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Pharmacokinetics and Tissue Residues of Tilmicosin in Normal and Experimentally *Mycoplasma Gallisepticum*-Infected Broiler Chickens

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A B S T R A C T

The present study was conducted to investigate the pharmacokinetics of tilmicosin (25 mg/kg b.wt.) following single and repeated oral administration (once daily for three consecutive days) as well as tissue residues in normal and experimentally *Mycoplasma gallisepticum*-infected broiler chickens. Following single administration of tilmicosin in normal chickens, the drug reached its maximum serum concentrations (C_{max}) after 2.45±0.01 h of administration with value of 0.97±0.04 µg/ml. Absorption half-life ($t_{0.5ab}$) of tilmicosin was 0.89±0.02 h and the elimination half-life ($t_{0.5el}$) was 14.73 ±1.24 h. The repeated oral administration of tilmicosin in normal and *Mycoplasma gallisepticum*- infected chickens revealed a lower significant serum tilmicosin concentration after all times of sampling in infected chickens compared to those of normal chickens. Tilmicosin residues was assayed in lung, kidney, liver, heart, breast muscle, thigh muscle, fat and skin after 24, 48, 72, 96, 120 and 144 h post last dose. The results showed that the highest residue values were recorded in lung followed by liver and kidneys while the lowest values were recorded in heart. Tilmicosin residues were not detected in all tested tissues except in lung till 5th day after sessation of drug administration. However, all the tested tissues were free from tilmicosin residues after 5th day after sessation of tilmicosin administration and this suggest a withdrawal period of five days for tilmicosin in broiler chickens. In conclusion, timicosin has rapid absorption, long elimination half-life, rapid and extensive penetration from blood into tissues especially lungs. Additionally, timicosin had a short withdrawal time.

Keywords: Pharmacokinetics, tissue residues, tilmicosin, Broiler chickens.

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1.INTRODUCTION

Tilmicosin is a semisynthetic, broad-spectrum, bacteriostatic macrolide antibiotic with a wide range of veterinary uses. It shows promising prospect of applications in clinical veterinary field. Tilmicosin is a useful drug for treatment and control of respiratory diseases because of its large volume of distribution, long half-life and preferential high accumulation in lungs (Debono *et al*., 1989). Tilmicosin is used for treatment and control of respiratory diseases caused by *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Ornithobacterium rhinotracheale and Pasteurella multocida* in broilers (Jordan & Horrocks, 1996; Kempf *et al.*, 1997; EMEA 1998; Prescott 2000; Varga *et al.*, 2001; Abu-Basha *et al.*, 2007). It has been also licensed for combating respiratory diseases in pigs, sheep and cattle (Moore *et al.*, 1996; Hoar *et al.*, 1998; Christodoulopoulos *et al.*, 2002).

The pharmacokinetics after parenteral administration of tilmicosin has been studied in cow (Ziv *et al.,* 1995; Modric *et al.,* 1998), goat (Ramadan, 1997), sheep (Modric et al., 1998), and elk (Clark *et al.,* 2004). However, limited studies are available concerning the disposition of tilmicosin following oral administration to animals including fowl, swine, broiler chicken and lactating goat (Keles *et al.,* 2001; Shen *et al.,* 2005; Elsayed et al., 2014; El-Komy et al., 2016).

Mycoplasma infection can create inconvenience and economic damage and continues to be an important cause of loss in poultry production that should be taken into consideration in the poultry industry, which is often not apparent, and it silently damages the effectiveness of investment (Mohammed *et al*., 1987; Kleven, 1990). At the poultry industry *Mycoplasma gallisepticum (Mg)* and

Mycoplasma Synovie (Ms) are of the greatest importance (Mohammed *et al.,* 1987 & Kleven *et al.,* 1997). Uses of recent anti-mycoplasmal drugs either in the prophylaxis and therapy is still recommended in the eradication programs than application of sanitary measures or usage of vaccine (Arzey and Arzey, 1992; Jordan *et al*., 1999). Tilmicosin has also been shown to be effective for the treatment of mastitis in cattle and sheep, pasteurellosis in rabbits, and *Mycoplasma gallisepticum* infections in chicken (McKay *et al.*, 1996; Kempf *et al.*, 1997; Croft *et al.*, 2000; Dingwell *et al.*, 2003).

Administering veterinary medications to animals without an appropriate withdrawal period may lead to violative residues in tissues. Despite the extensive use of tilmicosin in poultry industry, limited data is currently available about the disposition and tissues residues of tilmicosin in broiler chickens. Therefore, the main purpose of the present study was to investigate and provide an overview of the pharmacokinetic profile as well as tissue residues of tilmicosin in both normal and experimentally *Mycoplasma gallisepticum*-infected broiler chickens following single and repeated oral administration and to determine its withdrawal time.

2.MATERIALS AND METHODS:

1. Drug

Tilmicosin phosphate was obtained as an oral solution from ATCO Pharma for Pharmaceutical Industries, Cairo, Egypt, with a commercial name of tilmicoral®, a 25% oral solution, 240 ml. Each 100 ml contain tilmicosin phosphate 29.4 gm (Eq. to 25 gm tilmicosin base) which applied orally by intra-crop administration. Tilmicosin has the chemical name of 20-Deoxo-20-(3, 5-dimethyl-1-piperidinyl) desmycosin and molecular weight of 869.15 g/mol and with molecular formula of C46H80N2O13 (Debono *et al.,* 1989). Tilmicosin is produced by the removal of mycarose of tylosin A in an organic solvent such as butyl acetate (Debono *et al.,* 1989).

2. Experimental Chickens

Forty-one clinically healthy Hubbard chickens weighting about 1500 to 2000 g. Birds were chosen randomly from poultry farm in Qalubia government, Egypt. Chickens were of both sexes. Chickens were maintained at a suitable temperature and humidity according to their ages. The chickens had a free access to water and feed and the feed was free from antibacterial drugs.

3. Experimental design

Chickens were divided into 3 groups, group (1): five clinically healthy broiler chickens were orally intra-crop administered a

single dose of tilmicosin (25 mg/kg b.wt.). Blood samples (1ml) were collected from the brachial veins of each bird at 15 and 30 min. and at 1, 2, 4, 6, 8, 12 and 24 h after dosing for determination of tilmicosin concentration using microbiological assay.

Group (2): eighteen clinically healthy chickens were orally intra-crop administered of 25 mg tilmicosin/kg b.wt. once daily for three consecutive days, to determine pharmacokinetics parameters and tissue residues of tilmicosin in healthy chickens.

Group (3): eighteen experimentally *Mycoplasma gallisepticum*-infected chickens were orally intra-crop administered of 25 mg tilmicosin /kg b.wt. once daily for three consecutive days to determine pharmacokinetics and tissue residues of tilmicosin in infected chickens.

4. Experimental infection

Each chicken was experimentally infected with 100 µl aliquots of PPLO broth culture equal containing ($1x10^8$ CFU/ml of a pathogenic strain of *M. gallisepticum* which was given by automatic micropipette/chick intra-tracheal to induce infection according to Belah *et al.,* (2012). After appearance of the respiratory clinical symptoms as dyspnea, sneezing, nasal discharges, tracheal rales, lacrimal discharge, conjunctivitis and lack of appetite that beginning within 24 hours after infection and become intensive at 3rd day post infection with *M. gallisepticum*, each chicken was orally intra-crop administered tilmicosin at a dose rate of 25mg/kg b.wt. once daily for three consecutive days. After that blood and tissue samples were taken for assaying of pharmacokinetics and residues.

5. Collection of samples

5.1. Blood samples

About one ml of blood was taken from the brachial vein of five birds, following administration of tilmicosin. Blood samples were collected at 15 and 30 min. and at 1, 2, 4, 6, 8, 12 and 24 h after each dose for determination of tilmicosin concentration using microbiological assay. Blood samples were collected in sterilized test tubes and allowed to clot. Sera were separated by centrifugation at 600 *g* for 15 min. Sera were kept frozen at -20°C until assayed.

5.2. *Tissue samples*

For determination of tissue distribution and residual contents of tilmicosin, the microbiological assay technique (Petracca and Wanner, 1993) was used. Three chickens were randomly selected and slaughtered from group (2) and group (3) at 24, 48, 72, 96, 120 and 144 h after the last administered dose of tilmicosin. From each slaughtered chicken, tissue samples of lung, heart, liver, kidney, breast muscle, thigh muscle, fat and skin were collected for assaying of residues of tilmicosin. Samples were frozen at -20°C until assayed.

6. Analytical procedure

Tilmicosin was assayed in chicken's serum and distilled water by microbiological assay method using *Bacillus subtilis* ATCC 6633 as a test organism for tilmicosin (Arret *et al.,* 1971). The test organism was obtained from microbiology department, Animal Health Research Institute. Dokki, Giza, Egypt. Three plates were used for each sample. A well in each plate was filled with reference concentration (1 µg/ml of tilmicosin in distilled water or normal chicken's serum). The plates were incubated at 37º C for 18-24 h then the diameter of inhibitory zones was measured. The average diameter of inhibition zone of the samples was corrected by using the diameter of the reference concentration.

From the standard curve, the concentration corresponding to the correct values of the zone diameter were obtained.

For assay of tissue samples, two grams of tissue were homogenized by automatic homogenizer with 2 ml of distilled water. Mixtures were centrifuged at 600 *g* for 10 min. and supernatant fluid of each sample was aspirated and directly assayed microbiologically for tilmicosin concentration.

7. Pharmacokinetic analysis

The pharmacokinetic parameters were calculated according to Baggot, (1978 a & b). The pharmacokinetic analysis of data was done using noncompartmental analysis based on statistical moment theory as described by Gibaldi & Perrier (1982).

8. Statistical analysis

The data were calculated as mean ± standard error (S.E) of observation in PK and residue analysis. All statistical analysis was carried out according to (Snedecor and Cochran, 1980).

Differences of $P \leq 0.05$ * Significant, $P \leq 0.01$ ** highly significant and $P \leq 0.001$ *** very highly significant.

3.RESULTS

Time versus serum tilmicosin concentrations following a single oral administration of 25 mg tilmicosin/kg. b.wt. were illustrated in table 1 and shown in figure 1. Tilmicosin concentration was firstly detected in serum at 15 min. with a mean value of 0.25 ± 0.020 and the drug reached its maximum serum concentration of 1.23±0.062 µg/ml at about 2 h. The pharmacokinetic parameters following a single oral administration of tilmicosin were recorded in table 2. Tilmicosin was rapidly absorbed after its oral administration with an apparent first order absorption rate constant (K_{ab}) of 0.77 ± 0.019/h, while absorption half life time ($t_{0.5ab}$) was 0.89 ± 0.02 h. Tilmicosin was eliminated at rate (K_{el}) equal to 0.048 ± 0.003/h and the elimination half-life time ($t_{0.5el}$) was 14.73±1.24 h. The mean residence time (MRT) was 8.15 ± 0.15 h and the area under the serum tilmicosin concentration curve (AUC) was found to be 9.86 ± 0.59 µg•h/ml.

Time versus serum concentrations of tilmicosin cumulative following repeated oral administration of 25 mg tilmicosin/kg b.wt once daily for three consecutive days in normal and experimentally *M. Gallisepticum*-infected chickens were illustrated in table 3 and figure 2 & 3, respectively. Data revealed a significant decrease serum concentration of tilmicosin at all sampling times in infected chickens than in normal ones.

The pharmacokinetic parameters of tilmicosin following repeated oral administration of 25 mg tilmicosin/kg b.wt once daily for three consecutive days in normal and experimentally *M. Gallisepticum*-infected chickens were shown in table 4. Data revealed that the apparent first order absorption rate constant (K_{ab}) and the maximum serum concentrations (C_{max}) and the area under the serum tilmicosin concentration curve (AUC) were significantly decreased in infected chickens than in normal chickens following drug administration of all doses and showed that the absorption half-life ($t_{0.5ab}$) and the elimination half-life ($t_{0.5el}$) were significantly decreased in infected chickens than in normal ones after the first day of drug administration when compared to the second and third days of administration.

Tissue concentrations of tilmicosin in slaughtered normal and experimentally *M. Gallisepticum*-infected chickens following the repeated oral dosage regimen of 25 mg/kg b.wt. once daily for three consecutive days, were recorded in table 5. The data revealed a significant decrease in tissue concentrations of tilmicosin in experimentally *M. Gallisepticum*-infected chickens than in normal ones**.** Lungs had the highest concentrations of the drug followed by liver and kidney, while the lowest concentrations were determined in heart. This suggests that lung should be the target tissue for tilmicosin residues in broiler chickens.

Time after administration (h)	Chicken's number					_ (X±S.E.)
	(1)	(2)	(3)	(4)	(5)	
0.25	0.27	0.20	0.27	0.32	0.23	0.25 ± 0.020
0.5	0.43	0.38	0.42	0.48	0.37	0.41 ± 0.019
1	0.57	0.52	0.59	0.63	0.52	0.56 ± 0.021
2	1.22	1.06	1.32	1.42	1.16	1.23 ± 0.062
4	0.71	0.67	0.78	0.87	0.66	0.73 ± 0.039
6	0.66	0.65	0.71	0.75	0.61	0.67 ± 0.020
8	0.36	0.33	0.42	0.45	0.35	0.38 ± 0.022
12	0.28	0.27	0.34	0.34	0.23	0.29 ± 0.021
24	0.16	0.14	0.21	0.24	0.13	0.17 ± 0.021

Table (1): Serum concentrations of tilmicosin (µg/ml) in normal chicken following a single oral administration of 25 mg/kg b.wt. (n = 5).

Table (2): Pharmacokinetic parameters of tilmicosin (µg/ml) in normal chicken following a single oral administration of 25 mg/kg b.wt. (n =5).

Parameter	Unit	Chicken's number					_ (X±S.E.)
		(1)	(2)	(3)	(4)	(5)	
K_{ab}	**h^{-1}**	0.79	0.77	0.82	0.79	0.70	0.77 ± 0.019
$t_{0.5ab}$	**H**	0.87	0.89	0.84	0.87	0.98	0.89 ± 0.02
C_{max}	**µg/ml**	0.96	0.86	1.01	1.11	0.90	0.97 ± 0.04
T_{max}	**H**	2.39	2.50	2.47	2.45	2.45	2.45 ± 0.01
K_{el}	**h^{-1}**	0.049	0.053	0.042	0.036	0.058	0.048 ± 0.003
$t_{0.5el}$	**H**	13.93	12.87	16.27	18.77	11.83	14.73 ± 1.24
AUC	**µg• h/ml**	9.50	8.80	10.9	11.61	8.53	9.86 ± 0.59
AUMC	**µg• h/ml**	76.34	70.81	92.21	98.8	65.75	80.78 ± 6.32
MRT	**H**	8.03	8.04	8.48	8.50	7.70	8.15 ± 0.15

Table (3): Comparison of serum concentrations of tilmicosin (µg/ml) in normal (N) and experimentally *M. gallisepticum* infected (I) broiler chickens during repeated oral administration of 25 mg/kg b.wt. once daily for three consecutive days (n=5).

Days	**1st day (1st dose)**		**2nd day (2nd dose)**		**3rd day (3rd dose)**	
Time (h)	**N**	**I**	**N**	**I**	**N**	**I**
	(X±S.E.)	**(X±S.E.)**	**(X±S.E.)**	**(X±S.E.)**	**(X±S.E.)**	**(X±S.E.)**
0.25	0.25 ± 0.020	0.18 ± 0.01*	0.30 ± 0.02	0.21 ± 0.01**	0.38 ± 0.01	0.27±0.01 ***
	0.41 ± 0.019	0.28 ± 0.02**	0.44 ± 0.03	0.32 ± 0.03*	0.50 ± 0.04	0.37 ± 0.02*
1	0.56 ± 0.021	0.39±0.02***	0.64 ± 0.03	0.44 ± 0.04**	0.74 ± 0.06	0.52 ± 0.04*
2	1.23 ± 0.062	0.80±0.05***	1.40± 0.07	1.10 ± 0.06*	1.70 ± 0.09	1.30 ± 0.08*
4	0.73 ± 0.039	0.51±0.04**	0.82 ± 0.08	0.78 ± 0.07	1.00 ± 0.08	0.90 ± 0.06
6	0.67 ± 0.020	0.42±0.02***	0.70 ± 0.06	0.53 ± 0.05	0.86 ± 0.07	0.64 ± 0.04*
8	0.38 ± 0.022	0.34 ± 0.01	0.45 ± 0.03	0.40 ± 0.04	0.50 ± 0.07	0.46 ± 0.04
12	0.29 ± 0.021	0.26 ± 0.01	0.34 ± 0.02	0.29 ± 0.02	0.39± 0.03	0.35 ± 0.03
24	0.17 ± 0.021	0.14 ± 0.01	0.22± 0.02	0.20 ± 0.01	0.24 ± 0.01	0.22 ± 0.01

*→ Represent the significance in comparison with data of the normal group.
* $P < 0.05$ ** $P < 0.01$ *** $P < 0.001$

Table (4): Comparison of pharmacokinetic parameters of tilmicosinin normal (N) and experimentally *M. Gallisepticum* infected (I) broiler chickens during repeated oral administration of 25 mg/kg b.wt. once daily for three consecutive days (n=5).

Days	**1st day (1st dose)**		**2nd day (2nd dose)**		**3rd day (3rd dose)**	
Parameter	**N**	**I**	**N**	**I**	**N**	**I**
(unit)	**(X±S.E.)**	**(X±S.E.)**	**(X±S.E.)**	**(X±S.E.)**	**(X±S.E.)**	**(X±S.E.)**
K_{ab} (h^{-1})	0.77 ± 0.019	0.70 ± 0.04	0.82 ± 0.02	0.70 ± 0.03*	0.73 ± 0.04	0.70 ± 0.03
$T_{0.5\ (ab)}$ (h)	0.89 ± 0.02	0.68±0.03***	0.84 ± 0.05	0.98 ± 0.06	0.94 ± 0.04	0.97 ± 0.04
C_{max} (µg/ml)	0.97 ± 0.04	0.62±0.05 ***	1.07 ± 0.08	0.86±0.08	1.30 ± 0.09	1.01 ± 0.07 *
T_{max} (h)	2.45 ± 0.01	2.44±0.09	2.41 ± 0.12	2.66±0.07	2.47 ± 0.15	2.65 ± 0.08
K_{el} (h^{-1})	0.048±0.003	0.054±0.002	0.042 ± 0.001	0.040±0.003	0.044 ± 0.002	0.044 ± 0.002
$T_{0.5\ (el)}$ (h)	14.73 ± 1.24	12.7±1.06	16.20 ± 0.87	17.12 ± 0.73	15.53 ± 0.96	15.61 ± 0.82
AUC (µg•h/ml)	9.86 ± 0.59	7.44±0.61 *	11.25 ± 0.71	9.49 ± 0.63	13.16 ± 0.87	11.12 ± 0.64
AUMC (µg•h/ml)	80.78 ± 6.32	65.15±7.05	94.86 ± 8.42	82.54 ± 7.36	108.09 ± 8.07	95.57 ± 8.13
MRT (h)	8.15 ± 0.15	8.75 ± 0.31	8.43 ± 0.26	8.69 ± 0.38	8.20 ± 0.29	8.59 ± 0.27

*→ Represent the significance in comparison with data of the normal group.

* $P < 0.05$ ** $P < 0.01$ *** $P > 0.001$

Table (5): Comparison of tissue residue concentrations (µg/g) of tilmicosin following oral administration of 25 mg/kg.b.wt. once daily for three consecutive days in normal (N) and experimentally *M. Gallisepticum* infected (I) chicken (n=3).

	Time after the last dose (h)											
	24		**48**		**72**		**96**		**120**		**144**	
Tissues	**N**	**I**	**N**	**I**	**N**	**I**	**N**	**I**	**N**	**I**	**N**	**I**
Kidney	**4.53±0.12**	**3.88±0.17 ***	**3.12±0.21**	**2.65±0.11**	**1.67±0.07**	**1.32±0.07 ****	**0.35±0.01**	**0.28 ± 0.01 ****	-	-	-	-
Lung	**9.45±0.34**	**8.30±0.25 ***	**6.33±0.36**	**5.76±0.25**	**3.52±0.12**	**2.93±0.09 ****	**1.96±0.07**	**1.75 ± 0.09**	**0.51 ± 0.02**	**0.45± 0.01***	-	-
Heart	**4.24±0.17**	**3.41±0.16 ****	**2.15±0.17**	**1.84±0.09**	**0.57±0.01**	**0.48±0.01 *****	-	-	-	-	-	-
Liver	**5.32±0.16**	**4.56±0.14 ****	**3.28±0.12**	**2.73±0.13 ***	**1.85±0.07**	**1.53±0.05 ****	**0.42±0.02**	**0.33 ± 0.01 ****	-	-	-	-
Breast muscle	-	-	-	-	-	-	-	-	-	-	-	-
Thigh muscle	-	-	-	-	-	-	-	-	-	-	-	-
Fat	-	-	-	-	-	-	-	-	-	-	-	-
Skin	-	-	-	-	-	-	-	-	-	-	-	-

- Not detected

*→ Represent the significance in comparison with data of the normal group.

* P< 0.05 ** P< 0.01

*** P< 0.0

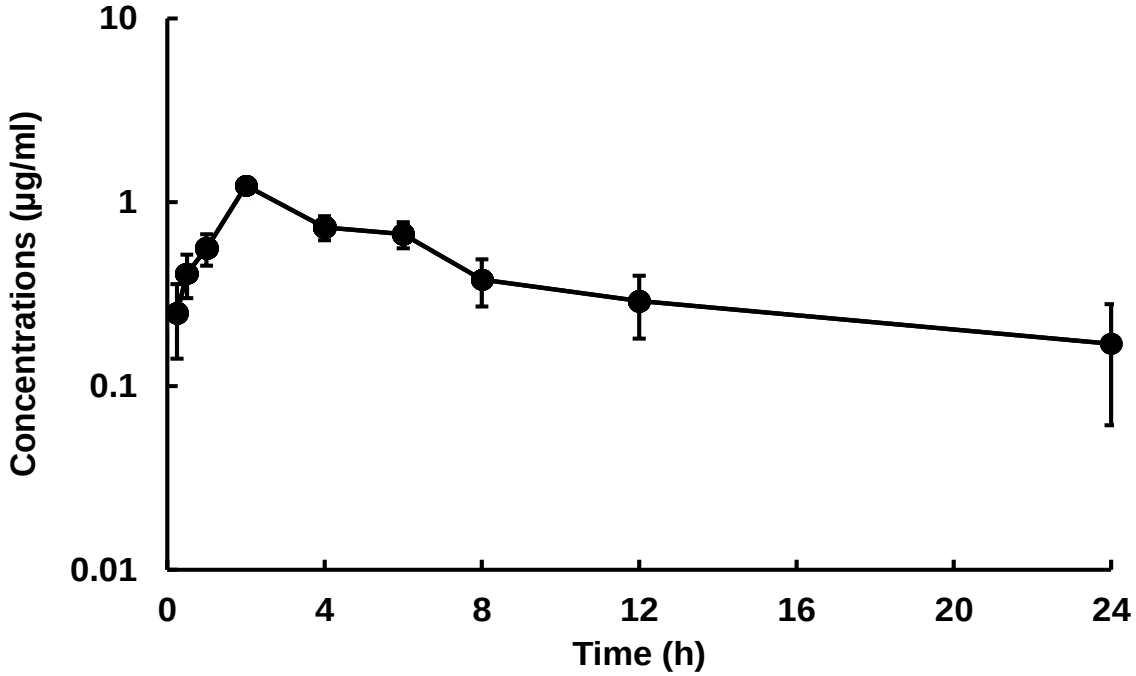

Figure (1): Semi-logarithmic graph depicting the time versus concentrations of tilmicosin in normal chicken following a single oral administration of 25 mg/kg b.wt. (n = 5).

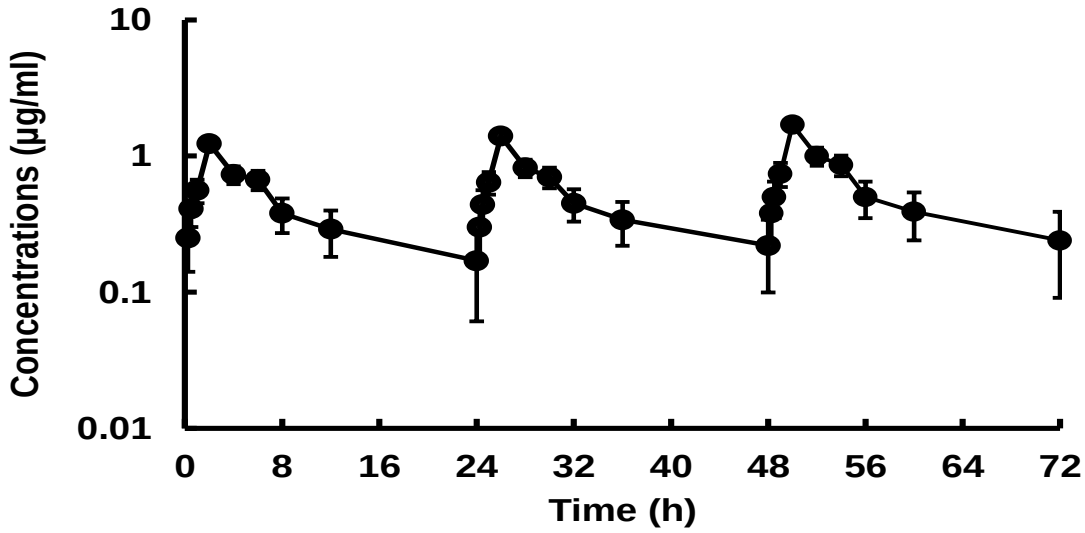

Figure (2): Semilogarithmic graph depicting the time course of tilmicosin in serum of normal chicken during repeated orally administration of 25 mg/kg b.wt. once daily for three consecutive days (n=5).

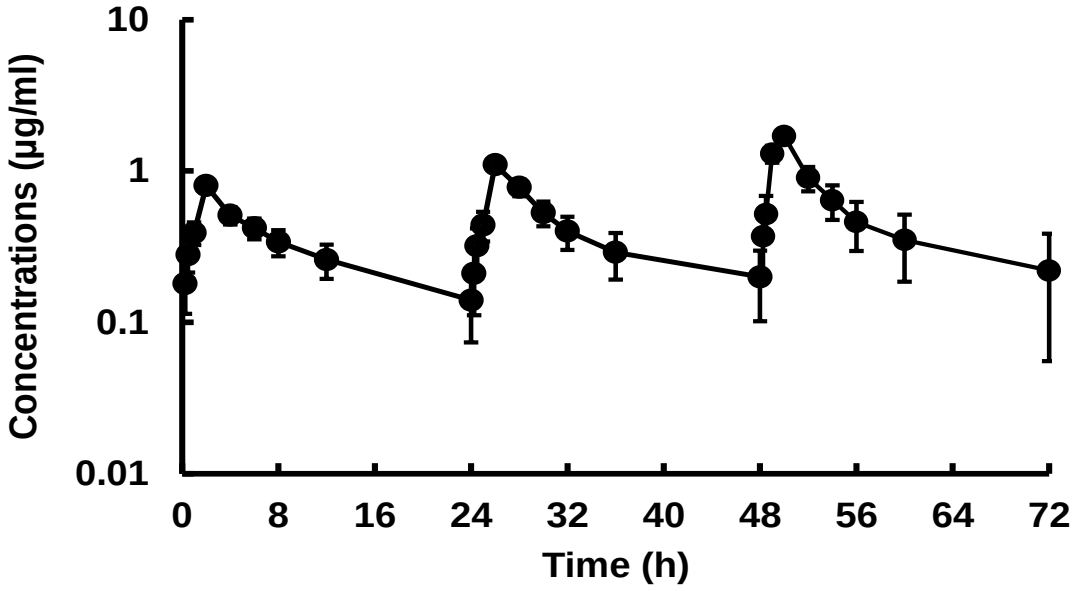

Figure (3): Semilogarithmic graph depicting the time course of tilmicosin in serum of experimentally *Mycoplasma gallisepticum*-infected chicken during repeated orally administration of 25 mg/kg b.wt. once daily for three consecutive days (n=5).

4.DISCUSSION

Pharmacokinetics of tilmicosin after intravenous administration were limited and unsuccessful due to its considerable cardiovascular adverse effects and deaths (Main *et al.*, 1996, Papich & Riviere 2001; Abu-Basha *et al.*, 2007).

In the present investigation, the pharmacokinetic properties of tilmicosin are similar to those of macrolides in general and characterized by low serum concentrations and large volumes of distribution, with accumulation and persistence in many tissues particularly lungs, which may concentrate the drug 20- 60-fold compared to serum (Ziv et al., 1995; Scorneaux and Shryock, 1999; Clark et al., 2004). Intracellular concentrations have been shown to be 40 times greater than that of serum (Ziv et al., 1995; Scorneaux and Shryock, 1999).

In the present study, following a single oral administration of 25 mg/kg b.wt. of timicosin, the drug reached its maximum serum concentrations (C_{max}) after 2.45±0.01 h of administration with value of 0.97±0.04 µg/ml. Tilmicosin could be detected in serum in a therapeutic level of 0.17 ± 0.021 µg/ml for 24 h after administration. The obtained result of C_{max} were consistent with those recorded for tilmicosin in calf (0.976 ± 0.06 µg/ml; Dimitrova et al., 2012a), in cows (0.86 ± 0.20 µg/ml; Avci and Elmas, 2014) and in lactating goat (1.07 ± 0.052 µg/ml; El-Komy et al., 2016). In contrast, the reported C_{max} in the present study were lower than those recorded for tilmicosin in goat (1.56 ug/ml; Ramadan,

1997), tilmicosin in sheep (1.19 ± 0.30 µg/ml; Atef et al., 1999), tilmicosin in fowl (1.28 ± 0.04 µg/ml; Keles et al., 2001), tilmicosin in swine (2.03 ± 0.28 µg/ml; Shen et al., 2005), tilmicosin in broiler chickens (2.09 ± 0.37 and 2.12 ± 0.40 µg/ml for Pulmotil® AC and Provitil®, respectively; Abu-Basha et al., 2007), tilmicosin in rabbit (1.31 µg/ml; Gallina et al., 2010) and tilmicosin in broiler chicken (1.25 ± 0.092 µg/ml; Elbadawy and Aboubakr, 2017). On the other hand, the obtained result of C_{max} was higher than those recorded for tilmicosin in adult sheep (0.44 µg/ml; Cochrane and Thomson, 1990) and for tilmicosin oral solution in broiler chickens (0.583 ± 0.03 µg/ml; Dimitrova et al., 2012b).

In the current study, tilmicosin reached its maximum serum concentration (T_{max}) at about (2.45 ± 0.01 h). This result was shorter than that reported for tilmicosin in goat (6.39 h; Ramadan, 1997), tilmicosin in sheep (3.9 h; Modric et al., 1998), tilmicosin in broiler chicken (3.99± 0.84, 5,82 ± 1.04 h; Abu-Basha et al., 2007) and tilmicosin in broiler chickens (3 h; Dimitrova et al., 2012b) and higher than those reported for tilmicosin in in cattle (0.5 h; Modric et al., 1998), tilmicosin in calf (1h; Dimitrova et al., 2012a). tilmicosin in cow (1h; Avci and Elmas, 2014) and tilmicosin in lactating goat (1.91 ± 0.19h and 1.46 ± 0.243h; for healthy and vaccinated ones, respectively El-Komy et al., 2016).

In the present work, following repeated oral administrations of tilmicosin, the obtained serum levels of tilmicosin in *Mycoplasma Gallisepticum*-infected broiler chickens were significantly lower than those in normal healthy ones. These lower serum concentrations of tilmicosin in experimentally *Mycoplasma Gallisepticum*-infected broiler chickens might be attributed the infection/inflammation further improves its tissue penetration (Modric et al., 1999) and was similar to data recorded by (Baggot, 1980; Naccari et al., 2001; Abo El-Ela et al., 2015; El-Komy et al., 2016).

The obtained results illustrated a significant decrease in the maximum serum concentration (C_{max}) in *Mycoplasma Gallisepticum*-infected broiler chickens than in normal broiler chickens following all doses. These results were similar with El-Komy et al., (2016) who found a significant decrease in C_{max} in experimentally *Pasterulla Multocida*-infected lactating goats than in healthy lactating goats following a single subcutaneous injection of 10 mg tilmicosin/kg b.wt. These existing differences are relatively common and are frequently related to or attributed to inter-species variation, used assay methods, dose of drug, chemical form of drug, amount of time between blood sampling and/or the health status, live body weight, age of the animal, climatic or other conditions related to experimental designs (Haddad et al., 1985).

In this experiment, the obtained results of serum and tissue residues of tilmicosin in slaughtered chickens following its repeated oral administrations of 25 mg/kg b.wt once daily for three consecutive days revealed a good distribution of tilmicosin in serum and in other tested tissues (lung, liver, kidney and heart). Tilmicosin appeared to be retained at higher concentrations and for longer times in the edible tissue than in serum. The concentrations of tilmicosin were high in tested tissues 24 h after stopping drug administration, then decreased slowly over time and tilmicosin residues were only detected in the lung till 5th day after sessation of tilmicosin administration. Lung had the highest concentration of tilmicosin followed by liver and kidney, whie the lowest concentration was determined in heart. This suggests that lung should be the target tissue for tilmicosin residues in broiler chickens.

Similar findings were previously reported for tilmicosin in fowl (Keles et al., 2001), tilmicosin in broiler chickens (Zhang et al., 2004) and tilmicosin in broiler chickens Elsayed et al., 2014).

The concentration of tilmicosin in rat's lungs was higher than the serum tilmicosin at all tested times and rats infected with *Mycoplasma pulmonis* had higher lung tilmicosin concentration than non-infected ones (Modric et al., 1999). This phenomenon was also seen in lung tissues of chickens, swine and cattle (Scorneaux and Shryock, 1998a, b, 1999). The high success rate of treatment is due to the prolonged presence of therapeutic concentrations of tilmicosin in the lung tissues (Papich and Riviere, 2001).

Using the microbiological assay technique, tilmicosin could not be detected in all tested tissues except in lung on the 5th day post last oral administration. In particular the high clearance of tilmicosin indicated the reduced possibility of finding residues of antimicrobial in broiler chickens a few days after treatment and necessity of shorter withdrawal time for this antimicrobial. The withdrawal period in this study was shown to be five days. The obtained results were similar to those recorded after oral administration of tilmicosin (4 days) in broiler chickens at 25 mg/kg b.wt. for 5 days (Elsayed et al., 2014) and after oral administration of tilmicosin in broiler chickens at 25 mg/kg b.wt. once daily for 5 days, withdrawal period of 6 days (Elbadawy and Aboubakr, 2017). On the other hand, the obtained result was shorter than that recorded after oral administration of tilmicosin to broiler chicken at 37.5 and 75.0 mg/l for 5 days, a pre-slaughter withdrawal time of more than 9 days is needed to ensure that the drug is eliminated from the tissues (Zhang et al, 2004).

5.CONCLUSION

Serum concentration of tilmicosin in normal and *Mycoplasma gallisepticum*-infected broiler chickens could be detected in a therapeutic level for 24 h following oral administration, exceed MIC of tilmicosin for *Mycoplasma gallisepticum*. Tilmicosin was rapidly absorbed and slowly eliminated after oral administration in broiler chicken.The highest concentration of tilmicosin was in lung tissue, suggesting that tilmicosin is suitable for treatment of respiratory infection in broiler chickens. The high concentration of tilmicosin in kidney tissue, suggest that tilmicosin was also suitable for treatment of urinary infection in broiler chickens. Tilmicosin withdrawal period of 5 days should be adopted.

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