Concurrent use of ciprofloxacin and metronidazole for controlling of some bacterial infections in broiler chickens

Sayed A. Abdel Ziz[1], Sabry M.A. Abdel Motaal[1], Osama E. Abd-Allah[2] and Marwa M.I. Sarhan[2]

[1] *Pharmacology Dept., Faculty of Vet. Med. Zagazig University*
[2] *Animal Health Research Institute, Zagazig Branch*

ABSTRACT

This study was carried out to investigate the efficacy of ciprofloxacin (8 mg/kg b.wt.) and/or metronidazole (30 mg/kg. b.wt.) in drinking water for 5 successive days for treatment of *E. coli* and/or *Cl. perfringens* each alone or both. Blood and tissue samples were collected from 5 birds from each group on the 5th, 12th and 19th days post-infection and treatment. This was done through studying the effect on growth Performance (body weight, weight gain, feed consumption and feed conversion rate), lesion score, mortality rate, some liver and kidney function parameters and some blood picture parameters. Infection with *E. coli* and/or *Cl. perfringens* induced the characteristic symptoms and lesions of the disease infection with *E. coli* induced mortality rate up to 20% which is reduced to 5% post- treatment with ciprofloxacin whereas, *Cl. perfringens* infection induced mortality up to 35% which is reduced to 12% post-treatment with metronidazole. Mixed infection with *E. coli* and *Cl. perfringens* induced 75% mortality which is reduced to 20% post-treatment with both drugs. Infection with *E. coli* and/or *Cl. perfringens* each alone or mixed together afforded a significant decrease in body weight, weight gain, feed consumption and increased feed conversion compared with normal control group. Whereas, treatment of infected birds with both drugs elicited a significant increase in body weight, feed consumption and significant decrease in feed conversion rate compared with infected non-treated groups. On blood picture infection with *E. coli* and *Cl. perfringens* or their mixed infection induced a significant decrease in total RBCs count, Hb % and PCV% whereas, treatment of infected birds with each drug alone or their combination induced a significant increase in the previous parameters along the course of the study compared with infected non-treated groups. Serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), uric acid and creatinine were significantly elevated in response to infection with each microbe alone or their combination compared with normal control group. Treatment of infected birds with each drug alone or their combination elicited a significant decrease in the previous parameters along entire period of the study compared with the infected non-treated groups. Taken together, our data could be framed within the view of the revealing high efficacy of metronidazole and ciprofloxacin toward infection with clostridium and /or *E. coli* in poultry.

Keywords: ciprofloxacin, metronidazole, broiler, clostridium, E. *coli*

(http://www.bvmj.bu.edu.eg) **(BVMJ-31(2): 83-92, 2016)**

1. INTRODUCTION

In many areas of the world, one of the most common causes of broiler mortality and economic losses is the enteric diseases inflicted by a mixed infection with *Escherichia coli (E. coli)* and *clostridium infection*. Necrotic enteritis which is an important sporadic disease of broiler chicken is caused mainly by *Cl. perfringens,* (Parish, 1961). *Clostridium perfringens* is a Gram-positive spore forming anaerobic bacterium present in the intestinal flora of humans and animals as well as in soil and water (Florence et al., 2011). *E. coli* is the most important agent causing secondary bacterial infection in poultry and may also be a primary pathogen (Gross, 1994). Ciprofloxacin, a third generation flouroquinolone antimicrobial and has an excellent activity against Gram-negative bacterium including *E. coli* (Andersson and MacGowan, 2003). Metronidazole is a nitroimidazole antimicrobial amoebicidal and antiprotozoal medication used particularly for anaerobic bacteria and protozoa. It is the drug of choice for first episodes of mild to moderate clostridium infection, (Merck Manual for Professionals, 2009). Controlling of mixed infections by using combinations of more than one drug is being performed by several veterinarians in

field. These combinations are carried for their synergistic effect, treating mixed infection, preventing drug resistance from developing and treating severe infection, when the organism is unknown (Werk and Schneider, 1988).

The current study was delineated to explore the plausible cross talk between ciprofloxacin and metronidazole that could be portrayed as alterations of efficacy and/or side effects.

2. MATERIALS AND METHODS

2.1. Drugs:

Ciprofloxacin (Ciprofloxacin® 20%): It is water soluble formulation obtained from Arabcomed Company, Obour City Egypt it was administered as concentration of 5 ppm. in drinking water (8 mg/kg b.wt.) *(Anadon et al.,* 2001*).* Metronidazole (Flagyl®, Susp.): It is water soluble formulation obtained from Arabcomed Company, it was administered in drinking water (30 mg/kg b.wt.) (Cybulski et al., 1996).

2.2. Chickens:

A total of 350, day-old Hubbard breed broiler chicks obtained from El-Kahera Poultry Company (Giza, Egypt) were used in this study.

2.3. Ration:

It was obtained from El-Kahera Poultry Company and used during our study.

2.4. Avain pathogens:

Cl. perferingens type C was supplied by Animal Health Research Institute (Dokki, Giza, Egypt). *E. coli* serotype O_{78} was obtained from poultry and Rabbit Diseases Dept. Fac. of Vet. Med. Zagazig University (Zagazig, Egypt).

2.5. Vaccines:

Gumboro vaccine was obtained from Rhone–Merieau Company (France). Hitchner Lasota live vaccine were obtained from Intervet Boxmeer Company (Holland).

2.6. A) In Vitro antibacterial sensitivity:

The antibiotic sensitivity test of the tested *E. coli* against some antimicrobial agents using disc diffusion method was carried out according to Baker and Breach (1980) using Moeller –Hinton agar plates, the results were interpreted according to Oxoid Manual Company (Oxoid, 1995).

2.7. B) In Vivo: Experimental design:

A total of 350, day-old Hubberd mixed broiler chicks were randomly classified into 7 equal groups as follows: Group 1: Non-infected, non-medicated (Control). Group 2: Infected with *Cl. perfringens* type C, non-medicated. Group 3: Infected with *Cl. perfringens* type C, and treated with metronidazole (30 mg/kg b.wt.) for 5 successive days. Group 4: Infected with *E. coli* serotype O_{78}, non-medicated. Group 5: Infected with *E. coli* serotype O_{78} and treated with ciprofloxacin (8 mg/kg b.wt.) for 5 successive days. Group 6: Infected with *Cl. perfringens* type C and *E. coli* serotype O_{78}, non-treated. Group 7: Infected with *Cl. perfringens* type C and *E. coli* serotype O_{78} and treated with combination of ciprofloxacin (8 mg/kg b.wt.) ml/L) and metronidazole (30 mg/kg b.wt.) for 5 successive days.

2.8. Pathogens infection:

2.8.1. E. coli O78.

It was obtained from Animal Health Research Institute. Zagazig, Egypt. Infection with *E. coli* strain O_{78} was done on 19th day of age by inoculating the chicks in the air sacs in between the 3rd and 4th ribs in the right side using special needle with 0.1 ml of concentration 10^6 c.f.u/ml (colony forming unit) (Akam and Ozkan, 1988).

2.8.2. Clostridium infection:

On the 15th, 17th and 19th days of age, chickens were given three oral inoculations of 2 ml, freshly prepared (24 hs.); Incubated thioglycolate broth culture of *Cl. perfringens* type C ($1.5x10^9$) organisms /ml on alternate days (Baha et al., 1997).

2.9. Drugs treatment:

Drugs were administered to birds one day post-infection in drinking water for five successive days. All chickens of each group were individually weighted at the beginning of experiment and weekly intervals, body weight gain, feed consumption, feed conversion rate was recorded.

2.10. Sampling:

2.10.1. Tissue sampling:

Samples were taken on the 25th, 32th and 39th days of age. Five birds from each group were scarified and specimens were collected from liver, kidney heart, lungs and intestine for histopathological examination.

2.10.2. Blood samples:

Five blood samples were collected from 5 birds of each group. Each blood sample was divided into two equal volumes in separate tubes, the first one was collected on EDTA (Coles, 1986) and used for haematological studies, [Total RBCs count. PCV% (Schalm, 1975) and Hb% (Lynch et al., 1969)] the second was collected in centrifuge tube, left to clot

and serum was carefully separated for serum biochemical studies.

2.11. Efficacy of the drugs:

2.11.1. Clinical symptoms:

Chickens infected with *Cl. perfringens* type C showed decreased feed consumption, ruffled feathers with bloody diarrhea (Sabina and Nicodemus, 2014). Chickens infected with *E. coli* serotype O_{78} showed decreased feed consumption followed by restlessness, standing about dejectedly with ruffled feathers labored rapid breathing, gasping and characteristic snacking (Sojka and Carnaghan, 1961).

2.11.2. Mortality rate:

The number of dead chicks in infected and treated groups was recorded and calculated as a percentage.

2.11.3. Lesion scores:

For evaluation of the efficacy of the tested drugs the method described by Amin and Jordan (1979) was performed. Chicks died during the experiments were necropsied on 5th, 12th and 19th days post treatment. Chicks were weighted, after that five chicks from each group were sacrificed and necropsied for detection of lesions of colibaillosis (air sacculitis, pericarditis and perihepatitis), *Cl. perfringens* (necrotic enteritis and hepatitis) were calculated as a percentage.

2.11.4. Body weight, weight gain and gain percent:

Chicks of each group were individually marked and weighed prior to infection and treatment and also weighted weekly at the 5th, 12th and 19th days post treatment). The gain in the body weight was calculated for each group from beginning and at end of at the 5th, 12th and 19th days. Gain percent was calculated.

2.11.5. Feed consumption and feed conversion rate:

Feed consumption and feed conversion rate for all groups were calculated weekly. The conversion rate was calculated as follows:

$$\text{Feed conversion rate (F.C.R.)} = \frac{\text{Feed consumption(g) period}}{\text{Weight gain (g) period}}$$

Some liver function parameters as serum transaminases (AST), (ALT) (Reitman and Frankel, 1957) and (ALP) (Belfield and Goldberg, 1971) Kidney function parameters as estimation of serum uric acid (Henry et al., 1957), serum creatinine (Husdan and Rapoport, 1968) were evaluated.

2.12. Statistical analysis:

The obtained data were statistically analyzed using one way ANOVA test on the bases of (Snedecor and Cochran, 1982).

3. RESULTS

Table (1) show the effect of oral administration of ciprofloxacin (8 mg/kg b.wt.) and Metronidazole (30 mg/kg) for 5 consecutive days to broiler chickens experimentally infected with *E. coli* O_{78} and Clostridium perfringens on mortalities % and lesion scores (A) and Sensitivity test for different antibiotics against *E. coli* and clostridium organisms (B). Effect of oral administration of ciprofloxacin and metronidazole given for 5 consecutive days on the Body weight, Body weight gain, Feed consumption (F.C) and Feed conversion rate (F.C.R.) of control and experimentally infected chickens with *Clostridium perfringens* type C and *E. coli are showed in* Table (2). Table (3) illustrate the effect of oral administration of ciprofloxacin (10 mg/kg B.wt.) and metronidazole (50 mg/Kg b.wt.) for 5 consecutive days on total RBCs count, Hb content, and PCV% in healthy and experimentally infected chicken with *Clostridium perfringens* type C and *E. coli*. Changes in serum AST, ALT, ALP, Uric acid and Creatinine of control and experimentally infected chickens with *Clostridium perfringens* type C and *E. coli* after oral administration of ciprofloxacin and metronidazole given for 5 consecutive days on are illustrated in table 4.

4. DISCUSSION

Ciprofloxacin is one of the second generation of quinolones with a wide spread application to control animals diseases. It has a rapid bactericidal activity against a broad spectrum of bacteria, Mycoplasma chlamydia and some Mycobacteria (Ridgway et al., 1984).

Metronidazole, a synthetic antimicrobial agent, is effective against Gr-ve, Gr+ve, anaerobic bacteria and some protozoans. It is the drug of choice for treatment of parasitic and protozoal disorders (Seddiek et al., 2014).

The present study was carried out to evaluate the efficacy of ciprofloxacin (8 mg/kg b.wt.), Metronidazole (30 mg/kg b.wt.) and their combination for 5 consecutive days in drinking water on *E. coli* O_{78} or *Cl. perfringens* each alone and their mixed infection for controlling such infection beside their effect on body performance as well as studying the effects of infections and drugs on some haematological, liver and kidney function parameters.

Table (1): The effect of oral administration of ciprofloxacin (8 mg/kg b.wt.) and Metronidazole (30 mg/kg) for 5 consecutive days to broiler chickens experimentally infected with *E. coli* O_{78} and Clostridium perfringens on mortalities % and lesion scores (A) and Sensitivity test for different antibiotics against *E. coli* and clostridium organisms (B). (n=10)

Groups	A						B		
	Mortality rate %	Lesion scores (%)					Sensitivity test of different of antibiotics against *E. coli* and clostridium organisms		
		Air sacullitis	Pericarditis	Peri-hepatitis	Necrotic enteritis	Liver (hepatitis)			
Normal Control	0	0	0	0	0	0	Antibiotic	Disc Conc. (mg)	Zone of inhibition (mm)
Cl. perfringens Infected non-medicated	30	0	0	0	80	50			
Cl. perfringens Infected metronidazole treated	10	0	0	0	20	20	Enrofloxacin	10	25±1.2
E. coli Infected non-treated	20	40	20	30	0	0	Ciprofloxacin	5	28±1.8
E. coli Infected ciprofloxacin treated	10	10	10	10	0	0	Amoxicillin	30	23±0.8
Cl. perfringes and *E. coli* Infected non-treated	70	50	20	60	90	70	Norfloxacin	10	28±1.5
Cl. perfringes and *E. coli* Infected treated with metronidazole+Ciprofloxacin	20	20	10	10	30	20	Metronidazole	15	25±1.6

Means carrying different superscripts in the same column are significant at $P<0.05$

Table (2): Effect of oral administration of ciprofloxacin and metronidazole given for 5 consecutive days on the Body weight, Body weight gain, Feed consumption (F.C) and Feed conversion rate (F.C.R.) of control and experimentally infected chickens with *Clostridium perfringens* type C and *E. coli*. (M+SE, n=5).

Groups	Time post treatment (days)											
	Body weight (gm)			Body weight gain (gm)			F.C. (gm)	F.C.R.	F.C. (gm)	F.C.R.	F.C. (gm)	F.C.R.
	5th	12th	19th	5th	12th	19th	5th		12th		19th	
Normal Control	810.2 ±6.5 ^{a}	1220.23 ±10.36 ^{a}	1683.95 ±14.95 ^{a}	299.68 +9.65 ^{a}	410.03 +13.61 ^{a}	463.72 +12.63 ^{a}	422.55 ±10.2 ^{a}	1.41 ±0.08 ^{b}	672.44 ±15.8 ^{a}	1.64 ±0.06 ^{b}	802.24 ±10.66 ^{b}	1.73 ±0.1 ^{b}
Cl. perfringens Infected non-medicated	730.62 ±9.62 ^{b}	995.59 ± 6.30 ^{b}	1168.62 ±14.83 ^{bc}	205.21 +8.20 ^{b}	264.97 +10.16 ^{b}	173.03 +6.41 ^{c}	359.12 ±15.6 ^{b}	1.75 ±0.12 ^{a}	506.09 ±20.2 ^{b}	1.91 ±0.1 ^{a}	362.06 ±8.39 ^{c}	2.10 ±0.08 ^{a}
Cl. perfringens Infected metronidazole treated	800.05 ±12.83 ^{a}	1290.15 ±19.63 ^{a}	1700.73 ±15.95 ^{a}	264.56 +9.21 ^{ab}	460.10 +20.53 ^{a}	509.85 +14.63 ^{a}	431.23 ±20.1 ^{a}	1.63 ±0.065 ^{a}	628.06 ±12.79 ^{a}	1.61 ±0.05 ^{b}	907.53 ±15.78 ^{a}	1.76 ±0.75 ^{b}
E. coli Infected non-treated	742.58 ±9.36 ^{b}	1006.63 ±7.63 ^{b}	1200.95 ±8.94 ^{b}	216.93 +7.06 ^{b}	264.05 +8.16 ^{b}	194.32 +9.80 ^{c}	377.45 ±6.85 ^{b}	1.74 ±0.11 ^{a}	501.69 ±18.6 ^{b}	1.90 ±0.08 ^{a}	410.01 ±10.8 ^{c}	2.11 ±0.1 ^{a}
E. coli Infected ciprofloxacin treated	809.15 ±9.51 ^{a}	1210.42 ±22.25 ^{a}	1684.63 ±19.63 ^{a}	278.53 +9.20 ^{a}	401.27 +12.92 ^{a}	474.21 +16.52 ^{a}	442.86 ±8.23 ^{a}	1.59 ±0.06 ^{ab}	650.05 ±22.5 ^{a}	1.62 ±0.1 ^{b}	858.32 ±16.85 ^{a}	1.81 ±0.1 ^{b}
Cl. perfringes and *E. coli* Infected non-treated	670.16 ±10.61 ^{bc}	900.84 ±5.84 ^{b}	950.73 ±9.93 ^{c}	140.16 +7.20 ^{c}	230.68 +11.63 ^{b}	49.89 +15.30 ^{d}	246.68 ±10.66 ^{c}	1.76 ±0.15 ^{a}	442.90 ±12.65 ^{c}	1.92 ±0.08 ^{a}	109.76 ±5.5 ^{d}	2.20 ±0.12 ^{a}
Cl. perfringes and *E. coli* Infected treated with metronidazole+Ciprofloxacin	785.23 ±9.14 ^{a}	990.36 ±14.17 ^{a}	1220.93 ±18.95 ^{b}	275.85 +11.10 ^{a}	294.8 +8.30 ^{a}	430.57 +19.82 ^{b}	460.66 ±10.4 ^{a}	1.67 ±10.4 ^{a}	507.00 ±15.95 ^{b}	1.22 ±0.09 ^{c}	415.03 ±9.59 ^{c}	1.80 ±0.15 ^{b}

Table (3): Effect of oral administration of ciprofloxacin (10 mg/kg B.wt.) and metronidazole (50 mg/Kg b.wt.) for 5 consecutive days on total RBCs count, Hb content, and PCV% in healthy and experimentally infected chicken with *Clostridium perfringens* type C and *E. coli* . (Mean ± S.E., n = 5).

Groups	Time post drug administration								
	Total RBCs count (10^6/mm^3)			Hemoglobin content (gm/dl)			Packed Cell Volume (PCV%)		
	5th day	10th day	20th day	5th day	10th day	20th day	5th day	10th day	20th day
Normal Control	2.82± 0.08 a	2.63± 0.07 a	2.91± 0.09 a	10.12± 0.19 a	10.11±0.17 a	10.11±0.18 a	28.51±0.50 a	29.40±0.40 a	29.51±0.51 a
Cl. perfringens Infected non-medicated	2.01±0.08 b	1.85±0.07 bc	1.85±0.09 b	8.01±0.19 b	8.09±0.17 b	8.10± 0.28 b	25.01±0.24 b	26.01± 0.23 b	24.02±0.99 bc
Cl. perfringens Infected metronidazole treated	2.03± 0.27 b	2.02±0.08 ab	2.85± 0.28 a	9.00± 0.32 b	9.81± 0.29 a	9.95± 0.32 a	18.61±0.22 b	18.98±0.78 c	26.44± 0.32 b
E. coli Infected non-treated	2.10±0.19 b	1.99±0.10 b	2.00±0.20 b	8.29±0.50 bc	8.17±0.31 b	8.18±0.38 b	26.70±0.24 a	27.70±0.21 b	24.92±0.54 b
E. coli Infected ciprofloxacin treated	2.69± 0.08 a	2.65± 0.09 a	2.87± 0.09 a	9.13± 0.25 b	9.12± 0.22 a	9.28± 0.28 a	26.70± 0.22 a	28.21± 0.34 a	29.49± 0.54 a
Cl. perfringes and *E. coli* Infected non-treated	2.00±0.12 b	1.80±0.21 bc	1.86±0.24 b	7.98±0.29 bc	7.82±0.28 b	7.94±0.48 b	25.18±0.19 b	25.17±0.88 b	24.18±0.99 bc
Cl. perfringes and *E. coli* Infected treated with metronidazole+Ciprofloxacin	2.26±0.08 b	2.61± 0.07 a	2.89± 0.11 a	9.10± 0.21 b	9.15± 0.11 a	9.63± 0.21 a	27.11± 0.25 a	29.20± 0.24 a	29.01± 0.24 a

Table (4): Effect of oral administration of ciprofloxacin and metronidazole given for 5 consecutive days on serum AST, ALT, ALP, Uric acid and Creatinine of control and experimentally infected chickens with *Clostridium perfringens* type C and *E. coli*. (M±SE, n=5).

Groups	Time post treatment (days)														
	Serum (AST) (IU /L)			Serum (ALT) (IU/L)			Serum (ALP) kind and king U/100 ml			Serum Uric acid (mg/d)			Serum Creatinine (mg/d)		
	5th	12th	19th	5th	12th	19th	5th	12th	19th	5th	12th	19th	5th	12th	19th
Normal Control	36.7 ± 0.56 c	36.7 ± 1.76 b	36.7 ±1.76 bc	26.43 ± 1.36 b	26.43 ± 1.36 c	26.43 ± 1.36 c	145 ± 3.2 c	153.00 ±3.60 b	156 ±0.3 a	1.68 ± 0.08 c	1.68± 0.08 d	1.68± 0.08 b	0.95± 0.07 bc	0.95 ± 0.07 b	0.95± 0.07 b
Cl. perfringens Infected non-medicated	50.73 ±3.8 a	50.81 ±2.9 a	51.9 ± 3.3 a	40.41 ±0.87 a	33.41 ±0.89 ab	35.01 ±1.12 a	164.3 ±1.5 a	171.1 ±1.1 a	158 ±4.2 a	3.01 ±0.36 a	2.99± 0.13 a	2.08± 0.15 a	2.01± 0.09 a	1.90 ± 0.09 a	1.89± 0.08 a
Cl. perfringens Infected metronidazole treated	40.01 ±1.05 b	38.0 ± 1.90 b	37.0 3 ± 1.42 b	30.11 ±1.03 b	29.9 ±1.02 bc	28.8 ±1.51 bc	158.4 ± 2.3 b	168.2 ± 3.1 a	156.0 ± 4.9 a	2.01 ± 0.14 b	1.08± 0.14 c	1.79± 0.15 ab	1.00± 0.04 bc	1.00 ± 0.05 b	0.97± 0.05 b
E. coli Infected non-treated	51.84 ±3.84 a	49.8 ±3.45 a	52.8 ±3.80 a	39.73 ±1.42 a	34.74 ±1.42 ab	34.75 ±1.32 a	165.5 ±3.2 a	172.3 ±1.2 a	158 ± 5.1 a	2.99 ±0.09 a	2.89± 0.07 a	2.07± 0.08 a	1.99± 0.08 a	1.89 ± 0.09 a	1.88± 0.09 a
E. coli Infected ciprofloxacin treated	42.21 ±2.03 b	39.0 ± 2.91 b	38.2 ±2.62 b	33.05 ±1.16 b	38.05 ±1.18 a	29.08 ±1.58 ab	158.2 ± 2.4 b	169.1 ±3.5 a	159 ± 4.8 a	2.1 ± 0.12 b	1.99± 0.12 b	1.69± 0.13 b	1.35± 0.15 b	0.98 ± 0.16 b	0.96± 0.17 b
Cl. perfringes and *E. coli* Infected non-treated	54.69 ±4.09 a	52.68 ±3.8 a	51.51 ±3.9 a	39.92 ±1.62 a	37.21 ±1.51 a	36.03 ±1.52 a	169.8 ±1.9 a	171.00 ±3.1 a	157 ±4.3 a	2.97 ±0.34 a	2.90± 0.05 a	2.14± 0.25 a	1.89± 0.08 a	1.95 ± 0.04 a	1.95± 0.03 a
Cl. perfringes and *E. coli* Infected treated with metronidazole+Ciprofloxacin	34.04 ±0.92 c	37.21 ±1.62 b	36.90 ±1.22 b	34.04 ±1.32 ab	27.05 ±1.21 c	27.01 ±1.31 bc	158.5 ± 1.8 b	168.00 ± 3.2 b	160.1 ± 4.1 a	2.15 ± 0.14 b	2.18± 0.19 b	1.89± 0.18 ab	1.13± 0.03 bc	1.00± 0.02 b	1.00± 0.03 b

In the present study, the disc diffusion test was used to compare the antimicrobial activity of ciprofloxacin and metronidazole with other antimicrobial (amoxicillin, enrofloxacin & norfloxacin). Disc diffusion test revealed more potent inhibitory effect when using ciprofloxacin on *E. coli* (28±1.8 mm) and metronidazole on Cl. perferingens (25±1.6 mm) inhibition zones than other antimicrobial agents. This indicates superior activity of ciprofloxacin and metronidazole than other antimicrobial agents. Such finding was compatible with those reported by EL-Kadeem (2005) who found that *E. coli* was sensitive to ciprofloxacin. Our results are also in accordance with those recorded by Zeng et al. (1996) who found that metronidazole is very active against *Cl. perfringens*.

In the present study, it has been noticed that, the most common clinical signs appeared in chickens infected with *Cl. perfringens* were loss of appetite, depression, dropping wings, diarrhea, ruffled feathers dehydration emaciation and elicited gross pathological lesions (necrotic enteritis and liver hepatitis) and high mortality rate up to 30%. Same signs and gross pathological lesions were observed by Abdalla and Emam (2006) in broiler chickens infected with *Cl. perfringens*. Our findings were reinforced with those recorded by (Magdy et al., 2012), who recorded that, infection with *Cl. perfringens* in broiler chickens induced high mortality rate, which might be due to effect of bacterial toxins (Olkowski et al., 2006). Infection in chickens with *Cl. perfringens* is associated with mortality rates up to 50% (Mc Devit et al., 2006).

In this work, infected chickens with *Cl. perfringens* type C treated with metronidazole displayed no clinical signs of illness as they were healthy and viable allover the experiment beside decreased mortality rate to 10%. Our results are in agreement with Sameh et al. (2005) who stated that oral administration of metronidazole to chickens infected with *Cl. perfringens* led to disappearance of clinical signs and reduction of mortality rate.

Metronidazole was effective in treatment of *Cl. perfringens* infection and reduction in mortality rate (Sabina and Nicodemus, 2014).

Our results revealed that the main clinical signs that appeared on chickens experimentally infected with *E. coli* were loss of appetite, depression, respiratory symptoms including sneezing, gasping, mild conjunctivitis with frothy exudates in their eyes and diarrhea and mortality rate reaching to 20%. Moreover, *E. coli* infection elicited gross pathological lesions as air saculitis, pericarditis, perihepatitis and enteritis. The same signs and gross pathological lesions were observed by Barnes and Gross (1997) in chickens infected with E. coli. Our finding coordinates with those recorded by (Youessf et al., 1982) they reported that *E. coli* infection was associated with air saculitis, hepatitis; salpingitis and respiratory manifestation. Our results were similar to that recorded by Roushdy (2007) who observed that mortality rate due *E. coli* infection was high

The obtained results revealed that treatment of *E. coli* infected chickens with ciprofloxacin resulted in complete disappearance of clinical signs and improved the health status of infected chickens as evidenced in this study by reduction of mortally rate to 10% and gross pathological lesions. These recorded results were similar to these recorded by Shawky (2006) in chickens infected with *E. coli* and treated with ciprofloxacin.

The obtained results in this work revealed that chickens exposed to mixed infection (*E. coli* and *Cl. perfringens*) showed severe respiratory signs and severe diarrhea with air saculitis, pericarditis, perihepatitis, severe enteritis and increase in mortality rate to 70% besides presence of lesions (air saculitis, pericarditis, perihepatitis, necrotic enteritis and hepatitis). These clinical signs and post mortem lesions agreed with those reported by Mark (2004).

In this study, it is clear that chickens experimentally infected with *E. coli* and/or *Cl. perfringens* either alone or mixed infection revealed significant decrease in body weight, weight gain, feed consumption and increased feed conversion rate throughout the experimental period. These results might be attributed to intestinal damage caused by microorganisms as proved in the histopathological study. This previous assumption is supported by Sameh et al. (2005). They found that *Cl. Perfringens* infected chickens showed poor digestion, poor feed efficiency and poor weight gain. Reduction of weight gain and a concomitant adverse effect on feed conversion rate may be due to reduction of feed intake (Sarkar et al., 2013). Our result agrees with that obtained by El-Gharbawy (2014) who found that, broiler chickens infected with *Cl. perfringens* showed a significant decrease in body weight, weight gain and increase in feed conversion rate.

On the other hand, the body weight gain of chicks infected with *E. coli* and *Cl. Perfringens* either alone or mixed infection and treated with ciprofloxacin ant/or metronidazole in their recommended doses either alone or in combination showed non-significant decrease in body weight gain at 5^{th} day post administration accompanied by non-significant increase in body weight gain at 12^{th} and 19^{th} days post administration. This change may be due to bactericidal effect of drug improving the general health condition (Kamel, 2004). This result coordinated with the findings of Papich (2002) who reported that metronidazole was effective against *Cl. perfringens* infection. Also these findings were

reinforced by John et al. (2004), they found that, using another quinolone (enrofloxacin) in *E. coli* treatment in chickens displayed better weight gain and feed conversion rate. Infected chicks with *E. coli* treated with another quinolone (danofloxacin) improved body performance (Bryan et al., 1998).

The infection with *Cl. perfringens* and/or *E. coli* each alone or mixed infection in chickens revealed significant decrease in RBCs count, Hb and PCV % all over the experimental period. Reduction in erythrogram parameters caused by *E. coli* infection could be attributed to bacterial endotoxins which cause intravascular destruction of erythrocytic cells and consequently lead to hemolysis with breakdown of hemoglobin (Karaivanov L., 1984). The change in blood picture in infected broiler chickens agreed with (Dagmar et al., 2002) who stated that infected bacteria produces cell damaging protein toxin (hemolysin) that causes changes in cell membrane permeability and formation of surface lesions causing RBCs destruction. In addition, Tserenpuntag et al. (2005) stated that *E. coli* lipopolysaccharide has direct effect as it inhibits bone marrow cells and its nephrotoxicity decrease erythropoietin in blood.

Our results are compatible with Kadry et al. (2009) in chickens infected with *Cl. perfringens*. These results may be due to breakdown of phospholipids of erythrocytes membrane causing hemolysis, damaging circulating erythrocytes by Clostridial toxin (Opengart, 2008). Alpha toxin is a principal lethal toxin of *Cl. perfringens* and is a multifunctional phospholipase produced by nearly all isolates toxin is hemolytic, necrotizing and potently lethal. The hydrolytic action of toxin on membrane phospholipids of erythrocytes results in lysis (Songer, 1996).

Infected chicks with *Cl. perfringens* and *E. coli* and medicated by ciprofloxacin and metronidazole in their recommended doses either alone or in combination for 5 consecutive days showed significant increase in RBCs, Hb and PCV% all over of the course of the study compared with infected non-treated groups.

Our results coordinate with those recorded by EL-Kadeem (2005) who recorded that treatment of *E. coli* infected chickens with ciprofloxacin improved blood picture which is reverted to the control values. Our recorded results are also in accordance with those reported by (Sameh et al., 2005)who reported that using metronidazole in treatment of chickens infected with *Cl. perfringens* showed improved blood picture (RBCs, Hb and PCV%)

Infected chickens with *Cl. perfringens* and/or *E. coli* either alone or mixed infection showed significant increase in serum enzymes (AST, ALT and ALP) activities all over the experimental period. The obtained data coincides with the results of Campell and Coles (1986), they stated that ALT is mostly of hepatic origin and so their high level in the serum was indicative to liver damage. Also, the elevation of serum AST level post infection was mainly due to damage in liver cells (Halliwell, 1981).

Our results were supported by the results obtained by Mwafy (2000) who noticed that chickens infected with *E. coli* exhibited elevation in liver enzymes (AST, ALT and ALP) activities. Our results were in complete harmony with those reported by Joan and Pannel (1981), who stated that the infection produced alteration in cellular permeability due to changes in cell membrane which allows the escape of these enzymes into serum in abnormal high level. Our results are in accordance with Shawky (2006) who found that *E. coli* infection in chickens resulted in significant increase in liver enzymes (AST, ALT and ALP) activities.

These findings were supported by those previously obtained by Allam et al. (2013) who reported that *Cl. perfringens* infection in duckling induce significant increase in liver enzymes. This could be due to the destructive effect of *Cl. perfringens* and Clostridial toxins on the liver cells and these results were in accordance with Oda (2012). Elevation in liver enzymes were reported previously by Kadry et al. (2009) in chickens infected with *Cl. Perfringens*

Medication of *E. coli* and *Cl. perfringens* infected broiler chickens with ciprofloxacin and metronidazole in their tested doses either alone or in combination for 5 consecutive days induced significant increase in liver enzyme activities at 5th day post treatment with insignificant increase on 12th and 19th day post-treatment. Our data coincides with Oda (2012), who found that, broiler chicken infected with *Cl. perfringens* and treated with metronidazole showed elevation in serum levels of liver enzymes. Also, broiler chicken infected with *E. coli* treated with therapeutic doses of ciprofloxacin induced significant elevation in serum liver enzymes (AST, ALT and ALP) activities (EL-Kadeem, 2005).

Regarding the effect on serum uric acid and creatinine, the obtained results revealed that experimental infection of broiler chickens with *E. coli* and *Cl. perfringens* induced a significant increase in uric acid and creatinine levels all over the experimental period. Elevation in uric acid could be attributed to the degenerative changes in renal tubules preventing excretion of uric acid and creatinine leading to an increase in their levels in serum (Kaneko, 1980). These results are confirmed by the pathological changes as the kidneys showed degenerative changes in some tubules (cloudy swelling and hydropic degeneration).

Our results were in accordance with that obtained by Abdalla and Adayel (2006). They found that serum uric acid concentration in infected chickens with *E. coli* showed a significant increase. These results were in accordance with that obtained by (El-Sayed, 2007) she found that uric acid and creatinine concentrations were significantly increased in broiler chickens infected with *E. coli*.

Our results were supported by the results obtained by Halliwell (1981), who reported that *Cl. perfringens* infection in broiler chickens induced significant increase in uric acid and creatinine attributing this elevation to kidney damage induced by bacterial toxins. Increase in uric acid and creatinine were recorded by Kadry et al. (2009) in broiler chickens infected with *Cl. perfringens*.

In the current work, the administration of ciprofloxacin and/or metronidazole in their tested doses either alone or in combination for 5 consecutive days to *E. coli* and *Cl. perfringens* infected broiler chickens either alone or mixed infection produced a non-significant increase in serum uric acid and creatinine level along the entire period of the study compared with normal control group. This result was in accordance with that obtained by Sameh et al. (2005), who stated that uric acid and creatinine concentration was significantly increased only at 1st day post treatment in broiler chicken infected with *Cl. perfringens* and treated with metronidazole. On the same ground, Kadry et al. (2009) and Allam et al. (2013) reported that uric acid and creatinine concentrations were non-significantly increased post treatment of broiler chickens infected with *Cl. perfringens* using metronidazole. Compared with normal control group and a significant decrease compared with infected non-treated groups.

The combination of both drugs elicited the best treatment of mixed infection than each drug alone indicating synergistic effect. These results provide a further support for efficacy of two drugs in controlling mixed infection (Hooper and Wolfson, 1985).

5. REFERENCES

Abdalla, O., Adayel, S., 2006. Concurrent use of kanamycin and spiramycin for controlling chronic respiratory disease in broiler chicks, In: Sci. Vet. Med. Zag., Hurghada, pp. 556-563.

Abdalla, O., Emam, E., 2006. compatibility of diclazuril and lincomycin in broiler chickens. Zag. Vet. J. 33, 147-161.

Akam, A., Ozkan, A., 1988. Isolation and identification of causative agent of Escherichia coli infection, the pathological finding and antibiotic sensitivity test. etik veteriner Microbidoji dergisi 3, 25-32.

Allam, H., Nahad, A., Halla, S., Abdulla, S., Dina, M., 2013. Immu-nobiochemical and pathological Studies on Necrotic Enteritis in Pekin Duckling. Mansoura Vet. Med J. 13, 25-36.

Amin, M.M., Jordan, F.T.W., 1979. Infection of the Chicken with a Virulent or Avirulent Strain of Mycoplasma-Gallisepticum Alone and Together with Newcastle-Disease Virus or Escherichia-Coli or Both. Vet Microbiol 4, 35-45.

Anadon, A., Martinez-Larranaga, M.R., Iturbe, J., Martinez, M.A., Diaz, M.J., Frejo, M.T., Martinez, M., 2001. Pharmacokinetics and residues of ciprofloxacin and its metabolites in broiler chickens. Res Vet Sci 71, 101-109.

Andersson, M.I., MacGowan, A.P., 2003. Development of the quinolones. J Antimicrob Chemother 51 Suppl 1, 1-11.

Baha, I., Ikemoto, T., Juk, T., Sasaik, K., McDougo, H., 1997. Closteridial population and the intestinal lesions in chickens infected with clostridium perifringes and Eimeria necatrix. Vet. Microb. 54, 301-308.

Baker, F., Breach, M., 1980. Medical Microbiology Techniques. Bulter worth Co., London,UK.

Barnes, H., Gross, W., 1997. Colibacillosis in clank, B., Barnes H., Beard C. Mc Douglas, L., and Said Y. Disease of pultry, 10th ed, Ames. Iowa State Univ 138-144.

Belfield, A., Goldberg, D.M., 1971. Revised assay for serum phenyl phosphate activity using 4-amine–antipyrine. Enzyme 12, 561-573.

Bryan, C., John, J., Ingrid, A., Robrecht, F., 1998. Comparison of the efficacies of three fluoroquinolone, one of antimicrobial agents, given as continous or pulsed-water medication, against. E coli infection in chickens. Anti Agent & Chem. 42 83-87.

Campell, T., Coles, J., 1986. Avian clinical pathology in Vet. Clin. Path, 4th ed. Sunders Comp, Philadelphia, London and Tornto.

Coles, E.H., 1986. Veterinary clinical pathology, 4th ed. W.B. Sounders Company, Philadelphia, London, Toronto, Mexico, Tokyo, Hong King.

Cybulski, W., Larson, P., Starmer, G., 1996. Principles of veterinary therapeutic. long man singapore publisher (Pte) Ltd.

Dagmar, J., Muhsin, O., Ntondo, B., 2002. Production and characterization E coli enterohemolysin and its structure effects of erythrocyte membranes. Cell Biolo Inter 26, 75-86.

El-Gharbawy, E. 2014. Concurrent use of amoxicillin and metronidazol for controling closterdial problems in broiler chickensM.VSc., Monefia University.

EL-Kadeem, A. 2005. pharmacological studies of gentamicin and ciprofloxacin in colibacillosis in chickensM.VSc., Zag. Uni. .

El-Sayed, A.A. 2007. efficacy of spiramycin on colibacillosis in chickensM VSc, Zag. Univ.

Florence, L.C.H., Hakim, S.L., Kamaluddin, M.A., Thong, K.L., 2011. Determination of toxinotypes of environmental Clostridium perfringens by Polymerase Chain Reaction. Tropical Biomedicine 28, 171-174.

Gross, W.G., 1994. Diseases due to Escherichia coli in poultry in: Escherichia coli in domestic animals and humans C.L. Gyles, ed. CAB Internationa l, Walling ford UK 237-259.

Halliwell, W., 1981. Serum chemistry profiles in health study of rapter disease of birds. In Recent advances in the study of rapter disease, Ed by cooper J. and Green W, Chiron. Pub. Lit west, York Shire, England.

Henry, R., Cannon, D., Winkelman, J., 1957. Clinical Chemistry Principles and Technique, 2nd ed. Harper Publishers, Hagerstown.

Hooper, D.C., Wolfson, J.S., 1985. The Fluoroquinolones - Pharmacology, Clinical Uses, and Toxicities in Humans. Antimicrobial Agents and Chemotherapy 28, 716-721.

Husdan, H., Rapoport, A., 1968. Estimation of creatinine by the Jaffe reaction. A comparison of three methods. Clin Chem 14, 222-238.

Joan, F., Pannel, P., 1981. Clinical chemistry in diagnosis and treatment, 3rd ed. Llayed-luke, London.

John, R., Charles, H., Greg, M., 2004. Comparative efficacy of enrofloxacin, oxytetracycline, and sulfadimethoxine for the controlling of mortality and morbidity caused by Escherichia coli in broiler chickens. Avian diseases 48, 658-662.

Kadry, M., Halla, M., Nesreen, A., Aly, S., 2009. Evaluation of lincomycin as a therapy of cl perfringens infection in chickens. Benha Vet Med J 20, 1-10.

Kamel, M. 2004. Interaction between danofloxacin and fofluperdone acetate in chickensMVSc, Zag. Univ.

Kaneko, J., 1980. Clinical biochemistry of domestic animals, 4th ed. Academic press Inc., New York, London.

Karaivanov L., 1984. Somatic antigens of Pasteurella multocida strains. Vet. Med. Nauki 21, 12-16.

Lynch, M., Raphae, S., Inwood, M., 1969. Medical laboratory technology and clinical pathology, 2nd ed. W. Saunders Co., Philadelphia, London.

Magdy, F., Eman, R., Hassan, A., Radwan, S., N., Rady, M., 2012. Effect of probiotic on nerotic enteritis in chicken with presence of Immunosuppressive Factors. Global Vet. 9, 44-51.

Mark, E., 2004. BVSe, MACVS, regional technical manager swine, Alpharma pharmaceuticals (Ltd, 5th preecha Bullding, 25.33 Sukhumvit roads), Prakhanong 10260, Thailand.

Mc Devit, R., Broker, J., Sparks, N., 2006. Necrotic enteritis a continuing challenge for the poultry industry world's. pouit Sci. J. 62, 221-247.

Merck Manual for Professionals, 2009. http//www.merch.com/ mmpe/lexicomp/metronidazole.html.

Mwafy, R. 2000. A possible deleterious effects on danofloxacin in ratsMVSc, Zag. Unv.

Oda, S., 2012. Histopathological and biochemcal alterations of characteri-dazole induced toxicity in male rats. Global Vet. 9, 303-310.

Olkowski, A.A., Wojnarowicz, C., Chirino-Trejo, M., Drew, M.D., 2006. Responses of broiler chickens orally challenged with Clostridium perfringens isolated from field cases of necrotic enteritis. Research in Veterinary Science 81, 99-108.

Opengart, K., 2008. Necrotic enteritis In: Y. Saif, Editor, Diseases of poultry. Disease of poultry, 12th ed. Black well.

Oxoid, 1995. 7th Ed. Unipath Limited Wade Roud. Bashing Stocke Hampshire RG, 24-spw.

Papich, M., 2002. Hand Book of Veterinary Drugs. Saunders Comp, Philadelphia.

Parish, W.E., 1961. Necrotic enteritis in the fowl. I. Histopathology of the examination of the causal clostridium perfringens. II. The experimental diseases. J. Comp. Pathol. 71, 377-404.

Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 28, 56-63.

Ridgway, G.L., Mumtaz, G., Gabriel, F.G., Oriel, J.D., 1984. The Activity of Ciprofloxacin and Other 4-Quinolones against Chlamydia-Trachomatis and Mycoplasmas Invitro. European Journal of Clinical Microbiology & Infectious Diseases 3, 344-346.

Roushdy, M. 2007. Some pharmacological studies on pefloxacin in chickensMVSc, Zag Unv.

Sabina, M., Nicodemus, M., 2014. Clostridium: Pathogenic roles, industrial uses medicinal prospect natural product as ameliorative agents against pathogenic spp. Jordan J. of Biol Sci. 7, 81 -94.

Sameh, M., Nasser, A., Gehan, G., 2005. Efficacy of metronidazole and probiotic in Clostridium perferingens infection in chickens. 4th Int. Sci. Conf. Masoura, 1393 -1205.

Sarkar, J., Ray, S., Mukhopadhayay, D., Niyogi, S., 2013. Study on Clostridium perferingens Type A infection in broiler of west Bengal. India IIOABJ 4, 1–3.

Schalm, O., 1975. Veterinary Haematology Ed, Bailliere, 3rd ed. Tindall and Cassel Ltd., London.

Seddiek, A., Mohamed, M., Hanem, F., Ali, M., 2014. Antitricho-monal efficacy of metronidazole against trichomonas gallinae infecting domestic pigeons. Parasitology Res 113, 319-329.

Shawky, N.A. 2006. Antibacterial efficacy of cefoperazone and its combination with sulbactam in chickensPh.D, Zag. Uni. .

Snedecor, G.W., Cochran, W.G., 1982. statistical methods, 8th ed. The Iowa State University, Press, Ames, Iowa, USA.

Sojka, W., Carnaghan, R., 1961. Escherichia coli infection in poultry. Res. Vet. Sci. 2, 340-350.

Songer, J., 1996. Clostridial enteric diseases in domestic animlal clinical microbiol. Reviews, 216-234.

Tserenpuntag, B., Chang, H., Morse, D., 2005. Hemolytic uremic syndrome risk and E. coli O157:H7. Infect Dis 11, 955-957.

Werk, R., Schneider, L., 1988. Infection 16 (1988) Nr.4© MM medizin veriag GmbH Munchen, Munchen.

Youessf, Y., Awaad, M., Hamoda, A. 1982. Antibiotic outbreak of coli septiemia in chicks caused by E. coli, Cairo University.

Zeng, Z., Liu, Y., Sun, Y., 1996. Efficacy of ofloxacin against colibacillosis in chickens. Chinese J. Vet. Sci. 16, 470-473.