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TREATMENT OF DUCKLING SALMONELLOSIS BY ENROFLOXACIN (With 4 Tables)

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استخدام عقار الأنروفلوكساسين فى علاج سالمونيلا البط

رفعت خضرى - إسماعيل السيد

السالمونيلا هي أحد الأمراض البكتيرية التي تهدد صناعة الدواجن وتؤدي إلى خسائر إقتصادية كبيرة. والأنروفلوكساسين هو أحد مركبات الفلوروكينولون الذي ثبت كفاعته فى علاج سالمونيلا الدواجن إلا أنه لا تتوفر معلومات عن كفاعته فى علاج البط . ولذا فى هذه الدراسة تم حقن الأنروفلوكساسين بجرعات ٥ ، ١٠ ، ٢٠ مجم/كجم من الوزن الذى تحت الجلد لمدة خمسة أيام متتالية فى ١٢٠ بطة بكينى عمر اسبوع واحد ومعدى معمليا بعثرة حقلية من السالمونيلا تيفموريم. أظهرت كل المجموعات المعالجة تحسن معنوى فى وزن الجسم المكتسب عند مقارنتها بالوزن فى البط المصاب والغير معالج. وقد نتج عن استخدام الدواء بجرعة ٥ مجم لكل كجم وزن حتى تقليل نسبة النفوق من ٤٠% فى البط المصاب والغير معالج إلى ٥% فى البط المصاب والمعالج بينما لم تسجل حالات نفوق فى البط المصاب والمعالج بأيا من الجرعتين ١٠ أو ٢٠ مجم لكل كجم وزن حتى. عند قياس مستوى الكالسيوم والفوسفور الغير عضوى والزلال فى مصل البط المصاب بالسالمونيلا تيفموريم والغير معالج كان هناك نقص معنوى فى مستواها مصحوبا بزيادة معنوية فى مستوى الألبيرتيت أمينوترانس فيريز والبروتين الكلى والجلوبولين الكلى وحمض اليوريك والكرياتين فى المصل. أحدث الأنروفلوكساسين عند استعماله بجرعة ٢٠مجم/كجم وزن حتى زيادة معنوية فى مستوى الإسبرتيت أمينوترانس فيريز وحمض اليوريك فى مصل البط المعالج. وقد لوحظ بعد إنتهاء العلاج بأسبوع أن الإختلال الذى حدث فى القياسات البيوكيمائية المذكورة انفاً قد اختفى فى المجموعتين المعالجتين بـ ١٠ و ٥ مجم / كجم وزن حتى بينما ظلت هذه القياسات مختلة فى المجموعة المعالجة بـ ٢٠مجم/كجم وزن حتى. وقد اثبتت الإختبارات المعملية أن الأنروفلوكساسين والدانوفلوكساسين وحمض الأكسولونك هي أفضل مضادات البكتريا تأثيرا على ميكروب السالمونيلا تيفمورم المعزول حقليا من البط المصاب. وقد

تم استنتاج أن الأنتروفلوكساسين يعتبر علاج فعال للإصابة بالسالمونيلا تيفيموريم في البط الصغير بجرعة لاتتعدى ١٠مجم/كجم وزن حى.

SUMMARY

Salmonellosis is a serious bacterial disease affecting poultry industry leading to severe economic losses. Enrofloxacin, a fluoroquinolone compound, was proved to be efficient for treating salmonellosis in chickens, however no data were available about its efficacy in ducks. In this study, Enrofloxacin was injected subcutaneously at a dose levels of 5, 10 and 20 mg/kg.b.wt. for five successive days into 120 ducklings of one week old experimentally infected with *Salmonella typhimurium*. All treated groups showed a significant ($P<0.05$) improvement of body gain when compared to infected, non treated ducklings. The drug at a dose level of 5mg/kg. b.wt. reduced mortality from 40% to 5%, whereas, at 10 and 20mg/kg. b. wt. no mortalities were recorded. Infected ducklings with *Salmonella typhimurium* showed a significant ($P<0.05$) decrease of serum calcium, inorganic phosphorous and albumin associated with a significant ($P<0.05$) increase of serum aspartate aminotransferase (AST), total proteins, total globulins, uric acid and creatinine. Enrofloxacin at a dose level of 20mg/kg. b. wt. induced a significant ($P<0.05$) increase of serum AST, creatinine and uric acid in treated ducklings. Moreover, these parameters remained disturbed one week post treatment. In infected ducklings, the forementioned disturbed biochemical parameters were corrected and returned back to its normality condition one week post treatment by Enrofloxacin at a dose levels of 5and 10mg kg.b.wt. Antibiogram study revealed that Enrofloxacin, Danofloxacin and Oxalinic acid were the most effective antibacterials against *Salmonella typhimurium* isolated from naturally infected ducklings.

Key words: Ducks - Salmonellosis - Treatment

INTRODUCTION

Salmonellosis is one of the most serious bacterial disease of poultry results in severe economic losses. The disease is commonly caused by *salmonella typhimurium* (S.t.). Infection of young birds with S.t. evokes a high mortality starting 2-3 days post-exposure (Williams, 1984). On the

other hand, S.t. was isolated from 30% of the ducks condemned at slaughter due to arthritis (Rasmussen, 1962).

In chickens, several antimicrobials are effectively used for control of Salmonellosis, whereas, treatment of duck Salmonellosis is hampered by the lack of approved antimicrobials (Balog, et al., 1992).

Enrofloxacin (Enr.) is a recent fluoroquinolone antibacterial agent developed specifically for therapeutic use in animals (Brander, et al., 1991). The drug had been used for control of salmonellosis in chickens (Redmann, et al., 1989; Mazurkiewicz, et al., 1990 and Minta, et al., 1990). However, no data were available about its use in ducks. Hence, the present study was designed to explore its efficacy in treating duckling Salmonellosis with regarding to its effects on growth rate, feed consumption and some liver and kidney functions.

MATERIAL and METHODS

I- Drug:

A- Enrofloxacin (Spectrama-Vet)[®] injectable solution 10% from Amoun company, Egypt.

B- Commercial sensitivity discs: from oxoid, England.

These discs were Enrofloxacin (10ug), Danofloxacin (5ug), Norfloxacin (10ug), Oxolonic acid (10ug), Flumequine (30 ug), Gentamycin (10ug), Streptomycin (10 ug), Tetracycline (30 ug), Doxacycline (30 ug), combination of Sulfamethoxazol and Trimethoprim (25 ug), and Spectinomycin (10ug).

II- Ducklings:

One hundred and twenty, one week old, white pekin healthy ducklings free from salmonella infection, were used. A commercial unmedicated duck starter was fed. Light was provided continuously. Feed and water were supplied ad libitum. All hygienic measures were followed. Ducklings were divided into equal six groups and housed separately as follow:

A) Control (non treated, non infected).

B) Experimentally infected in the left leg with 0.1 cc of a tryptic-soy broth culture containing 10^8 c.f.u/ml of s.t. (Balog, et al., 1992). The used strain of S.t. was a field strain isolated from infected private farm in Sharkia Governorate. The strain was biochemically identified (Kauffmann, 1974).

C) Non infected, injected subcutaneously in the nape of the neck with Enr. (10 mg/kg. b.wt.) for 5 successive days.

D, E and F groups were infected with S.t. and injected subcutaneously with Enr. (5, 10, and 20mg/kg. b. wt. respectively) 24 hours post infection with S.t. for 5 successive days.

Ducklings were observed for three weeks post-treatment. Mortality%, weight gain and feed consumption were recorded. Using MacConkey's media, and Brilliant green agar, re-isolation of S.t. from liver of dead and slaughtered ducklings at end of treatment was carried out.

The sensitivity of the isolated S.t. against some antimicrobials using disc-diffusion method was detected (Ibrahim, and Shihata, 1989). The minimal inhibitory concentration (MIC) of Enr. on isolated S.t. was determined using the serial dilution tube technique (Gould, 1960).

At the end of treatment and one week post treatment, five ducklings from each group were slaughtered and the sera samples were collected for biochemical analysis. Serum uric acid (Caraway, 1955), creatinine (Husdan and Rapoport, 1968), aspartate aminotransferase (AST) after Reitman and Frankel (1957), albumin (Doumas, *et al.*, 1971) and total proteins (Doumas, 1975) were determined. Moreover, total globulins were calculated. Statistical analysis was carried out using analysis of variance according to Snedecor and Cochran, (1969).

Media:

Tryptic-soy broth, MacConkey-agar, Brilliant green agar and nutrient agar (oxid).

RESULTS

As shown in Table (1), ducklings experimentally infected with field isolate of S.t. and received no medication showed 40% mortality, reduction in body gain and consumed less feed/bird/day when compared to other groups.

Salmonella typhimurium was isolated from the livers of dead ducklings. No S.t. was isolated from non infected, non treated ducklings at necropsy. Post-mortem lesions in infected ducklings were congested liver, caseous plugs in ceca and impaction of the rectum.

On the other hand, subcutaneous administration of Enr. at a dose levels of 5, 10 and 20mg/kg.b.wt. for 5 successive days induced a significant ($P < 0.05$) increase of body gain when compared to infected, non treated

ducklings (Table, 1). Enrofloxacin at a dose level of 5mg/kg. b. wt. reduced mortality from 40% to 5% compared to infected, non treated ducklings. Meanwhile, no mortalities were recorded at a dose levels of 10 and 20 mg/kg. b. wt. (Table, 1). However, a significant increase of total proteins and total globulins coupled with a significant decrease of albumin at both dose levels was detected (Table,3). Moreover, administration of the drug at a dose level of 20 mg/kg. b. wt resulted in a significant decrease of serum calcium and inorganic phosphorous associated with a significant increase of AST, serum uric acid and creatinine (Table, 3). Measurement of serum calcium and inorganic phosphorous, AST, total proteins, total globulins, albumin, uric acid and creatinine, one week post treatment by 5 and 10mg/ kg.b.wt. revealed a significant improvement of these parameters toward the normal levels (Table, 4).

Results of antibiogram study revealed that Enrofloxacin in addition to Danofloxacin, Norfloxacin and Oxolonic acid were the most effective antibacterials against s.t. isolated from naturally infected ducklings (Table,2). The MIC of Enrofloxacin against s.t. was determined as 0.02 ug/ml.

DISCUSSION

Enrofloxacin has a broad-spectrum antibacterial activity against most Gram-negative and some Gram-positive bacteria (Scheer, 1987a). Similar to other fluoroquinolones, Enr. achieves its rapid bactericidal activity by inhibition of bacterial DNA gyrase Inzyme, which is essential for supercoiling of DNA (Crumplin *et al.*, 1984 and Dudley, 1991).

In the present study, Enr. at a dose level of 5mg/kg. b.wt. reduced mortality rate from 40% to 5%, whereas the drug at 10 and 20 mg/kg. b.wt. completely prevented mortality in ducklings infected with s.t. This finding confirmed the in-vitro study of Watts *et al.* (1993), who reported that Enr. was the most active drug against *salmonella spp* isolated from ducks. Moreover, our antibiogram study revealed that s.t. isolated from naturally infected ducklings was highly sensitive to Enr. In addition, the MIC of the drug was determined as a value (0.02 ug/ml) below the average concentration of the drug (1.0 ug/ml) in serum of chickens after oral dosing of 2.5 mg/kg. b. wt. (Scheer, 1986a).

The obtained result was inconsistent with that of Steinberger, (1986). The author determined the MIC of Enr. for *salmonella* species as 0.06 ug/ml. This variation in the value of MICs might be attributed to the

variation in the type of strains used in the test. Furthermore, in-vitro and in-vivo studies revealed that Enr. was more effective than Gentamycin, Neomycin, Flumequine, Streptomycin and Ampicillin against 25 *Salmonella* species (Abd El-Galil and EL-Naenaey, 1993).

Infected non treated ducklings exhibited high mortality, poor weight gain and low feed consumption, whereas in all treated groups the feed consumption was increased in comparison to the infected, non treated ones.

As previously demonstrated in chickens (Stipkovits, 1988 and Wieliczko *et al.*, 1991) and geese (Tomczyk, 1992), Enr. had a beneficial effect on weight gain of ducklings infected with s.t. and treated with 5, 10 and 20 mg/kg. b.wt. of the drug. The increase in body gain of treated ducklings may be attributed to the bactericidal effect of the drug that consequently improves the metabolic activity of the bird (Coates *et al.*, 1951).

Although the drug at a dose level of 20mg/kg. b.wt. succeeded in preventing mortality due to s.t. infection, however a significant decrease and increase in serum albumin and AST respectively of treated ducks were recorded. The elevated level of AST is an indication of hepatic disease and necrosis in other tissues (Zimmerman, 1984). In chickens, the same finding was reported by Helal *et al.*, (1995). They found that Enr. at a dose level of 20 mg/kg. b.wt. evoked a significant increase in serum AST level. The recorded hypoalbuminaemia was another reflection of hepatic disease, as inability of the liver to synthesize albumin is a prominent feature in this case (Altman, 1979).

The significant increase of serum total proteins in s.t. infected ducklings may be attributed to the recorded increase of total globulins in response to infection, whereas the significant increase in all treated groups could be explained on the basis of immunostimulant effect elicited by the drug and resulting in an increase of total globulins (Helal, 1995).

Regarding the effect of Enr. on serum calcium and inorganic phosphorous, hypocalcemia and hypophosphatemia were evident in all treated groups and infected one. This effect might be attributed to metabolic disturbance and requires a further investigation.

In the present study, the significant increase of serum uric acid and creatinine in ducklings treated by 20mg/kg b.wt. could be attributed to degenerative changes in the kidney. This proposition was supported by the results of Helal *et al.*, (1995). They reported a degenerative changes in renal

tubules of chickens treated by Enr. Moreover, the authors reported a significant increase of serum creatinine in the treated chickens.

Measurement of the forementioned liver and kidney functions, one week post treatment with 5 and 10 mg/kg. b. wt. of Enr., revealed an improvement of altered parameters towards the normal levels. These results denoted that the liver and kidney tissues was not severely damaged.

It could be concluded that Enrofloxacin is an effective antibacterial agent for treatment of duckling salmonellosis at a dose level not exceeds 10mg/kg. b. wt.

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Table (1): Effect of subcutaneous administration of Enrofloxacin (5, 10 and 20, mg./kg. b. wt.) for five successive days on feed consumption and body gain in healthy and *Salmonella typhimurium* infected ducklings. (n=5)

Parameters Groups	One week old		Two weeks old			Three weeks old			Body gain (gm)		Feed: gain	Mortality
	Body weight (gm.)	Feed/bird/day (gm.)	Body weight (gm.)	Body gain (gm.)	bird / day (gm.)	Body weight (gm.)	Body gain (gm.)	Feed/bird / day (gm.)	Total body gain	Daily body gain		
Non infected, non treated	87.50 a	51.70 a	307.7 a	220.2 a	64.30 a	535.0 a	227.06 a	123.04 a	447.30 a	32.40 a	3.80	0
Non infected, treated by 10 mg./kg b. wt.	84.30 a	52.20 a	247.60 b	163.18 b	58.20 b	438.02 c	190.40 c	80.90 c	333.80 c	27.20 bc	2.97	0
Infected, treated by 20 mg./kg b. wt.	84.80 a	54.00 a	223.40 c	138.80 c	41.00 d	384.00 d	160.24 d	90.40 b	299.04 d	22.90 d	3.95	0
Infected, treated by 10 mg./kg b. wt.	85.90 a	83.10 a	245.04 b	159.20 b	48.20 c	428.00	183.00 c	118.02 a	342.06 c	26.20 c	4.5	0
Infected, treated by 5mg./kg b. wt.	85.20 a	52.80 a	257.3 b	172.06 b	54.50 b	466.06 b	209.00 b	85.70 bc	381.02 b	29.80 ab	2.88	5
Infected, non treated	83.70 a	50.20 a	192.80 d	109.30 d	32.20 e	347.00	154.12 d	53.60 d	263.06 e	22.00 d	2.43	40

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

Table (2): In-vitro susceptibility of *salmonella typhimurium* to some antibacterials.

Antibacterial disc (potency)	Degree of suceptibility
Enrofloxacin (10 ug)	+++
Danofloxacin (5 ug)	+++
Norfloxacin (10 ug)	+++
Oxolonic acid (10 ug)	+++
Flumequine (30 ug)	++
Gentamycin (10 ug)	++
Streptomycin (10 ug)	+
Tetracycline (30 ug)	-
Doxacycline (30 ug)	-
Sulfamethoxazole + Trimethoprim (25 ug)	-
Spectinomycin (10 ug)	-

Table (3): Effect of subcutaneous administration of Enrofloxacin (5, 10 and 20, mg./kg. b. wt.) for five successive days on serum calcium, inorganic phosphorous and liver and kidney functions in healthy and *Salmonella typhimurium* infected ducklings at the end of treatment. (n=5)

Parameters Groups	Calcium (mg./100ml)	Inorganic phosphorous (mg./100ml.)	AST (u./ml.)	Total proteins (mg./100ml.)	Albumin (mg/100ml)	Total globulins (mg./100ml)	Uric acid (mg./100ml)	Creatinine (mg./100ml)
Non infected, non treated	11.50 a	8.95 a	46.00 c	2.69 d	1.48 a	1.21 f	6.76 b	0.77 c
Non infected, treated by 10 mg./kg. b. wt.	11.30 a	7.68 b	48.30 c	3.01 c	1.30 b	1.71 d	7.46 b	0.79 c
Infected, treated by 20 mg./kg. b. wt.	8.60 a	6.37 c	57.20 b	3.84 b	1.10 c	2.84 b	8.86 a	0.94 b
Infected, treated by 10 mg./ kg. b. wt.	9.12 c	7.64 b	49.80 c	3.65 b	1.33 b	2.32 c	6.83 b	0.79 c
Infected, treated by 5mg./kg. b. wt.	10.00 b	7.93 b	49.75 c	2.87 cd	1.35 b	1.52 e	6.86 b	0.82 c
Infected, non treated	7.20 d	5.4 d	63.5 a	4.59 a	1.02 c	3.57 a	8.33 a	1.12 a

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

Table (4): Effect of subcutaneous administration of Enrofloxacin (5, 10 and 20, mg./kg. b. wt.) for five successive days on serum calcium, inorganic phosphorous and liver and kidney functions in healthy and *Salmonella typhimurium* infected ducklings at one week post treatment. (n=5)

Parameters Groups	Calcium (mg./100ml)	Inorganic phosphorous (mg./100ml.)	AST (u./ml.)	Total proteins (mg./100ml.)	Albumin (mg/100ml)	Total globulins (mg./100ml)	Uric acid (mg./100ml)	Creatinine (mg./100ml)
Non infected, non treated	11.95 a	8.93 a	47.37 c	2.85 c	1.52 a	1.33 d	6.86 c	0.77 b
Non infected, treated by 10 mg./kg.b. wt.	10.9 ab	8.00 b	48.20 c	3.05 c	1.35 bc	1.70 c	7.36 c	0.79 b
Infected, treated by 20 mg./kg. b. wt.	7.60 c	7.03 c	58.68 b	3.84 b	1.05 d	2.78 b	8.96 b	0.83 b
Infected, treated by 10 mg./kg. b. wt.	10.60 b	7.73 bc	49.10 c	3.05 c	1.26 c	1.79 c	6.98 c	0.79 b
Infected, treated by 5mg./kg.b.wt.	11.00 ab	7.43 bc	48.56 c	2.76 c	1.39 b	1.37 d	6.66 c	0.81 b
Infected, non treated	6.60 d	5.60 d	65.13 a	4.94 a	0.96 d	3.98 a	10.00 a	1.09 a

Different letters in the same column indicate significant changes (P<0.05).

