثالثة بعقار السبيراميسين (١سم/١٠كجم وزن حي) حقناً بالعضل والمجموعة الرابعة بعقار الكاناميسين بمعدل (١٠سم/كجم وزن حي) بالحقن بالعضل وتم علاج المجموعة الخامسة بالدوائين معاً بنفس الجرعات السابقة حقناً بالعضل واستمر العلاج لمدة ٣ أيام متتالية.

واستناداً إلي الأعراض التنفسية ونسبة النفوق – الصفة التشريحية وإعادة عزل الميكروبات والتغيرات البيوكيميائية في مصل الدجاج المصاب والغير معالج ومقارنتها بالدجاج المصاب والمعالج بالإضافة إلي الوزن المكتسب في كل المجاميع بعد نهاية العلاج بأسبوعين يمكن القول بأن حقن الاسبيراميسين مع الكاناميسين بالجرعات العلاجية يمكن التغلب علي المشاكل التنفسية الناتجة عن الإصابة المزدوجة بالميكروب القولوني والميكوبلازما بشكل أفضل من استخدام كلاً منهم علي حدة.