Pharmacokinetics aspects and tissue residues of Marbofloxacin in healthy and *Mycoplasma gallisepticum*–infected chickens

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Abstract

The pharmacokinetics of marbofloxacin in healthy and *Mycoplasma gallisepticum* - experimentally infected broiler chickens was investigated after single intravenous (I.V.) and oral administrations at a dose of 5 mg/kg body weight. Tissue residues of marbofloxacin in healthy chickens after three successive daily oral doses were also determined. Marbofloxacin concentrations were determined in Plasma and tissue by using high-performance liquid chromatography. Plasma concentrations of marbofloxacin were significantly higher in healthy than in infected chickens. In healthy chickens, marbofloxacin was eliminated slower ($t_{1/2\beta}$, 6.8 ± 0.34h) than in infected chickens (5.66±0.33 h).The mean residence time was 9.67±0.5 h in healthy *vs*

8.03±0.45 h in infected chickens. After oral administration the drug achieved its maximum plasma concentrations (C_{max}) of 1.37±0.08 µg/ml at maximum time (t_{max}) of 1.95±0.04 h in healthy and C_{max} 0.95±0.02 µg/ml at t_{max} of 2.08±0.05 in diseased chickens. In conclusion marbofloxacin at dose of 5 mg/kg administered intravenously or orally at 24 h intervals may provide successful treatment of chicken infected with *Mycoplasma gallisepticum*. Based on the tissue concentration in edible organs a 5 days withdrawal time is suggested.

Key words: Marbofloxacin, Pharmacokinetics, *M. gallisepticum*, tissue residues, HPLC

1. Introduction

Marbofloxacin is a broad-spectrum fluoroquinolone of the 3rd generation. It is developed for veterinary use only (Martinez et al., 2006).. Marbofloxacin has been used in the treatment of diseases caused by various microorganisms such as *Staphylococcus*, *Pseudomonas spp*. (Martín Barasa et al., 2000 and Anadón et al., 2002), *Escherichia coli, Enterobacter spp*, (Cotard et al., 1995), *Mycoplasma hyopneumoniae* and *Pasteurella multocida* (Dossin et al., 1998 and Thomas et al., 2000). The pharmacokinetic properties of marbofloxacin have been studied in several avian species such as Japanese quails and common pheasants (Haritova et al., 2013, Lashev et al., 2015), mallard ducks (Garcia-Montijano et al., 2012), adult

Eurassian Griffon vulture (Garcia-Montijano et al., 2011), Muscovy ducks (Goudah and Mouneir, 2009), turkeys (Haritova et al., 2006), ostriches (de Lucas et al .,2005) and Eurasian buzzards (Garcia-Montijano et al., 2001). In spite of some published investigating the pharmacokinetics of marbofloxacin in healthy and *M. gallisepticum*-infected broiler chickens (Anadón et al., 2002; Ding et al., 2013), little information about pharmacokinetics and bioavailability of marbofloxacin after oral administration in *M. gallisepticum* experimentally infected chickens are available. Most strains of *M. gallisepticum* are highly sensitive to fluoroquinolones of which marbofloxacin has some advantages over other members, such as a longer elimination half-life and higher serum concentrations (Haritova et al., 2006). Accordingly, the main purpose of this study is to compare the pharmacokinetics in healthy and *M. gallisepticum*-experimentally infected chickens as well as to determine its tissue residues and withdrawal time in healthy broiler chickens.

2. Material and methods

Marbofloxacin (Marbocyl ®; Vetoquinol) 10% injectable aqueous solution purchased from Veterinary Pharmaceutical Laboratory, Lure, France, was used for i.v. treatment. The same sterile solution was diluted with sterile pyrogen-free water to 1% weight /volume (w/v) and then used for oral administration (Haritova et al., 2006).

2.1. Mycoplasma gallisepticum strain:

Mycoplasma gallisepticum field isolate strain (EIS-C3-09) was obtained from Mycoplasma department, Animal Health Research Institute, Dokki, Giza, Egypt is used for experimental infection. The virulence of the used strain was increased by passage via yolk sac of SPF eggs and the yolk and allantois fluid were collected and used for inoculation (Yoder, 1984).

2.2. Chickens

Forty-eight apparently healthy Mycoplasma free chickens of 1.5±0.2kg were used. Chickens were obtained from El Arabia poultry breeding farm, Egypt. They were housed in cages, fed on balanced drug free-ration for two weeks before the experiment and supplied with water *ad-libitum*. They were reared in room maintained at 12 h lighting cycle and at constant temperature and relative humidity of 45 to 65%. They were allocated into five groups. The first 2 groups (of six chickens each) were kept healthy and assigned for the pharmacokinetic study following IV and oral administration of marbofloxacin, respectively. The 3rd and 4th group (of six chickens each) was infected with *M. gallisepticum* before IV and oral administration of marbofloxacin, respectively. A fifth group of 24 apparently healthy chickens was used for determination of marbofloxacin residues in liver, kidney, gizzard, breast muscle and thigh muscle after 5 successive days of oral administration. Marbofloxacin was used in a recommended dose of 5 mg/kg body weight.

2.3. Experimental infection

At the age of three weeks, *Mycoplasma* free chickens were inoculated by intraocular / intranasal route with 0.1 ml containing 10^7 colony forming units (CFU) of Mycoplasma culture and 0.1 ml of the inoculum by intratracheal route as described by Gharaibeh and Hailat (2011). The chickens were observed till the appearance of the clinical signs (OIE, 2008). Infection was confirmed by clinical symptoms as characterized by conjunctivitis, sneezing, sinusitis, serum plate agglutination as well as by molecular detection of *Mycoplasma gallisepticum* in tracheal swab by PCR. The *Mycoplasma gallisepticum* primers consist of the following sequences:(MG-14F: 5'-GAG-CTA-ATC-TGT-AAA-GTT-GGT-C-3') , (MG-13R: 5'-GCT-TCC-TTG-CGG-TTA-GCA-AC-3'). The Agar gel electrophoresis of *M. gallisepticum* was illustrated in figure.

2.4. Blood samples

Marbofloxacin was administered either by intravenous or oral route in a dose of 5 mg/kg body weight for both healthy and diseased chickens. After intravenous injection, blood samples were collected from the left brachial vein of each chicken into heparinized tubes at 5, 10, 15 and 30 minutes and 1, 2, 4, 6, 8, 10, and 24 hours post injection. After oral administration, samples were collected at 15 and 30 minutes and 1, 2, 4, 6, 8, 10 and 24 hours. All blood samples were centrifuged at 3500 rpm for 10 minutes, and plasma was harvested and stored frozen at -20 ºC until analyzed for marbofloxacin within a week.

2.5. Tissue samples

At the end of the third day of oral administration of marbofloxacin (5 mg/kg) in healthy chicken, six chickens were slaughtered after 24, 72, 120 and 168 hours. Tissue samples from liver, kidney, gizzard, breast and thigh muscle were taken from each slaughtered bird for drug assay. Samples were frozen and stored at -20°C until assayed.

2.6. *Assay of marbofloxacin*

The concentrations of marbofloxacin were determined by HPLC (Agilent series 1200 quaternary gradient pump, series 1200 auto sampler, series 1200 UV Vis detector , eclipse XDB C18 column (5um, 4.6mm, 250mm) according to Ding et al., (2013). The mobile phase consisted of 12% acetonitrile, 0.75% formic acid and 0.4% triethylamine. UV detector was operated at a wave length of 295 nm; Flow rate was 1ml/min; injection volume was 50 µl. All reagents were of HPLC grade. Perchloric acid 72%, formic acid 85%, triethylamine and phosphoric acid were of analytical grade.

2.6.1. Preparation of standard curves of marbofloxacin

for establishment of standard curve of marbofloxacin, a stock solution of 1000 µg/ml of marbofloxacin in distilled water was prepared. Standard concentrations were obtained by further dilution in drug free healthy chicken plasma or in healthy chicken homogenized tissues (liver, kidney, gizzard, thigh and breast muscle) to obtain

concentrations of 0.01, 0.1, 0.5, 1, 2 and 5µg/ml (Mahmood et al., 2012). Drug free healthy chicken plasma or drug free healthy chicken tissues were spiked with marbofloxacin from the previously prepared concentration.

Marbofloxacin was extracted from chicken plasma according to Mahmood et al., (2012). In an eppindorf tube**,** 200 µL aliquot of chicken plasma and 100uL of 20% perchloric acid (ClHO4) were added. The mixture was vortexed and then centrifuged at 4500 rpm for 10 min. A 50µL aliquot of the supernatant solution was added to the auto-sampler vial for analysis.

Marbofloxacin was extracted from tissue samples according to (Strelevitz et al., 1996 and Ding et al., 2013). Pooled tissue samples (3.0 g) were homogenized with 15 mL extraction solution (methanol, 0.015 mol/L phosphoric acid, and 0.015 mol/L perchloric acid solution, 50:50 v/v) and centrifuged at 15000 rpm for 8 min. The sample was then hydrolyzed for 90 min in 50 °C water bath. After cooling to room temperature and centrifugation at 5000 rpm for 10 min, a 50 µl aliquot of the supernatant solution was added to the auto-sampler vial for analysis.

2.7. Pharmacokinetic analysis

The pharmacokinetic parameters were calculated by PKSolver: An add-in program for Microsoft Excel, version 2 (Zhang et al., 2010).

Statistical analysis

The data were calculated as mean ± standard deviation. The pharmacokinetic parameters of the drug in healthy chickens were compared to those of infected chicken by the Student's (t) test.

3. Results

3.1. Validation of the method of analysis

The method of analysis of marbofloxacin was tested for accuracy and sensitivity by determination of the limit of detection (LOD) and limit of quantification (LOQ). The limit of detection for the method of analysis of marbofloxacin concentration in plasma was 0.001µg/ml and in tissues was 0.002µg/gm. The limit of quantification for the method of analysis of marbofloxacin concentration was 0.01µg/ml in plasma and 0.01 µg/gm in tissues. The recovery percent of marbofloxacin ranged from 97-99 %**.** from spiked plasma and from 91-94%**.** From spiked tissue samples.

3.2. Pharmacokinetics of marbofloxacin in chickens following i.v. administration

Following single intravenous injection of marbofloxacin (5 mg /kg b.wt.), the drug was detected 24 hours after administration with mean values of 0.19 ± 0.02 and 0.13 ± 0.01 µg/ml in healthy and *M. gallisepticum*– infected chickens respectively (figure 2).

The plasma concentration-time data of marbofloxacin following intravenous injection in healthy and diseased chickens was best fitted to a two compartments open model. The pharmacokinetic parameters of marbofloxacin after a single intravenous injection were recorded in Table 1. The results revealed a rapid distribution of marbofloxacin in healthy ($t_{½\alpha}$ = 0.18±0.04 h) and *M. gallisepticum* – infected ($t_{½\alpha}$ = 0.2 ±0.03 h) chickens. The volume of distribution at steady state (V_{dss}) was 0.35±0.02 and 0.41±0.01L/kg in healthy and diseased chickens, respectively. The elimination half – life ($t_{½\beta}$) was 6.8 ± 0.34 and 5.66±0.33 h in healthy and *M. gallisepticum* – infected chickens, respectively. The MRT of marbofloxacin in *M. gallisepticum* – infected chickens was significantly shorter than that in healthy ones.

3.3. *Pharmacokinetics of marbofloxacin in chickens following oral administration*

Following single oral administration of marbofloxacin (5 mg /kg body weight), the drug was detected after 24 hours at a concentration of 0.26±0.02 and 0.14±0.01µg/ml in healthy and *M. gallisepticum*– infected chickens respectively (figure 3). Following single oral administration of marbofloxacin, the drug achieved its maximum plasma concentrations (C_{max}) of 1.37±0.08 and 0.95±0.02 µg/ml in healthy and *M. gallisepticum* – infected chickens, respectively. The maximum concentration was attained at t_{max} of 1.95±0.04 h and 2.08±0.05h in healthy and diseased chickens, respectively. The pharmacokinetic parameters following single oral administration of

marbofloxacin into healthy and *M. gallisepticum* – infected chickens are recorded in Table 2. The mean systemic bioavailability (F %) following oral administration of marbofloxacin was 80.20 ± 6.27% and 72.66±6.30 % in healthy and *M. gallisepticum*-infected chickens, respectively.

3.4. Tissue residues of marbofloxacin in healthy chickens

Tissue concentrations of marbofloxacin in slaughtered healthy chickens following oral administration of 5mg / kg body weight once daily for 3 consecutive days are recorded in Table 3. The present data revealed that Marbofloxacin concentration in breast muscle, thigh muscle and gizzard 24 hours after the last dose was 0.17 ± 0.02, 0.13 ± 0.01and 0.18 ± 0.015µg / g, respectively. It disappeared after 48 hours of the last dose. In the liver and kidney marbofloxacin was detected after 24 hours of the last dose at a concentration of 1.52± 0.04 and 1.74 ± 0.04015µg / g respectively. Its concentration was then decreased to 0.02± 0.004 and 0.03 ± 0.005 µg / g repectively, at the 5th day following last oral dose and diappeared after that.

4. Discussion

Although some studies have been published regarding the pharmacokinetics of marbofloxacin in healthy or infected animals (Anadón et al., 2002; Huang et al., 2003; Abo-El-Sooud and Goudah, 2010), there is a paucity of systematic information about pharmacokinetics of marbofloxacin in infected chickens. In the present

study, pharmacokinetics of marbofloxacin after a single IV and oral administration of 5mg/kg in healthy and *M. gallisepticum* infected chickens were determined. The reported mean plasma concentration of mabofloxacin 24 hours after I.V. administration and in healthy (0.19 ± 0.02 µg/ml) and *M. gallisepticum*– infected (0.13±0.01 µg/ml) chickens was above the MIC for marbofloxacin against most susceptible organisms; MIC ≤1 µg/ml (Belew et al . (2015), suggesting a single dose at 24 hours interval would be sufficient for treating diseases caused by mycoplasma and other susceptible microorganisms.

Disposition of marbofloxacin after IV and oral administration in chickens was best fitted to a 2-compartment open model. This was indicated by the calculated AKaiKe's information criterion (Yamaoka et al., 1978). Moreover, the disappearance of marbofloxacin from the plasma of chickens was characterized by an initial rapid distribution followed by slower elimination phase confirming the distribution according to the 2-compartment open model. Previously, the 2-compartment open model was reported to be the best to describe the disposition of marbofloxacin in chickens (Anadón, et al., 2002) and ducks (Garcia-Montijano et al., 2012). However, the non-compartmental analysis was used to describe the disposition of marbofloxacin in ostriches (De Lucas et al., 2005). The reported half-life alpha in chickens (0.18±0.04 h) was closely similar to that previously reported avian species **(**Anadón et al., 2002 and Goudah and Hasabelnaby, 2010). In mammals, similar values were reported in

foals; 0.27 (Tohamy and El-Gendy, 2013) and buffalo; 0.31 h (Elzoghby and Aboubakr, 2015). However, much longer half-life of distribution was reported in Vulture; 2.53 h (Garcia-Montijano et al., 2011) and rabbits; 1.42 h, (Marin et al., 2013). The lower plasma concentration (µg/ml) of marbofloxacin in *M. gallisepticum*- infected chickens after single intravenous injection than that in healthy ones indicates that marbofloxacin disappeared more rapid from blood of diseased than healthy chickens. This is confirmed by the rapid transfer of the drug from the central to the peripheral compartment in diseased (K_{12}, 1.04 h^{-1}) than in healthy (1.29 h^{-1}) and the wide distribution of the drug in diseased (V_{dss}; 0.41±0.01 L/kg) as compared to healthy (V_{dss}; 0.35±0.02 L/Kg) chickens. Moreover, the increased elimination rate of marbofloxacin from the body of diseased than healthy chickens (Cl; 51.04±3.67 in diseased vs 36.37±3.41 mg/(µg/ml)/h) could be a further factor for the observed lower plasma concentration of marbofloxacin in plasma of diseased chickens. This is in parallel with the short elimination half-life in diseased (5.66 h) than in the healthy birds (6.8 h). In correlation to the lower plasma concentration in the diseased chickens, the AUC (16.24µg/ml.h) was also smaller than that in healthy ones (22.85; µg/ml.h). The short elimination half-life in diseased chickens correlates with the decreased persistence of the drug in their bodies (MRT; 8.03h) than healthy chickens (9.67 h). From the previous discussion it could be suggested that marbofloxacin is widely distributed in diseased birds and rapidly eliminated from their bodies than in healthy ones. The volume of the central compartment distribution (V_1) was 0.23± 0.01 L/kg, which is nearly equal to the

assumed total extracellular fluid volume (approximately 0.2 L/kg) but less than the assumed total body water (approximately 0.55 L/kg), suggesting that marbofloxacin moderately crosses biological membranes. This is also confirmed by the reported low value of the volume of the peripheral compartment distribution (V_2, 0.12± 0.007 ml/kg) and by the moderate volume of distribution at steady state (V_{dss}, 0.35±0.02 L/kg). Once again the value of V_{dss} (0.35 L/kg) is in between the assumed total extracellular fluid volume (0.2 L/kg) and the assumed total body water (0.55 L/kg) indicating that marbofloxacin is of moderate tissue penetration ability. The obtained value was close to that recorded in Muscovy ducks; 0.57 L / kg; following administration of 2 mg/kg (Goudah and Hasabelnaby, 2010) but slightly lower than that reported in broiler chickens; 0.77 L / kg (Anadón et al., 2002), mallard ducks; 1.78±0.37 L/kg, (Garcia-Montijano et al., 2012), adult Eurassian Griffon vulture; 1.51±0.22L/kg (Garcia-Montijano et al., 2011) and turkey; 1.41 L / kg (Haritova et al., 2006) following administration of 2 mg/kg. Moreover, the reported V_{dss} was much lower than those reported in ostriches, 3.22 L / kg (De Lucas et al., 2005) following administration at similar dosage level. In comparison to other quinolones, extremely higher values of volume of distribution were reported for pefloxacin, 3.74 L/kg (Dimitrova et al., 2008; and danofloxacin, 5.41 L / kg following administration of 5mg/kg (Goudah and Mouneir, 2009). The elimination half-life of marbofloxacin ($t½_{\beta}$) after IV administration (6.8±0.34h) was similar to that reported for marbofloxacin intravenously administered in foals; 6.4 h (Tohamy and El-Gendy, 2013) and approaching to that reported

in broiler chickens; 5.26 h (Anadón et al., 2002). However, it was much longer than that reported in ostriches; 1.47 ± 0.31 h (De Lucas et al., 2005) following administration of similar doses. Moreover, a half-life of 5.92 h, 4.11 h, 2.43 and h 10.8 h were reported in lactating sows (Petracca et al., 1993), Eurasian buzzards (Garcia-Montijano et al., 2001), mallard ducks (Garcia-Montijano et al., 2012) and dogs (Lefebvre et al., 1998), respectively following administration of 2mg/kg. The elimination half-life of marbofloxacin in diseased chicken (5.66 h) was shorter than healthy chickens (6.8 h), that correlates to a short MRT in diseased chickens (8.03 h) as compared to healthy ones (9.67 h). The short half-life of elimination in diseased may be related to the low degree of plasma protein binding (<30%) as assumed by Petracca et al. (1993) which allows a larger amount of free drug for elimination. However, longer half-life has been reported in *Mannheimia haemolytica* infected calves (Ismail and El-Kattan, 2007), in *Mycoplasma* and *E. coli*-infected chicken (Ding et al., 2013) and in *Pasteurella mutocida* infected rabbits (Abo-El-Sooud and Goudah, 2009) following administration of marbofloxacin in a dose of 2mg/kg. The difference in the diseased conditions, species and /or doses may explain these variations.

When given orally, marbofloxacin was rapidly and efficiently absorbed in chickens. The reported short half-life of absorption (0.62±0.05 h) was similar to that previously reported in chickens (0.60± 0.05h; Anadón et al., 2002) following administration of 2mg/kg but shorter than that reported in turkey; 7.73 h (Haritova et al.,

2006) following administration of marbofloxacin in a dose of 2mg/kg. Marbofloxacin achieved a maximum concentration (C_{max}) of 1.37±0.08 µg/ml at t_{max} of 1.95±0.04 h which is nearly similar to that previously reported in mallard ducks; 1.34±0.27 µg/ml (Garcia-Montijano et al., 2012) but slightly higher than that reported in chickens; 1.05ug/ml (Anadón et al., 2002) following administration of 2 mg/kg. A nearly similar C_{max} was reported in buffalo calves, 1.19-0.04µg/mL given a dose of 1mg/kg orally (Baroni, et al., 2007). It has been shown that C_{max} was increased by increasing the dose in dogs ranged from 0.831±0.263 µg/ml after oral administration of 1 mg/kg to 2.927±0.581µg/ml following oral dose of 4 mg/kg (Schneider et al., 1996). The lower C_{max} in diseased compared to healthy chickens correlates with the general lower plasma concentration in diseased birds. Oral bioavailability of marbofloxacin (80.198%) was nearly similar to that reported in turkey; 84.34% (Haritova et al, 2006) and lactating sow; 77.95 (Anadón, et al., 2002) following administration of 2mg/kg. However, it was higher than that recorded in broiler chickens; 56.82% (Anadón, et al., 2002) and quails; 50.1% (Lashev et al., 2015) receiving similar dose. The oral F% of marbofloxacin in chickens was slightly lower than that reported in ostrich; 95.03% (De Lucas et al., 2005) at similar dosage levels. These differences are probably because of the differences in the doses used which reflect the difference in the AUCs and consequently the F%. The high oral bioavailability reflects the rapid rate and efficient extent of absorption of marbofloxacin. In the present study the body clearance of marbofloxacin in healthy chickens (Cl_2; 0.3 ± 0.05 l/h/kg) was similar

to that previously reported in chickens; 0.17±0.03 l/h/kg (Anadón et al., 2002), in ducks; 0.16 l/h/kg (Goudah and Hasabelnaby, 2010), in turkey; 0.16 L/kg/h (Haritova et al., 2006) receiving 2mg/kg and in ruminant calves; 0.31 L/kg/h and 0.28L/kg h, (Thomas et al., 1994 and Baroni et al., 2007, respectively) following a dose of 1mg/kg. Higher values were reported in ostriches; 2.19 L/ h/kg (De Lucas et al., 2005) receiving 5mg/kg. Fluoroquinolones are excreted by renal tubular secretion, and biliary or hepatic metabolic pathways (Neuman, 1988).

The residue testing revealed high concentrations of marbofloxacin in kidney (1.74±0.04µg/g) and liver (1.52±0.04µg/g) 24 hours after the last dose. These levels were higher than that previously reported in broiler chickens kidney; 0.985µg/kg and liver; 0.735 µg/kg (Anadón et al., 2002) who administered a dose of 2mg/kg which is less than half the used dose in the present study. On the other hand, the concentrations of marbofloxacin residues in kidney and liver at the 5th days after the last dose were very low (0.02 and 0.03 µg/g) as compared to the MRL of marbofloxacin suggested by European Union; 150µg/kg **[**(Yang et al., 2014). Moreover, marbofloxacin could not be detected in other organs after 24 hours of the last administration day. Therefore, a 4 days withdrawal time of marbofloxacin is suggested. Similar conclusion has been previously suggested (Yang et al., 2014).

In Conclusion, Marbofloxacin at dose of 5 mg/kg administered intravenously or orally at 24 h intervals may provide successful

treatment of chicken diseases caused by *Mycoplasma gallisepticum* and/or other susceptible microorganisms. Short elimination half-life and MRT and lower C_{max} in diseased birds are the main differences. Following oral administration of marbofloxacin, a 5 days withdrawal period after the last dose is suggested.

Conflict of Interest: The authors declare that there is no conflict of interest.

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Table 1: Mean pharmacokinetic parameters of marbofloxacin in healthy and *M. gallisepticum*- infected chickens after a single intravenous injection of 5mg/kg body weight. (Mean ± SD, n = 6).

Parameters	Units	Mean ± SD	
		Apparently healthy	*M. gallisepticum* infected
A	µg/ml	1.23±0.08	0.97±0.15**
α	h^{-1}	3.93±0.74	3.46±0.45
B	µg/ml	2.29±0.11	1.95±0.06***
β	h^{-1}	0.1±005	0.12±0.007***
k_{10}	h^{-1}	0.16±0.005	0.18±0.007***
k_{12}	h^{-1}	1.29±0.28	1.04±0.13
k_{21}	h^{-1}	2.59±0.47	2.36±0.36
$t_{1/2\alpha}$	h	0.18±0.04	0.20±0.03
$t_{1/2\beta}$	h	6.8±0.34	5.66±0.33***
C^0	µg/ml	3.5±0.18	2.92±0.2***
V_1	(mg)./(µg/ml).	234.58± 11.91	283.38± 19.19***
CL	(mg)./(µg/ml)./h	36.37 ± 3.41	51.04± 3.67***
V_2	(mg)./(µg/ml).	115.74± 7.03	125.36±7.82*
CL_2	L/kg/h	0.30 ±0.06	0.29± 0.03
AUC $_{0\text{-}t}$	µg/ml.h	20.88±1.73	15.38±1***
AUC $_{0\text{-}inf}$	µg/ml.h	22.85±2.16	16.235±1.17***
AUMC	µg/ml.h^2	221.89±32.69	130.81±16.32***
MRT	h	9.67±0.5	8.03±0.45***
V_{dss}	L/kg	0.35.32±0.02	0.41±0.01***

A and B; zero-time intercept of the distribution and elimination phase; α and β; distribution and elimination rate constant; k10, k12 and k21; first -order rate constants. t1/2α, t1/2β and t1/2ab; half-life of distribution, elimination and absorption. AUC 0-t, area under the plasma drug concentration versus time curve; AUC0-inf, total area under the concentration–time curve from zero to infinity; AUMC, area under the first moment curve; MRT, mean residence time. * $P \leq 0.05$ **$P \leq 0.01$ ***$P \leq 0.001$

Table 2: Mean pharmacokinetics parameters of marbofloxacin in healthy and *M. gallisepticum*- infected chickens after single oral dose of 5mg/kg body weight (Mean ± SD, n=6).

Parameters	Units	Mean ± SD	
		Healthy chickens	*M. gallisepticum*-infected chickens
A	µg/ml	3.2 ± 0.64	2.39±0.4*
α	h-1	0.88 ± 0.08	0.82±0.09
B	µg/ml	1.55 ± 0.10	1.12±0.03***
β	h^{-1}	0.07±0.005	0.09±0.01***
K_{ab}	h-1	1.12± 0.09	1.02±0.09
K_{10}	h-1	0.11±0.008	0.11±0.01
k_{12}	h-1	0.22± 0.02	0.19±0.01**
k_{21}	h-1	0.63±0.08	0.6±0.1
$t_{1/2\alpha}$	h	0.79±0.07	0.85±0.09
$t_{1/2\beta}$	h	9.39±0.26	8.44±04***
$t_{1/2ab}$	h	0.62±0.05	0.69±0.06
V1/F	mg/(µg/ml).	393.25± 21.39	557.06 ±21.56***
CL/F	mg/(µg/ml)./h	40.83±3.29	63.28 ± 3.8***
V2/F	mg/(µg/ml).	139±23.8	180.93 ±28.28*
CL2/F	mg/(µg/ml)./h	86.74±4.76	106.06± 4.81***
T_{max}	h	1.95±0.04	2.08±0.05***
C_{max}	µg/ml	1.37±0.08	0.95±0.02***
AUC 0-t	µg/ml.h	16.75±1.21	11.17±0.51***
AUC_{0-inf}	µg/ml.h	20.32±1.63	13.08±0.78***
AUMC	µg/ml.h^2	284.4±29.53	166.26±17.07***
MRT	h	13.98±0.37	12.69±0.56***
F	%	80.20± 6.27	72.66±6.30

K_{ab}; absorption rate constant, $t_{1/2ab}$; half-life of absorption, T_{max}, the time to maximum plasma concentration; C_{max}, the maximum plasma concentration; F, bioavailability. * $P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$

Table 3: Concentration (µg/g). of marbofloxacin in some edible organs of healthy chickens at various intervals after last oral administration of 5 mg/kg b.wt. once daily for 3 consecutive days (Mean ± SD, n=6).

Tissue	Days post administration			
	1st	3rd	5th	**7th**
Liver	1.52± 0.04	0.14 ± 0.01	0.02± 0.004	ND
Kidney	1.74 ± 0.04	0.22 ± 0.01	0.03 ± 0.005	ND
Breast muscle	0.17 ± 0.02	ND	ND	ND
Thigh muscle	0.13 ± 0.01	ND	ND	ND
Gizzard	0.18 ± 0.01	ND	ND	ND

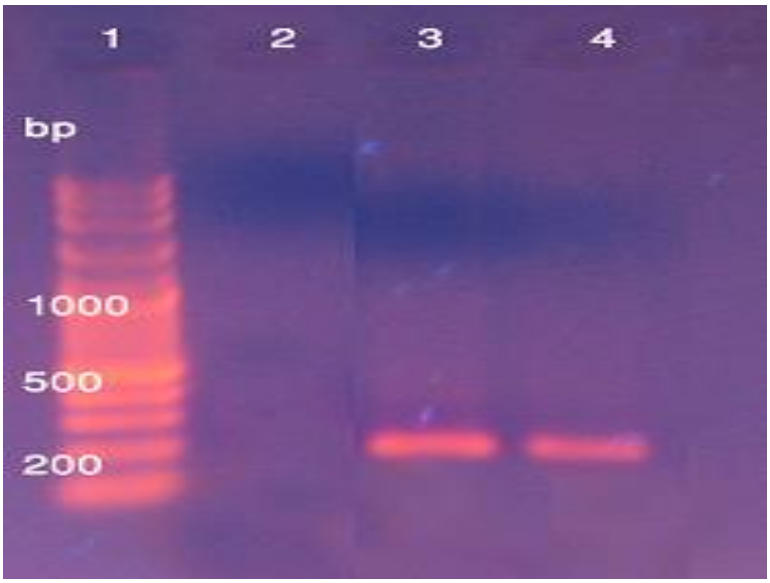

Figure 1: Agar gel electrophoresis of *M. gallisepticum* (MG).; Lane (1): 100 bp DNA marker, Lane (2): negative control, Lane (3): positive control MG, Lane (4): sample positive to MG

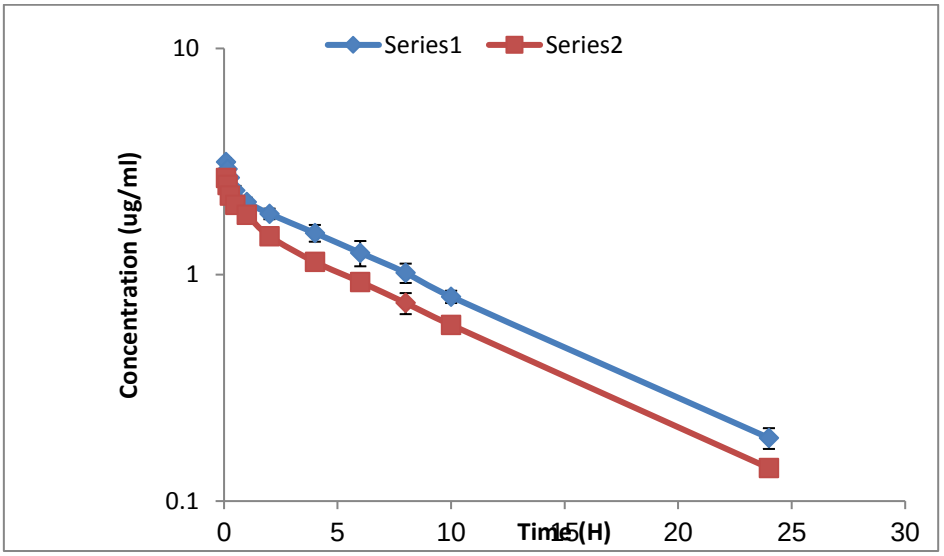

Figure 2: Mean plasma concentrations of marbofloxacin in apparently healthy and M. gallisepticum infected chickens after intravenous of 5mg/kg body weight. (n = 6).

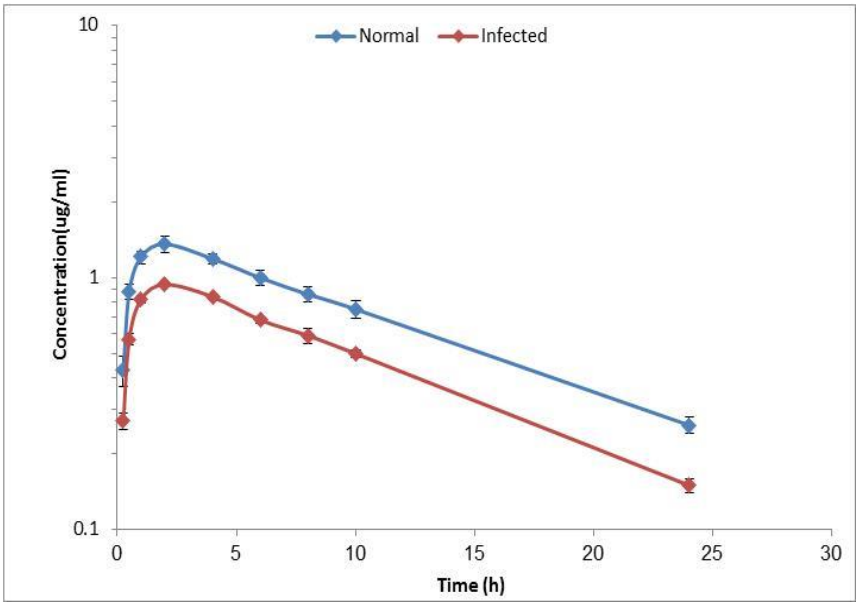

Figure 3: Mean Plasma concentrations of marbofloxacin in apparently healthy and *M. gallisepticum* - infected chickens after oral administration of 5mg/kg body weight (n=6).