EFFICACY AND SIDE EFFECTS OF THIAMPHENICOL IN DUCKLING INFECTED WITH SALMONELLOSIS

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Abstract

The activity of Thiamphenicol in-vitro against freshly isolated Salmonella typhimurium (S.t.) was tested. Antibiogram study revealed that gentamycin, enrofloxacin thiamphenicol, spectinomycin and neomycin were the most effective antimicrobials against tested Salmonella ty. isolated from naturally infected duckling. The minimal inhibitory concentration MIC of thiamphenicol against Salmonella was2. 0 µ/ml. In-vivo, studies were carried out on one hundred Muscovy duckling . They were divided into 4 equal groups, group A served as control (non infected, non treated), group B infected experimentally with Sal. by injection I/M with 0.1cc of a tryptic – soy broth culture containing 108 c. f. u./ml of Salmonella.,and served as infected, non treated group. Group c infected experimentally as above and treated orally with thiamphenicol (25mg/kg. b. wt.) for 5 successive days. Group D remain non infected and treated with thiamphenicol (25mg/kg. b. wt.)for 5 successive days. Treatment start 24 hours post infection. At the end of treatment and one week post medication, the obtained results concerning clinical signs, mortality rate, body weight , feed consumption and feed conversion rate showed that, thiamphenicol was effective agent in the control of the experimental infection. The study revealed that the drug had reversible adverse effect on liver and kidney enzymes also on erythrocytic count, haemoglobin and packed cell volum which returned to its normal level after one week post medication.

INTRODUCTION

Salmonellosis is one of the most serious disease of poultry, results in severe economic losses .The disease is commonly caused by Salmonella typhimurium (Sal. ty.) evokes a high mortality starting 2-3 days post-exposure (William,1984). On the other hand Salmonella. ty. was isolated from 25% of duckling suffering from enteritis (Asawy, et al. 2004). Chloramphenicol is an old drug commonly used to control disease outbreaks, nevertheless, resistance to this antimicrobial agent had been recorded (Chou-cinching, et al. 2003).In addition, the most serious adverse effect associated with chloramphenicol treatment is bone marrow toxicity (bone marrow suppression), aplastic anemia(Mcintyre, et al.2004),therefore continuous research had led to development of new generation with high efficacy and low toxicity as thiamphenicol or florfenicol.

Thiamphenicol is an antibiotic used for the treatment of infectious diseases. The chemical structure of thiamphenicol is analogue of chloramphenicol, except for the

substituent in the "Para" position of the benzene ring .Chloramphenicol has a nitro group at that position, whereas thiamphenicol has a sulfonylmethyl group to limit the toxicity (Kitamura, et al.1997, Ferrari, 1981, Drago, et al. 2000) .

Thiamphenicol is active against both G+ ve &G- ve bacteria, anaerobes and chlamydiae. The antibacterial mechanism of thiamphenicol is through the inhibition of protein synthesis binding to50s ribosomal subunits of bacteria (Cannon, et al.1990) and displays broad-spectrum antibiotic activity (Van Beers et al., 1975, SUTTER and Finegold 1976). Thiamphenicol is used extensively in veterinary medicine for the treatment and control of respiratory and intestinal infections in cattle and poultry (Bishop, 1998, Schwarz and Chaslus 2001) .Thiamphenicol has been previously investigated at a dose of 8 to 9 mg/kg in humans, 30 to 60 mg/kg for calves, 20 to 40 mg/kg for pigs, 15 to 67 mg/kg for poultry and 30 mg/kg in dairy cows (Nau et al., 1981).

Kitamura et al. (1997) indicated thăt thiamphenicol is neither toxic nor carcinogenic, for any organs or tissues of F334 rats when given continuously at the levels of 125 or 250 ppm in drinking water for 2 years. Thiamphenicol treatment in mice with the dose of 200 mg/kg for 7 days did not cause any changes in the biochemical parameters related to dose and time.(Hismiogullari , et al. 2011).

The present study was designed to explore its efficacy in treated duckling salmonellosis with regarding to its effects on growth rate, feed consumption and some serum biochemical parameters and liver and kidney functions.

MATERIALS AND METHODS

Drug :

Thiamphenicol (Tirsan 200)®: is an oral 20% solution from Fatro-pharmaceutical Vet. Industry, 40064 ozzano Emilia (BO) Italy, distributed by Agromed Co. for Agri. & Vet.

Salmonella typhimurium strain was isolated and identified biochemically and serologically according to Edward's and Ewing (1972) from duck farms infected with salmonellas in Salhia city ,Sharkia Governorate.

Commercial sensitivity discs®

Thiamphenicol (Th. 25µg), Enrofloxacin (En. 5µg) ,Gentamycin (Gn. 10µg), Neomycin (N10), Doxycycline (DO30), Ampicillin (AM30), Spectinomycin (Spt) and Sulpha &Trimethoprim.

Media :

MacConkey agar, MacConkey broth, Muller- Hinton agar and nutrient broth (Oxoid, LTD, England) were used .

The isolated strain of Salmonella was tested for its sensitivity to different antimicrobial agents using disc-diffusion method (Quinin et al. 1994). Minimum inhibitory concentrations (MICs) of thiamphenicol and other antimicrobials were determined using macrodilution (tube) broth method : Serial dilutions of the antibiotic are made in a liquid mediun which is inoculated with a standardized number of organisms and incubated at35±2ċ for16 to 20 hours , comparing the amount of growth in the tubes containing the antibiotic with the amount of growth in the growth- control tubes (no antmicrobial agent) used in each set of tests when determining the growth end point. The lowest concentration (highest dilution) of antibiotic preventing appearance of turbidity is considered to be the minimal inhibitory concentration (MIC).. it was tested according to Cruick Shank et al.(1975).

Birds :

One hundred , ten- day old female Muscovy duckling from farms in Sharkia Governorate weighing 150-200 gm were employed for this study, duckling were free from salmonella infection. A commercial unmedicated duck starter was fed, light was provided continuously. Feed and water were supplied at libitum. All hygienic measures were followed.

Experimental design :

Duckling were divided into equal four groups(25 birds/group) and housed separately as follow :

A : Control (non treated-non infected).

B :Experimentally infected in the left leg with 0.1cc of a tryptic-soybroth culture containing 108 c. f. u./ml of Salmonella typhimurium (Balog et al. 1992)

C : Non infected orally treated with thiamphenicol (25mg/kg. b. wt)

D: Infected as group B and treated orally with thiamphenicol (25mg/kg. b. wt.)

Treatment of duckling start 24 hours after infection and continue for 5 days in drinking water. Birds were examined daily for clinical signs of disease and mortality, post-mortem lesions in dead duckling were recorded and re isolation of Salmonella typhimurium from liver and heart of infected group were conducted using Mac Conkey broth and agar.

Total feed consumption and body gain were recorded before and post treatment.

At the end of treatment (the 1st.day post treatment and the 7th. day post treatment), five duckling from each group were sacrificed and two blood samples

were collected, the first sample was collected on sodium EDTA for haematological studies. Erythrocytic count was performed using an improved Neubauer haemocytometer and Nutt and Herrick solution as a special diluent for chicken "blood (Hassanin 1993). Haemoglobin estimation was performed by the test-kit (Zinki 1986). The packed cell volume (PCV) was estimated by the micro haematocrite capillary method (Schalm 1975).

The second blood samples were collected and the serum was separated for evaluation of serum activities of hepatotoxicity markers as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) according to Reitman and Frankel, (1957)as well as serum concentrations of total protein, according to Doumas et al. 1981,Albumin (Doumas 1971), Creatinine (Bartels (1971) ,Uric acid (Trinder 1969), Calcium (Goldenberg 1966) and inorganic phosphorous (Glinder and King 1972).

RESULTS

Results of antibiogram study revealed that thiamphenicol in addition to gentamicin, neomycin and spectinomycin were the most effective antibacterials against Salmonella typhimurium.

Table (1) The MIC of thiamphenicol against Salmonella typhimurium was2. 0 µg/ml. Table (2).The clinical signs of diseased duckling were general signs of illness, diarrhoea, pasty vents and loss of body weight.

Post mortem examination of both freshly dead and sacrificed infected duckling revealed congested liver, cascous pluges in ceca with sever enteritis .These observed symptoms and p. m. lesions were completely disappeared after treatment with thiamphenicol for 5 successive days .Infected and non medicated duckling showed 48% mortalities, reduction in body gain and reduction in feed consumption/ bird in comparison with other groups (Table 3).

Oral administration of thiamphenicol induced a significant increase of body weight ($p < 0.05$) in comparison to infected non treated group , table (3).Thiamphenicol reduced mortality from 48% to 12% in comparison to infected non treated duckling

Alteration of some serum haematological and biochemical values in infected duckling and non infected treated duckling with thiamphenicol was detected, table 4,5 and 6.Treatment of infected duckling with thiamphenicol improved these parameters toward its normal levels after seven days post medication.

Table 1. In- vitro susceptibility of isolated Sal.ty.from infected duckling to thiamphenicol and commonly used antimicrobial drugs

Antimicrobial agent	**Disc potency µg**	**Standard degree Of sensitivity**	**Mean zone of Inhibition (m.m.)**
Thiamphenicol	Th30	<18	20
Enrofloxacin	EN5	<22	21
Gentamycin	GN10	<15	23
Neomycin	N10	<15	18
Doxycycline	Do30	<18	12
Sulpha and Trimethoprim	SXT25	<16	13
Ampicillin	AM30	<16	13
Spectinomycin	Spt	<14	18

Table 2. M I.Cs of thiamphenicol and other commonly used antimicrobial drugs against isolated Sal. y. (µg/ml)

Antimcrobial agent	M.I.C. µg/ml
Thiamphenicol	2.0
Enrofloxacin	0.06
Danofloxacin	0.01
Florfenicol	2.0
Doxy cycline	8.0
Sulpha and trimethoprin	250
Chloramphenicol	16.0

Table 3. Mortality rate percent, mean body weight gain, feed consumption and feed conversion rate (FCR) on the 1st and 7th day post oral medication with thiamphenicol at dose 25mg/kg for 5 successive days

Group	Mortality		Body Weight at the beginning of exp.(gm)	1st day post medication (17day old)			7th day post medication (24day old)		
	No.	%		Body weight (gm)	Feed consumption gm/bird / week	F.C.R.	Body weight (gm)	Feed consumption gm/bird/week	F.C.R
1- Control Non Infected Non Treated	NIL	NIL	187a	290a	230a	2.25a	468a	411a	2.35a
2- Infected Non Treated	12	48	183d	192d	125c	3.1c	280d	245d	2.8d
3- Non infected and treated with Thiamphenicol	NIL	NIL	185a	307a	265b	2.15b	490a	450a	2.35a
4- Infected and treated with Thiamphenicol	3	12	181a	257b	250a	2.4a	428b	410a	2.0a

Different letters in the same column indicate significant changes ($p<0.05$)

Table 4. Effect of oral administration of thiamphenicol at dose 25mg/kg for five successive days on some serum biochemical values in expermintally infected duckling with Sal. ty. at the end of treatment (Mean± S.E) (n=5)

Group	AST IU/L	ALT IU/L	Total Protein Gm/dl	Albumin Gm/dl	Creatinine Mg/dl	Uric acid Mg/dl	Calcium Mg/100ml	Inorganic Phosphorous Mg/100m
1-Control non inf.	47.00c	10.00c	2.65 d	1.49 a	0.80 b	6.76b	11.60 a	8.91 a
2-Inf. Non tr.	65.00a	16.75a	4.59 a	1.01 c	1.13 a	8.33a	6,80 d	5.70 c
3-Non inf. Treat. thiamphenicol	49.7 c	11.30b	3.01 c	1.29 b	0.89 c	6.83b	10,6 b	7.4 b
4-Inf. Treat. thiamphenicol	57.20b	13.5 b	3.99 c	1.33 b	0.94 b	7.46b	10.60 b	7.73 b

Table 5. Effect of oral administration of thiamphenicol at dose 25mg/kg for five successive days on some serum biochemical values in experimentally infected duckling with Sal. ty. at one week post treatment (Mean ± S.E) (n=5)

Group	AST IU/L	ALT IU/L	Total Protein Gm/dl	Albumin Gm/dl	Creatinin Mg/dl	Uric acid Mg/dl	Calcium Mg/100ml	Inorganic Phosphorous Mg/100ml
1- Control non inf. Non tr.	46.37 c	8.80 c	2.85 d	1.52 a	0.79 b	6.81 c	11.91 a	8.93 a
2- Inf. Non tr.	67.13 a	18.20 a	4.94 a	0.98 d	1.19 a	10.20 a	6.80 d	5.60 d
3- Non inf. tr.thiamphenicol	56.56b	11.74 c	2.95 c	1.35 b	0.81 b	7.36 b	10.81 ab	7.73 b
4- Inf. and tr. thiamphenicol	59.10 c	13.60b	3.05 c	1.39 b	0.89 b	8.96 b	10.15 b	8.60 b

Different letters in the same column indicate significant changes ($p<0.05$)

Table 6. Effect of oral administration of thiamphenicol at a dose 25mg/kg for five successive days on some haematological parameters in experimentally infected duckling with Sal. ty. (Mean±_S. E) (n=5)

Group	1st day post medication			7th day post medication		
	RBCs 106/ul	H,b. Gm/dl	P.C.V. %	RBCs 106/ul	H.b. Gm/dl	P.C.V. %
1-Control Non inf. Non treat.	3.07 ±0.3	11.1 ±0.35	37.5 ±1.09	3.05 ±0.31	11.1 ±0.35	39.1 ±2.60
2-Inf. Non treated	2.48 ±0.13	8.78 ±0.85*	31.8 ±1.26*	2.1 ±0.18*	9.1 ±0.70*	32.8 ±1.10*
3-Non inf. treated	2.61 ±0.36	9.96 ±0.22*	35.1 ±0.51	2.95 ±0.11	10.1 ±0.66	36.7 ±1.72
4- inf.ected treat.	2,85 ±0.21	9.29 ±0.42*	33.9 ±1.81	2.81 ±0.18	10.2 ±0.96	36.7 ±2.53

*Significant at ($p<0.05$)

DISCUSSION

Thiamphenicol is a broad-spectrum antibiotic closely related to chloramphenicol. The chemical structure of thiamphenicol differs that of chloramphenicol in having sulpho. group instead of nitro group. It is active against both G- ve & G+ ve bacteria., acting by inhibition of bacterial protein synthesis binding to 50s ribosomal subunits of susceptible bacteria.

Antibiogram of tested Salmonella typhimurium to thiamphenicol and other commonly used antimicrobials was performed, the results obtained indicated that Salmonella typhymurium was highly sensitive to gentamicin, enrofloxacin,thiamphenicol and spectinomycin. These results are constant with that reported by Ablini et al. (1999) they reported that Salmonella spp. were more sensitive to thiamphenicol. In addition the MIC of thiamphenicol was determined as 2.0 µg /ml .

The clinical signs observed on the infected and non treated duckling were depression, loss of appetite , pasty vents and loss of body weight. Similar symptoms were previously recorded by (Asawy et al.2004). The treatment of infected duckling with thiamphenicol reduced mortality rate from 48% to 12% , also reduced clinical signs of the disease.

These finding indicating the effectiveness of thiamphenicol in treatment of Salmonella ty. infection. Numerous reports have indicated that the effectiveness of thiamphenicol of treatment of Salmonella infection (Bishop 1998, Schwarz and Chaslus 2001).

In the present study administration of thiamphenicol with therapeutic dose of 25mg/kg.body weight for 5 successive days resulted in a significant increase in the body weight, feed consumption and feed conversion rate on 7th day post treatment and the body weight returned to the normal level in infected treated duckling , this may be attributed to the antimicrobial effect of the drug which consequently improves the metabolic activity of the bird Alexander (1985) .Our results are in agreement with Yang Hong et al. (2002) who recorded that no difference in live weight gain was found among the duckling experimentally infected with E. coli and treated with florfenicol and the healthy non infected duckling . The significant increase in the liver enzymes (AST &ALT) in the present study in the infected duckling may be due to hepatitis induced by bacterial infection and its toxins. Campbell and Coles (1986) revealed the increased activity of AST and ALT to hepatocellular damage of ducks.

Treatment with thiamphenicol at a dose of 25mg/kg.for 5 successive days showed a significant increase in serum AST & ALT on 1st day post medication ,this changes was reversible returned to normal level after one week post treatments , the

same results was reported in mice (Hismiogullari et al.2011) . Abdalla et al.(2005) reported that the treatment with florfenicol, a structural analogue of thiamphenicol cause reversible significant increase of liver enzymes in treated healthy chickens. Nevertheless hypoalbumenia , a significant increase of serum total proteins in infected group in our study was detected .Similar findings were mentioned by Campbell and Coles 1986 ,this may be due to destructive effect of bacteria and its toxin on the liver cells which is the main sources of albumin and protein synthesis in the body (Mcpherson 1984). Elizabeth Moreira et al.(2008)recorded a rise in serum total protein caused by elevated globulin fraction in acute or chronic conditions in Chinese goose. Our results on 7th day post medication revealed improvement the level of total protein and albumin, this may be due to decrease liver cell destruction. The biochemical findings of the kidney function test of the infected non treated duckling in this study denote very highly significant increase in the level of serum uric acid and creatinine as compared with healthy control group. These may be attributed to renal damage which could be due to bacteria and its toxins , these parameters showed significant decrease post treatment with thiamphencol in comparison with infected, non treated duckling but is failed in returning to the normal levels. The hypocalcaemia in the infected non-treated duckling may be attributed to decrease calcium re absorption from damaged renal tubules (Coles 1986) .In the same time the hypophosphataemia in this investigation is always associated with hypocalcaemia and renal damage as the metabolism of calcium and phosphorous is closely related to each other. No significant changes were detected in serum levels of calcium and inorganic phosphorous in infected duckling treated with thiamphenicol in all duckling. A significant fluctuations within the physiological ranges in the levels of plasma calcium and inorganic phosphorous in duckling treated with thiamphenicol was recorded .

The results of haematological study in infected non treated duckling revealed that the RBCs count , Hb concentration and PCV were decreased significantly ,this may be due to haemorrhagic effect of Salmonella and its toxins which cause intra vascular destruction of erythrocytic cells in the body tissues. These nearly similar results were reported by Hassanin1993. Although ,the drug at therapeutic dose decrease mortality percent in infected treated ducklings, However administration of drug produced anaemia manifested by a decrease of RBCs count , PCV.and a significant decrease in Hb concentration these results were similar to those of (Ando et al. 1997) they reported that the minor changes in haematological and biochemical parameters were insignificant while Ferrari 1981reported that thiamphenicol act as hematopoietic suppressant in a dose dependant manner after repeated dosing. On the otherhand Turton et al (2002) reported that thiamphenicol caused decrease in

RBC,HCT and Hb., Marrow cell count were reduced, marrow was hypocellular and anaemia .

Based on these results, it could be concluded that Thiamphenicol at the recommended therapeutic regimen had reversible adverse effect on the liver and kidney function of duckling and return to normal levels after one week post the end of treatment. These results denoted that the liver and kidney tissues was not severely damaged.

The drug is an effective antibacterial agent for treatment of duckling salmonellosis however reversible bone marrow suppression, manifested by anaemia were occurred.

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الكفاءة والآثار الجانبية لعقار الثيامفينيكول في كتاكيت البط المصابه بالسالمونيلا

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عقار الثيامفينيكول احد المضات البكتيريه المشابهه في تركيبها للكلورمفينكول ولكنه ذو كفائه وفاعليه اعلى منه. وفى هذه الدراسه تم عزل ميكروب السالمونيلا من احد مزارع البط المسكوفى في محافظه الشرقيه.وبعمل اختبار الحساسيه باستخدام اقراص الحساسيه للثيامفينكول وبعض مضادات الميكروبات شائعه الاستخدام اتضح كفائته العاليه كذلك تم تحديد الحد الادنى القاتل من الدواء للميكروب وجد انه ٢ ميكروجرام لكل ملىبالاضافه لما سبق فقد تم تقييم فاعليته الدوائيه في كتاكيت البط المسكوفى المصابه تجريبيا بميكروب السالمونيلا وذلك بتقسيم عدد١٠٠ كتكوت بط مسكوفى عمر ١٠ ايام الى اربع مجموعات متساويه على النحو التالى :المجموعه الاولى :مجموعه ضابطه وغير معالجه، المجموعه الثانيه: بط مصاب تجريبيا با لسا لمونيلا وغير معالج ،المجموعه الثالثه :بط مصاب تجريبيا با لسا لمونيلا ومعالج بعقار الثيامفينكول بمياه الشرب بمعدل٢٥مج/كج وزن حى لمده ٥ ايام متتاليه، المجموعه الرابعه :بط سليم ومعالج بعقار الثيامفينيكول بمياه الشرب بمعدل ٢٥ مج/كج وزن حى لمدع ٥ ايام متتاليه.واستنادا الى الاعراض الاكلينيكيه ونسبه النفوق والصفه التشريحيه وومعدل استهلاك العلف ومعدل التحويل الغذائى بالاضافه الى الوزن المكتسب في كل المجاميع ومقارنتها بالبط السليم الغير معالج يمكن الحكم بكفائه عقار الثيامفينيكول. ولقد لوحظ ارتفاع معنوى مؤقت في انزيمات الكبد والكلى وتغير معنوى في صوره الدم (انيميا) في البط السليم المعالج ولكنها تراجعت لمعدلاتها الطبيعيه بعد اسبوع من نهايه العلاج خلاصه القول ان هذه النتائج اثبتت كفائه العقار العلاجيه في التغلب على العدوى المعمليه لميكروب السالمونيلا مع ملاحظه حدوث انيميا اثناء العلاج .