Efficacy Of Pefloxacin Against Salmonellosis In Balady Chicks

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ABSTRACT

Six Salmonella (Sal.) isolates were recovered from 110 diseased Balady chicks aged 1- 3o days-old collected from diffferent localities at Sharkia Governorate. These isolates were serotyped into two *Sal. enteritidis* (S.E.) and one *Sal typhimurium* and three untyped isolates .

Antibiogram of isolated *Sal. enteritidis* to pefloxacin and other common antimicrobials agents was performed .Pefloxacin and other fluoroquinolones (Ciprofloxacin and Enrofloxacin) were the most effective antimicrobial agents against S.E. The minimal inhibitory concentration (MICs) of pefloxacin against tested S. E. was o .3 ug / ml .

In-vivo studies were carried out on 300 Balady chicks. They were divided into 6 equal groups; Group 1 served as control (non infected, non treated), the other 5 groups were injected intramuscularly with 0.25ml of broth containing $10^{3.3}$ c.f.u./ml. S.E. at the age of one week. Group 2 remain infected, non treated . Group 3 and 4 treated orally with pefloxacin at a dose level of 5mg /kg . b.wt for 3 and 5 successive days respectively. Groups 5 and 6 were treated orally with pefloxacin at a dose level of 10 mg / kg b.wt. for 3 and 5 successive days respectively . Treatment start twenty four hours post infection. At the age of 3 week, the obtained results concerning clinical signs, mortality rate, reisolation of S.E. and the body gain showed that pefloxacin at a dose level of 5 and 10 mg /kg b.wt for 5 successive days was highly effective agent in the control of the experimental infection.

Moreover. chicks infected with S. E. serum AST, ALT, creatinine, uric acid, globulin, and inorganic phosphorous were significantly increased but albumin and calcium levels were decreased significantly. These parameters were improved towards the normal levels in chicks infected with S.E .and treated with pefloxacin for 5 successive days.

INTRODUCTION

Avian Salmonellosis is one of the most serious bacterial diseases affecting poultry industry, where it causes great economic loses due to high rate of mortality, retardation of growth and low productivity ***(1)***. Infection of young birds with Salmonellosis evokes high mortality starting 2-3 days post exposure ***(2)***.

Fluoroquinolones are a group of antimicrobial agents widely used in clinical veterinary practice because of their widespectrum antibacterial activity and high degree of bioavailability ***(3)*** where they inhibit the activity of bacterial DNA gyrase (bacterial topoisomerase II) ***(4)***.

Pefloxacin is one of a third generation of fluoroquinolones with broad spectrum activity against G+ve, G-ve bacteria and Mycoplasma ***(5)***.

Fluorinated quinolones including pefloxacin were active against all of the Salmonella strains ***(6)***. This study was planned to study the activity of pefloxacin against recent isolates of Salmonella spp. *in vitro* and *in vivo*. Also to investigate the optimum effective dose and duration of therapy for the control of these spp. of Salmonella as well as its effect on body weight gain, feed conversion ratio and some liver and kidney functions.

MATERIAL AND METHODS

Samples and cultivation

One hundred and ten specimens from liver, small intestine and large intestine received by our laboratory Zagazig Vet. Research Institute were collected from both diseased and freshly dead Balady chicks with an average age 1- 30 days-old from private farms from different localities at the Sharkia Governorate.The specimens were cultured on peptone broth and Selenite F. broth (Oxoide) for 24 hrs at 37°C, then a loopfull was streaked onto MacConkey agar plates and incubated at 37°C, for 24 - 48 hrs. Bacterial colonies were selected for further morphological and biochemical identifications ***(7,8)***.

Media

MacConkeys agar and broth, nutrient agar and broth, Selenite F. broth, and heart infusion agar ***(8)***.

Drug

Pefloxacin (pefloxacin 10%) oral solution was obtained from United Co. For Chem and Med. Preperations (UCCMA), Cairo, Egypt. Each ml contain 100 mg of pefloxacin base.

***In–vitro* antibacterial activity**

The antibiotic sensitivity test of the isolated Salmonella against some antimicrobial agents using disc diffusion method was carried out ***(9)*** using, Mueller-Hinton agar plates. The results were interpreted according to Oxoide Manual Company ***(10)***.

The minimal inhibitory concentration values (MICs) of pefloxacin and other antimicrobials were determined against isolated Salmonella using a serial broth dilution method ***(11)***.

Experimental evaluation of pefloxacin

Three-hundred and five, one-week-old balady chicks were obtained from private hatchery, five chicks were randomly sacrificed and bacteriologically examined to confirm that free from Salmonellae. Group 1 chicks (non infected, and non treated) where 2nd group was infected with Sal. and remain non treated (positive control), 3rd and 4th group were infected with Sal. and treated orally with pefloxacin at the dose level 5mg /kg b.wt for 3 and 5 successive days respectively, 5th and 6th groups were infected with Sal. And treated orally with pefloxacin at the dose level of 10 mg/kg ..wt for 3 and 5 successive day respectively. The route of infection: each chicks of infected groups received *Salmonella enteritidis* by I/M injection of infective dose of 0.25 ml of $10^{3.3}$ c.f.u. ***(12)***. Treatment started 24hrs post infection and the period of experiment lasted to the age of 3 weeks. All chicks were individually weighted, feed consumption, feed conversion, mortality, rate clinical symptoms and P/ M lesions were recorded for all groups at the end of experiment. At the end of experimental period chicks were sacrificed and blood samples were obtained and sera were collected.

Biochemical analysis

The collected serum samples were used for estimation of AST, ALT, ***(13)*** total protein ***(14)***, albumin ***(15)***, globulin was calculated as difference between total protein and albumin, uric acid ***(16)***, Creatinine ***(17)***, calcium ***(18)*** and inorganic phosphorous ***(19)***.

Statistical analysis

The obtained data were statistically analysed using student's (t) test as described by ***(20)***.

RESULTS

Bacteriological examinations

Bacteriological examinations results of 110 diseased and dead Balady chicks revealed the presence of 6 strains of Salmonella spp. with ratio of 5.5% which were identified as two *Sal. Enteritidis*, one *Sal. typhimurium* and three untyped Salmonellae .

Results of antibiogram study revealed that pefloxacin in addition to enrofloxacin ciprofloxacin, kanamycin, aminosidine were the most effective antimicrobials against Sal .E isolated from naturally infected balady chicks (Table 1) The M I C of pefloxacin against Sal .E. was 0.3 ug / m l.

Experimental infected chicks with Sal. E. showed clinical signs manifested by depression, loss of appetite, pasty vents and loss of body weight. Post mortem examination of both freshly dead and sacrificed experimentally infected chicks revealed congested liver with caseous pluges in ceca with severe enteritis. These observed symtoms and P/M lesions were milder with pefloxacin therapy for 3 successive days (gps, 3, 5) and completely disappeared after treatment for 5 successive days (gps 4,6).

Infected and non-medicated chicks (positive control) showed 38% mortalities, reduction in body gain and reduction in feed consumption / bird in comparison with other groups (Table 2). Oral administration of pefloxacin at a dose levels of 5 and 10 mg /kg.

b. wt for 3 or 5 successive days induced a significant ($p<0.05$) increase of body weight gain in comparison to infected non treated group (Table 3).

Pefloxacin at a dose level of 5 mg/ kg. b.wt. for 3 days reduced mortality from 38 % to 6 % in comparison to infected non-treated chicks. Meanwhile, no moralities were recorded with after administration of pefloxacin at a dose levels 5 and 10 mg/ kg.b. wt for 5 successive days.

Alteration of some serum biochemical values in all groups were detected. Infected chicks with Salmonella denoted a significant, increase in the activities of AST, ALT, Creatinine, uric acid, globulin and inorganic phosphorus but albumin and calcium levels were decreased significantly compared with control group.

These parameters were reverted to nearly their normal levels in infected treated chicks with pefloxacin (Table 4).

Table 1. *In - Vitro* susceptibility of *Sal. enteritidis*. of avian origin to pefloxacin and some commonly used antimicrabials.

Chemotherapy	Disc potency (ug)	Mean zone of inhibition (m.m)	M. I .C (ug) / ml
Pefloxacin (PEF)	(5)	23	0.3
Enrofloxacin (EF)	(10)	24	0.15
Ciprofloxacin (C. P)	(5)	22	0.1
Kanamycin (K)	(30)	19	2.5
Aminosidine (AM)	(30)	21	0.6
Gentamycin (GM)	(10)	16	0.6
Ampicillin (A)	(30)	14	32
Colistin (C)	(25)	15	16

Table 2. Mortality rate and reisolution of *Sal. enteritidis* from infected Balady chicks and pefloxacin treated chicks 3 weeks old n = 50

Parameter / Group	Mortality		Frequency of resolution
	No	%	
(1) Control	O	O	O
(2) Inf . non treated	19	38	100%
(3) Inf. treat 5 mg for 3 days	3	6	15%
(4) Inf .treat 5 mg for 5 days	1	2	10%
(5) Inf .treat 10 mg for 3 days	O	O	0%
(6)Inf. treat. 10 mg for 5 days	O	O	0%

Table 3. Effect of oral administration of pefloxacin at a dose levels of 5 and 10 mg / kg .b. wt for 3 and 5 successive days on mean body gain, feed conversion rate (F. C R) of *Sal. enteritidis* - infected Balady chicks (N =50).

Parameter / Groups	One week old #				Three week old			
	Body weight (gm)	Body Gain (gm)	Feed intake gm/ bird	F.C.R	Body Weight gm/ bird	Body Gain Gm/ bird	Feed Intake gm/ bird	F.C.R
(1)Control	51± 5.4	26± 0.98	47	1.8	145 ± 3.8	94± 2.87	175	1.86
(2) Inf. non treated	52± 1.04	28± 0.07	44	1.78	120±2.0***	68± 3.96 ***	136	2.00
(3) Inf.treat 5 mg for 3 days	51± 1.30	25± 0.84	46	1.84	140± 0.7*	89± 0.72	170	1.91
(4) Inf .treat 10 mg for 5 days	53 ± 1.27	29± 0.93	51	1.75	153± 0.4	100± 1.0	182	1.82
(5) Inf .treat.10 mg for 3 days	50 ± 1.30	26± 0.69	46	1.76	146± 1.5	96± 1.73	174	1.83
(6) Inf .treat – 10 mg for 5 days	52± 1.31	27± 86	48	1.77	155± 1.8	103± 1.1*	185	1.8

*p < 0.05 **p < 0.01 ***p < 0.001 # Start of infection and treatment

Table 4.Effect of oral administration of peflioxacin at a dose levels of 5 and 10 mg / kg. b . wt for 3 and 5 successive days on some biochemical parameters of *salmonella* infected Balady chicks n = 50

Parameter / Group	A S T (u/ L)	A L T (u / L)	Total protein (gm / dl)	albumin (gm / dl)	Globulin (gm / dl)	Uric acid (mg/ dl)	Creatinine (mg/ dl)	Calcium (mg/ dl)	Inorganic Phosphorus (mg/ dl)
(1)Control	93 ± 5.72	12 ± 1.2	3.34 ± 0.15	1.35 ± 0.12	1.89 ± 0.14	3.8 ± 0.38	0.64 ± 0.09	11.18 ±0.41	8.11± 0.48
(2) Inf. nontreat	146± 8.34	31± 2.5	3.96± 0.06	0.80± 0.03	2.18± 0.42	7.94± 0.36***	0.81± 0.91*	8.5± 0.48**	13.1± 0.46**
(3) Inf. treat 5 mg for 3 day	112± 9.46*	19± 1.8*	3.25± 0.22	1.34± 0.04	1.91± 0.32	5.13± 0.45*	0.56± 0.07	10.0± 0.69	10.28± 0.68
(4) Inf. Treat 5 mg for 5 day	89± 3.62	10.6± 2.49	3.29± 0.12	1.27± 0.27	1.92± 0.32	4.1± 0.62	0.51± 0.06	10.5± 0.69	9.13± 1.89
(5)Inf. treat 10mg for 3 day	112± 12.37*	17± 0.69	3.01± 0.37	1.18± 0.25	1.93± 0.18	4.95± 0.72	0.56± 0.20	11.1± 0.92	7.96± 0.93
(6) Inf.treat 10mg for 5 day	95.25± 6.59	11± 2.56	3.03± 0.26	1.29± 0.13	1.84± 0.27	4.23± 0.92	0.54± 0.12	11.13± 0.98	8.85± 0.86

*p < 0.05 **p < 0.01 ***p < 0.00

DISCUSSION

Avian Salmonellosis is a problem of economic concern to all phases of the poultry industry from production to marketing *(21)*.

Bacteriological examination of 110 diseased and dead Balady chicks aged 1-30 days- old collected from Sharkia province revealed 6 Salmonella isolates with isolation rate (5.5 %). These isolates were identified into two *Sal. enteritidis*, one *Sal. typhimurium* and three unidentified isolates. These finding were similar to the results were obtained *(22)*.

Antibiogram of isolated *Sal. enteritidis* to pefloxacin and other commonly used antimicrobials was performed. The results obtaind indicated that the isolated *Sal. enteritidis* was highly sensitive to pefloxacin. These results are constant with the previously cited result ***(23)***. Which reporting that Salmonella spp. showed 100% sensitivity to pefloxacin. Also, other members of quinolone group are still effective in inhibiting Salmonella infection, several investigators ***(24,25)*** reported that ciprofloxacin, enrofloxacin, norfloxacin were 100% active against all tested Salmonella isolates.

In addition, the MIC of pefloxacin was determined as 0.3 ug ml as reported by ***Sharma, et al (26)***.

The clinical signs observed on the infected and non-treated chicks were depression, loss of appetite, pasty vents and loss of body weight. Similar symptoms were previously recorded ***(24)***.

The treatment of infected chicks with pefloxacin at a dose levels of 5 mg and 10 mg/ k g.b.wt. for 3 successive days partly reduced clinical signs and decreased mortality rate from 38 % to 6 % and 2 % respectively. Meanwhile using of pefloxacin at a dose levels of 5 mg and 10 mg /kg b.wt for 5 successive days ameliorate clinical symptoms and reduce mortality rate to 2 %. These finding indicating the effectiveness of pefloxacin in both treatment and prevention of Salmonella infection. Numerous reports have indicated that the effectiveness of pefloxacin in the treatment of Salmonella infection ***(6,27)***.

The authors found that all new quinalone derivatives including pefloxacin and ciprofloxacin were very active against all of the Salmonella strains. In addition, trails for reisolation of *Sal. enteritidis*. from infected and treated chicks when compared with infected non treated group confirmed the efficacy of pefloxacin in treatment of avian Salmonellosis.

These results are consistent with that reported by several investigators ***(25, 28 ,29).*** The authors reported that no Salmonella was found in feces or cloacal swabs after fluoroquinolone therapy.

The obtained results revealed that the administration of pefloxacin in a dose level of 5 and 10 mg / 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 pefloxacin at a dose level of 5 and 10 mg/ kg. b. wt for 3 successive days partly improved the performance of chicks infected with Salmonella.

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 ***(2)***. On the other hand the body gain of chicks infected with Salmonella increased after treatment with pefloxacin compared with infected non treated chicks ***(30)***. The improvement of body gain in infected and treated chicks might be attributed to bactericidial effect of drug on the infection and consequently improved the general health condition ***(4,31).*** These results provide a further reason for efficacy of pefloxacin in control of Salmonella infection.

Biochemical analysis of serum from chicks infected with *Sal. enteritidis* showed that serum AST, ALT and globulins were significantly ($p<0.05$) increased. These parameters were improved towards the normal levels as a result of treatment of infected chicks with pefloxacin.

The increase in serum ASTand ALT activity after infection suggest a hepatocellular damage ***(32).***

Treatment with pefloxacin at doses level of 5 mg and 10mg / kg. b. wt ,for 5 successive days displayed non significant changes in serum AST, ALT as well as total protein and albumin. Recent investigation recorded similar results ***(33, 34, 35).*** The authors mentioned that pefloxacin had no adverse effect on liver enzymes, where there was a raise with high dose of pefloxacin treatment 1- day post treatment and recur to

normal 7- days after pefloxacin treatment in chicks.

On the contrary the administration of pefloxacin at a dose levels of 5 mg and 10 mg. kg .b . wt. for 3 successive days showed insignificant increase in liver enzymes and failed to return to normal levels indicating that the period of treatment is not enough for full cure.

Creatinine and uric acid levels were significantly increased in infected non-treated chicks with Salmonella. This finding was disagreed with the results obtained by ***Abdallah (2)*** .Infected chicks with Sal and treated with pefloxacin for 5 successive day showed significant decrease in creatinine and uric acid levels which returned to normal levels .These results coincides with ***(36)*** who reported that fleroxacin had no significant nephrotoxicity in rats. The treatment with pefloxacin for 3 successive days resulting significant decrease in creatinine and uric acid in comparison with infected, non treated chicks but is failed in returning to the normal levels.

The hypocalcaemia in the infected non-treated chicks may be attributed to decrease calcium reabsorption from damaged renal tubules ***(37)***. In the same time the hyperphosphataemia 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 chicks treated with pefloxacin in all experimental chicks. A significant fluctuations within the physiological ranges in levels of plasma calcium and inorganic phosphorous in chickens treated with pefloxacin was recorded previously ***(38)***.

Finally it could be concluded that the medication of *Sal. enteritidis* infected Balady chicks at a dose of 5 mg and 10 mg /kg .b.wt. for 5 successive days has a superior activity and efficacy than administration for 3 successive days .

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الملخص العربى

كفاءة بفلو كساسين ضد السالمونيلا فى الكتاكيت البلدى

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معهد بحوث صحة الحيوان بالزقازيق

عقار بفلو كساسين أحد المضادات البكتيرية الحديثة لمجموعة الفلوروكينولون فى مجال الدواجن وفى هذه الدراسة تم عزل ٦ معزولات بكترية من ميكروب السالمونيلا من عدد ١١٠ كتكوت بلدى مريض عمرها ١- ٣٠يوم بنسبة ٥,٥ % حضرت إلى المعمل للتشخيص.

وبعمل اختيار الحساسية لميكروب السالمونيلا انتراتيديس المعزول من الدجاج المريض لعقار بفلو كساسين وبعض مضادات الميكروبات شائعة الاستخدام اتضح كفاءته العالية كذلك تم تحديد الحد الأدنى القاتل من الدواء للميكروب وجد أنه ٠,٣ ميكروجرام لكل ملى .

وبالإضافة لما سبق فقد تم تقييم فاعلية الدواء فى الدجاج البلدى المصاب صناعيا بميكروب السالمونيلا وذلك بتقسيم عدد ٣٠٠ كتكوت بلدى إلى ستة مجموعات متساوية على النحو التالى:-

المجموعة الأولى: مجموعة ضابطة سليمة وغير معالجة.

المجموعة الثانية: كتاكيت مصابة وغير معالجة.

المجموعة الثالثة: كتاكيت مصابة ومعالجة بعقار بفلو كساسين بمياه الشرب بمعدل ٥ ملجم / ك وزن حى لمدة ٣ أيام متتالية .

المجموعة الرابعة: كتاكيت مصابة ومعالجة بعقار بفلو كساسين بمياه الشرب بمعدل ٥ ملجم / ك وزن حى لمدة ٥ أيام متتالية

المجموعة الخامسة: كتاكيت مصابة ومعالجة بعقار بفلو كساسين بمياه الشرب بمعدل ١٠ مليجرام / ك وزن حى لمدة ٣ أيام متتالية.

المجموعة السادسة: كتاكيت مصابة ومعالجة بعقار بفلو كساسين بمياه الشرب بمعدل ١٠ مليجرام /ك وزن حى لمدة ٥ أيام متتالية.

واستنادا إلى الأعراض الظاهرة للمرض – ونسبة النفوق وإعادة عزل الميكروب من الكتاكيت المصابة الغير معالجة والمعالجة ومقارنتها بالكتاكيت السليمة الغير معالجة بالإضافة إلى الوزن المكتسب يمكن الحكم بكفاءة عقار بفلو كساسين بالجرعة ٥ مليجرام او ١٠ ملجم / ك وزن حى لمدة ٥ أيام متتالية.

علاوة على ذلك فقد لوحظ ارتفاع معنوى فى أنزيمات الكبد والكلى والجلوبيولين والفوسفور الغير عضوى فى مصل الدجاج المصاب فى الكتاكيت المصابة والغير معالجة بينما تحسن معدل هذه القيم فى الكتاكيت المصابة والتى تم علاجها لمدة ٥ أيام.

وخلاصة القول أن هذه النتائج أثبتت كفاءة عالية لعقار بفلو كساسين على العدوى المعملية بميكروب السالونيلا بالجرعة ٥ ملى ١٠ملجم / ك وزن حى شريطة أن يستمر العلاج لمدة لا تقل عن ٥ أيام. وبهذه الطريقة يمكن التحكم وعلاج ميكروب السالمونيلا أنتراتيديس فى الدواجن.