International Journal of Pharmacology and Toxicology, 5 (1) (2017) 11-16

International Journal of Pharmacology and Toxicology

Website: www.sciencepubco.com/index.php/IJPT
doi: 10.14419/ijpt.v5i1.7084
Research paper

Pharmacokinetics, tissue residues of tilmicosin phosphate (tilmicoral®) and its *in vitro* and *in vivo* evaluation for the control of *Mycoplasma gallisepticum* infection in broiler chickens

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Abstract

The aim of present study was to determine the pharmacokinetics and tissue residues of tilmicosin phosphate (tilmicoral®) as well as its *in vitro* and *in vivo* evaluation for control of *Mycoplasma gallisepticum* (MG) infection in broiler chickens. Pharmacokinetics (single oral dose) and tissues residues (daily for five days) of tilmicosin (25 mg/kg b.wt) in broilers were investigated. Peak plasma concentration of tilmicosin was 1.25±0.0.09 µg/mL and achieved at 3.15±0.34 h. Elimination half-life was long (44.3±7.22 h) and V_{darea} was large (1.25±0.082 L/kg). Residue study revealed a good distribution and penetration of tilmicosine in lung, liver, kidney and muscles. Tilmicosin could not be detected in all tested tissues (except in lung) at 6 days after last administration. The MIC of tilmicosin and tylosin against MG were 0.054 and 0.319 µg/mL, respectively. MG infected chickens and treated by tilmicosin or tylosin showed a significant ($p<0.05$) improvement in mean body weights gain and a significant ($p<0.05$) decline in mean clinical signs score, air sac lesion score and mortality rate, however tilmicosin was a superior drug. *In conclusion*, timicoral® was a very effective medication for controlling MG infection in broiler chickens due to its rapid absorption, long elimination half-life, rapid and extensive penetration from blood into tissues especially lungs and air sacs. Additionally, tilmicoral® had a short withdrawal time. Moreover, its superior efficacy (*in vitro* and *in vivo*) against MG.

Keywords: *Broilers; Efficacy; Mycoplasma; Pharmacokinetics; Residues; Tilmicosin.*

1. Introduction

Tilmicosin is a semisynthetic, broad-spectrum, bacteriostatic macrolide antibiotic synthesized from tylosin with a wide range of veterinary uses. It shows promising prospect of application in clinical veterinary practice. Tilmicosin is a particularly useful drug for treatment and control of respiratory diseases due to its high volume of distribution, long half-life and preferential accumulation in lung. Tilmicosin is used for treatment and control of respiratory diseases caused by *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Pasteurella multocida* and *Ornithobacterium rhinotracheale* in broiler chickens (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, cattle and sheep (Moore et al. 1996, Hoar et al. 1998, Christodoulopoulos et al. 2002). The pharmacokinetics after parenteral administration of tilmicosin has been investigated 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, few studies are available on the pharmacokinetics of tilmicosin after oral administration to animals including fowl and swine (Keles et al. 2001, Shen et al. 2005).

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, a few information is currently available about the disposition and tissues residues of tilmicosine as well as its efficacy in broiler chickens. Therefore, the main purposes of the present study were to investigate and provide an overview of the pharmacokinetic profile and tissue residues of tilmicosin (tilmicoral)® in broiler chickens after oral administration to determine its withdrawal time. Moreover, the *in vitro* and *in vivo* evaluation of the efficacy of tilmicoral® in comparison with tylosin for the control of *Mycoplasma* infection in broiler chickens were examined.

2. Materials & methods

2.1. Drugs

1) Tilmicosin phosphate was obtained as an oral solution from ATCO Pharma for Pharmaceutical Industries, Cairo, Egypt under a trade name of tilmicoral®, a 25% oral solution. Each mL contains 250 mg of tilmicosin as tilmicosin phosphate. Tilmicosin has the chemical name of 20-Deoxo-20-(3, 5-dimethyl-1-piperidinyl) desmycosin with molecular formula of $C_{46}H_{80}N_2O_{13}$ (Fig.1) and of molecular weight: 869.13.

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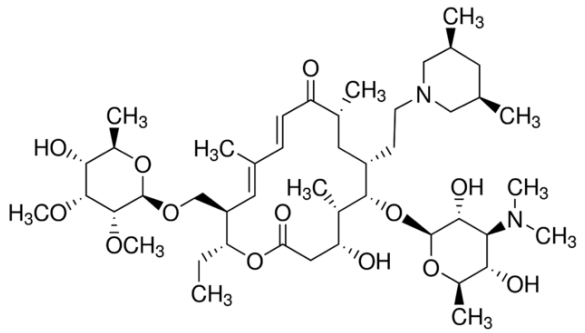

Fig. 1: Structural Formula of Timicosin ($C_{46}H_{80}N_2O_{13}$).

2) Tylan soluble®, a water soluble powder was obtained from (Elanco Animal Health, UK) and dispensed as tylosin tartrate 100 g.

2.2. Experimental chickens

Three hundred and seventy, healthy Hubbard chickens of different ages and weights were used in the pharmacokinetics, tissues residues and clinical efficacy studies. Chickens were of both sexes and purchased from a local poultry farm. The chickens were maintained at a suitable temperature and humidity according to their ages. The chickens had a free access to water and feed. The feed was free from antibacterial drugs. The experiments were performed in accordance with the guidelines set by the Ethical Committee of Faculty of Veterinary Medicine, Benha University, Egypt.

2.3. Experimental design

2.3.1. Pharmacokinetic study

Ten chickens of 45-50 days old weighing about 2-2.5 kg were used. Feed was withheld 12 h before giving drug and was offered 5 h after drug administration. Each chicken was given a single oral (directly into crop using gavage) dose of tilmicosin phosphate (tilmicoral®) at a level of 25 mg/kg body weight. This dose was selected according to the manufacturer's approved daily dose, which falls within the range applied by some researchers on efficacy studies of tilmicosin in the control of *Mycoplasma* infection in broilers (Jordan & Horrocks 1996, Kempf et al. 1997). Blood samples (1-1.5 mL) were collected from left wing vein or other veins into heparinized tubes at 0 (before dosing), 15 and 30 minutes, and at 1, 2, 4, 6, 8, 10, 12, 24, 48, and 72 h after dosing. The samples were centrifuged at 1000 *g* for 5 minutes and then plasma was collected and stored at -20°C until analysis.

The pharmacokinetic analysis of data was done using non-compartmental analysis based on statistical moment theory as described by Gibaldi & Perrier (1982), with the help of the WinNonLin program (version, 3.3, Pharsight, USA). The parameters calculated were: area under plasma concentration-time curve (AUC) using linear trapezoid method; mean residence time (MRT), where MRT = AUMC/AUC; volume of distribution (V_{darea}), where V_{darea} = (dose/AUC)×β; elimination rate (k_{el}), was determined by least-squares regression analysis of terminal log-linear phase of the plasma concentration–time profile (k_{el} = 2.303× slope); elimination half-life ($t_{1/2\beta}$), where $t_{1/2\beta}$ = 0.639/k_{el}; total body clearance (CL_{tot}), where CL_{tot} = dose/AUC. The maximum concentration (C_{max}) and the corresponding peak time (t_{max}) were determined by inspection of the individual drug plasma concentration–time profiles.

2.3.2. Tissue residue study

Sixty broiler chickens of 45-50 days age and of 2-2.5 kg weight were used. During acclimatization for 2 weeks, they were fed drug free balanced rations *ad libitum* with free access to water. The birds were administered tilmicosin orally in drinking water at a dose level of 25 mg/kg body weight (corresponding to 1 mL of tilmicoral® per liter of drinking water) daily for five days. Ten chickens were slaughtered on 1, 2, 4, 6, 10, and 14 days after the last administered dose. Samples of lung, liver, kidneys, heart and breast muscles, were taken for assay of tilmicosin.

Microbiological assay: Tilmicosin concentrations in both plasma and tissue samples were assayed using microbiological method of antibiotic using *Bacillus subtilis* (ATCC 6633) as a test organism for tilmicosin (Arret et al. 1971). Standard curves were constructed using distilled water and antibacterial free serum collected from chicken. Six wells, 8 mm in diameter, were cut at equal distances in standard Petri dishes containing 25 mL seeded agar. The wells were filled with 100 µL of either the test samples (serum or tissues) or tilmicosin standards. The plates were kept at room temperature for 2 h before being incubated at 37°C for 18 h. Zones of inhibition were measured using micrometer, and tilmicosin concentrations in the test samples were calculated from the standard curve. The lower detectable limit of tilmicosin was 0.025 µg/mL in both plasma and tissues. Semi logarithmic plots of inhibition zone diameter versus standard tilmicosin concentrations in serum and distilled water were linear between 0.025 and 50 µg/mL. Two grams of each tissue was minced in test tube with 2 mL distilled water. Mixtures were homogenized in homogenizer, centrifuged at 1000 *g* for five minutes, and the supernatant fluid of each sample was taken and directly assayed microbiologically for tilmicosin concentration.

2.3.3. Efficacy study

2.3.3.1. *In vitro* sensitivity

To investigate the effects of tilmicosin phosphate (timicoral®) along with other effective drugs (tylosin, doxycycline, enrofloxacin and erythromycin) on MG in broiler chickens, the minimum inhibitory concentrations (MIC) of these drugs against MG was determined by micro-broth dilution method as described by Whithear et al. (1983). A 24-h broth culture of MG in Frey's broth medium (Frey et al. 1986) was used as inoculum. An aliquot of about one mL was added into four mL of Frey's broth and incubated at 37°C until *Mycoplasma* growth turned the medium into orange color (pH 6.8-7.0). A two mL amount of this broth was added to 18 mL of Frey broth and homogenized. This gave sufficient inoculum to set up two replicate plates. Stock solutions of the tested drugs were aseptically prepared, filtered and stored at -20°C. Solutions were diluted in Frey's broth to the required concentrations. After that, 150 µL of the broth of pH 7.8, containing the desired density of the MG was inoculated into each well with a multichannel dispenser. Control plate containing Frey's broth with MG and did not contain any antibiotic was included in all tests. The inoculated plates were incubated aerobically at 37°C for 14 days. The MIC was taken as the lowest concentration of drug that completely prevent the growth (prevented color change in the medium). The MIC (µg/mL) was read when the phenol red indicator in the culture control had just changed from red to orange yellow. This was determined visually by comparing with the color of sterile Frey broth.

2.3.3.2. *In vivo* evaluation of the efficacy of tilmicosin and tylosin

This clinical trial was carried out on a 300 Hubbard, one day-old chicks taken from *Mycoplasma* positive breeder flocks and the presence of MG was confirmed serologically by detection of antibodies using Serum Plate Agglutination (SPA) test (Kempf et al. 1994) in a three trials each of 25 chick. The commercial stained MG antigen (Nobilis) produced by Intervet International B.V. Boxmeer, Holland, was used. These chicks were underwent routine vaccination program. Chicks were fed *ad libitum* with a commercial ration free of antibacterials, coccidiostats, and growth promoters. Water was provided *ad libitum*. At 22 days of age, respiratory signs of sneezing, nasal and lacrimal discharge and conjunctivitis as well as first mortalities were appeared in chicks.

At this point, chicks were divided into three groups, each of 100 bird. The first group was kept infected without treatment (infected untreated). The second group was treated with tilmicosin at a dose of 25 mg/kg body weight (corresponding to 1 mL of tilmicoral® per liter of drinking water) daily for 3 successive days. Third group was treated with tylosin orally in drinking water at a dose of of 50 mg/kg B. wt. corresponding to 500 mg tylan souble® per liter of drinking water daily for 3 successive days. Just after appearance of the first clinical signs and mortalities, the efficacy was evaluated by recording clinical signs, mortalities, post-mortem lesions and the average body weights and cumulative feed conversion of chickens in each group. All chickens (treated and untreated) were daily observed during and after treatment until end of the experiment (6 weeks old). The respiratory signs were individually observed in chickens and were taken scores according to Kempf et al. (1998) as following: 0 = no respiratory signs; 1 = slight symptoms (sneezing and few tracheal rals); 2 = moderate symptoms (sneezing and tracheal rals); 3 = severe symptoms (sneezing or frequent tracheal rales, dyspnea). The number of dead chickens in untreated and treated groups was recorded daily during and after treatment until end of experiment. Twenty chickens from each of treated and untreated chickens were sacrificed weekly after appearance of clinical signs until end of experiment. The typical air-sacs lesions of MG of dead and slaughtered chickens during and after medications were recorded and scored according to Kleven et al. (1972) as following: 0 = no air sac lesion observed, the air sac membranes were completely clear without gross alterations; 1 = the membranes were slightly cloudy without marked alterations; 2 = the membranes were slightly thickened and usually with small accumulations of cheesy-like substances; 3 = the membranes were clearly thickened and meaty in consistency with marked accumulation of clotted exudates confined to a single air sac; 4 = the membranes were with gross remarkable pathological alterations as score No. 3 but lesions were found in two or more air sacs. The average body weights and cumulative feed conversion of chickens in each group were measured after appearance of clinical signs at 22 days old and then weekly (at 29, 36 and 42 days of age).

2.4. Statistical analysis

The obtained data were statistically tested and analysed using the method of Snedecor & Cochran (1980). Differences of $p<0.05$ were considered significant.

3. Results

Tilmicoral® was well tolerated by birds and there were no unexpected incidents that could have influenced the outcome of the study. The concentrations of tilmicosin in chicken plasma were determined for 72 h after oral administration. The mean concentration-time profile of tilmicoral® is shown in Table 1 and Fig. 2. The drug was readily absorbed from gastrointestinal tract and was measurable at 60 minutes in all chickens. The pharmacokinetic parameters are recorded in Table 2. The mean AUC_{0-72} was 23.7 ± 4.15 µg·h/mL. The peak concentrations (C_{max}) in chicken plasma were 2.09 ± 0.37 µg/mL and achieved at (t_{max}) 3.15 ± 0.34 h. The elimination half-life ($t_{1/2\beta}$), elimination rate constant (k_{el}), total body clearance (Cl_{tot}), volume of distribution (V_{darea}) and mean residence time (MRT) of tilmicoral® were also determined and summarized in Table 2.

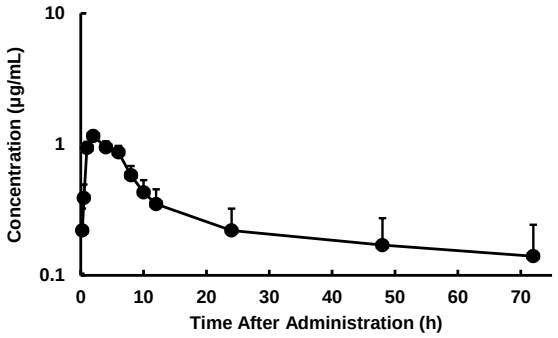

Fig. 2: Semilogarthimic Plot Showing the Plasma Concentration-Time Curve of Tilmicosine (25 Mg/Kg Body Weight) after Its Single Oral Administration to Chickens. Each Point and Vertical Bar Represents the Mean and Standard Error, Respectively (n = 10).

Table 1: Plasma Concentrations of Tilmicosin (µg/mL) in Chickens after Administration of a Single Oral Dose of 25 Mg/Kg Body Weight. Values are Mean ± SE (n = 10)

Time post administration (h)	Plasma concentration (µg/mL)
0.5	0.654 ± 0.0821
1	0.941 ± 0.371
2	1.16 ± 0.262
4	0.682 ± 0.171
6	0.873 ± 0.293
8	0.584 ± 0.142
10	0.432 ± 0.0912
12	0.351 ± 0.0611
24	0.223 ± 0.0323
48	0.312 ± 0.0521
72	0.233 ± 0.0323

Table 2: Pharmacokinetic Parameters of Tilmicosin in Chickens after Administration of a Single Oral Dose of 25 Mg/Kg Body Weight. Values are Mean ± SE (n = 10)

Parameter	Mean value ± SE
C_{max} (µg/mL)	1.25 ± 0.0921
t_{max} (h)	3.15 ± 0.344
AUC_{0-72} (µg·h/mL)	23.7 ± 4.15
$t_{1/2\beta}$ (h)	44.3 ± 7.22
k_{el} (h^{-1})	0.0251 ± 0.00381
Cl_{tot} (mL/(min.kg))	20.1 ± 3.03
V_{darea} (L/kg)	1.25± 0.0821
MRT (h)	68.4 ± 13.5

C_{max} = maximum plasma concentration; t_{max} = time to maximum plasma concentration; AUC_{0-72} = area under the plasma concentration–time curve from zero time to 72 h; $t_{1/2\beta}$ = elimination half-life; k_{el} = elimination rate constant; CL_{tot} = total body clearance; V_{darea} = volume of distribution area; MRT= mean residence time.

Tissues concentrations of tilmicosin following repeated oral administration of 25 mg/kg body weight once daily for 5 consecutive days in broiler chickens were recorded in Table 3. The data revealed a good distribution and penetration of tilmicoral® in lung, liver, kidney and muscles. The drug could not be detected (except in lung) by microbiological assay in all tested tissues at 6 days after last administration.

Table 3: Tissue Concentrations (µg/G) of Tilmicosin Following Oral Administration of 25 Mg/Kg Body Weight Once Daily for Five Consecutive Days in Broiler Chicken (n = 10).

Time (h)	Mean ± SD (n=10)				
	Lung	Liver	Kidney	Heart	Breast muscles
24	8.45±0.35	4.55±0.12	3.63±0.15	3.25±0.12	2.99±0.16
48	5.96±0.25	2.95±0.13	2.75±0.072	2.15±0.091	2.15±0.11
96	2.35±0.12	1.25±0.072	1.18±0.073	1.15±0.042	1.12±0.07
144	0.95±0.052	–	–	–	–
240	–	–	–	–	–
336	–	–	–	–	–

– = not detected.

The MICs for tilmicosin, tylosin, doxycycline, enrofloxacin and erythromycin, were determined and were 0.054, 0.319, 1.12, 0.875, 0.849 µg/mL, respectively. Tilmicosin had the smallest MIC followed by tylosin while doxycycline had the largest MIC which indicated that MG had a resistance to this antibiotic.

The tested diluted sera of one day-old chicks showed positive results to SPA test against stained antigen of MG in 4/25 (16%), 5/25 (20%) and 8/25 (32%) in trial 1, 2 and 3, respectively.

Table 4 showed the mean clinical score in MG infected untreated and infected treated groups. The first clinical signs of MG infection (sneezing and/or rales) were detected at 22 days of age with a mean clinical score of 1.98. Significant ($p<0.05$) difference in mean clinical score was noticed between MG infected untreated group and treated groups during experiment. Chickens in tilmicosin and tylosin treated groups showed a gradual and significant ($p<0.05$) decline in the mean clinical score to reach their lowest values (1.015) and (1.055) in tilmicosin and tylosin treated chickens, respectively during 33-42 days of age.

The results of mortality rate in MG infected untreated and infected treated groups were presented in Table 5. From the table, it could observe that the first recording of mortalities was associated with appearance of clinical signs at 22 days of age and mortality rate was (4%). Although there was no significant ($p<0.05$) difference in mortality rate between tilmicosin and tylosin treated groups but there was a difference ($p<0.05$) between infected untreated group and treated ones at all intervals of study. Gradual decline in mortality rate was observed in treated groups to reach the least values (1.10%) and (2.25%) in tilmicosin and tylosin treated group, respectively at the last interval of the study (33-42 days of age).

Table 4: The Mean Clinical Score in *Mycoplasma gallisepticum* Infected Untreated and Infected Treated Groups.

Group	Before treatment	During treatment	After treatment	
	(day 22)	(day 22-24)	(day 25-32)	(day 33-42)
Infected untreated	1.98±0.35[a]	2.57±0.46[a]	2.32±0.43[a]	2.13±0.27[a]
Tilmicosin treated	1.98±0.35[a]	1.79±0.27[c]	1.15±0.14[c]	1.015±0.021[c]
Tylosin treated	1.98±0.35[a]	1.87±0.32[c]	1.21±0.19[c]	1.055±0.052[c]

* Within a column, values followed by different lowercase letters are significantly different ($p < 0.05$).

Table 5: The Mortality Rate in *Mycoplasma gallisepticum* Infected Untreated and Infected Treated Groups.

Group	Before treatment	During treatment	After treatment	
	(day 22)	(day 22-24)	(day 25-32)	(day 33-42)
Infected untreated	4/100 (4%)[a]	8/96 (8.33%)[a]	6/88 (6.82%)[a]	4/82 (4.88)[a]
Tilmicosin treated	4/100 (4%)[a]	3/96 (3.13 %)[c]	2/93 (2.15%)[d]	1/91 (1.10%)[d]
Tylosin treated	4/100 (4%)[a]	4/96 (4.17%)[c]	3/92 (3.26%)[d]	2/89 (2.25%)[d]

* Within a column, values followed by different lowercase letters are significantly different ($p < 0.05$).

Table 6 illustrated the mean air-sac lesion scores in MG infected untreated and infected treated groups. Dead as well as slaughtered chickens in MG-infected untreated group showed significant ($p<0.05$) elevations in mean air-sac lesion score in comparison with treated ones along the different intervals of study. Moreover; there was a significant ($p<0.05$) difference between tilmicosin treated and tylosin treated birds. The lowest mean air-sac lesion score was observed in chickens treated with tilmicosin.

Table 6: The Mean Air-Sac Lesion Score in *Mycoplasma gallisepticum* Infected Untreated and Infected Treated Groups.

Group	Age (days)		
	(22-29)	(30-36)	(37-42)
Group 1: infected untreated	2.85±0.22[a]	3.41±0.32[a]	3.04±0.012[a]
Group 2: tilmicosin treated	1.46±0.23[c]	1.12±0.20[b]	0.83±0.10[c]
Group 3: tylosin treated	1.97±0.34[b]	1.31±0.23[b]	1.02±0.22[b]

* Within a column, values followed by different lowercase letters are significantly different ($p < 0.05$).

Table 7 presented the results of average body weights and cumulative feed conversion of surviving chickens in MG infected untreated and infected treated groups. There were significant ($p<0.05$) differences in average body weights between infected untreated birds and treated ones at different weeks intervals of study. Although there were no significant ($p<0.05$) differences between tilmicosin treated group and tylosin treated one, but tilmicosin treated group showed the best average body weights. The results of cumulative feed conversion clarified that, tilmicosin treated chickens showed the best feed conversion (1.85) followed by tylosin treated one (1.97) while the infected untreated chickens showed the lowest (2.87) feed conversion.

Table 7: The Average Body Weights (G) and the Cumulative Feed Conversion (CFC) of Surviving Birds in *Mycoplasma gallisepticum* Infected Untreated and Infected Treated Groups.

Group	The average body weights					
	Before treatment	After treatment			Total feed intake	CFC
	(day 22)	(day 29)	(day 36)	(day 42)		
IU	430.5±115.3[a]	625.3±38.6[a]	705.3±33.4[a]	975.1±45.3[a]	2800±25.3[a]	2.87[a]
Til. T	650.7±12.6[b]	1350.4±25.2[c]	1350.2±50.1[c]	1755.4±60.2[c]	3250±30.3[c]	1.85[c]
Tyl. T	645.4±17.8[b]	975.5±33.3[c]	1250.4±40.9[c]	1631.0±55.1[c]	3215±38.3[c]	1.97[c]

IU= Infected Untreated; Til. T= Tilmicosin treated; Tyl. T= tylosin treated

* Within a column, values followed by different lowercase letters are significantly different ($p < 0.05$).

4. Discussion

Pharmacokinetics of tilmicosin after intravenous administration were limited and unsuccessful due to its considerable cardiovascular adverse effects and deaths (Abu-Basha et al. 2007, Main et al. 1996, Papich & Riviere 2001). Few studies are available on the pharmacokinetics of tilmicosin after oral administration. The pharmacokinetics and tissue concentrations of tilmicosin after a single oral dose (50 mg/kg body weight) in fowl was investigated (Keles et al. 2001). Tilmicosin was slowly eliminated from both serum and lung with mean half-life of 30.2 ± 2.38 h and 75.7±3.67 h, respectively. The mean C_{max} of tilmicosin was 6.2 times greater in lung (7.96±0.30 µg/mL) than that in serum (1.28±0.04 µg/mL) and was achieved at 4.66±2.0 h and 17.8±7.51 h, respectively (Keles et al. 2001).

In the present study, mean peak plasma concentration (1.25±0.09 µg/mL) of tilmicosin phosphate (tilmicoral®) is higher than MICs for *Ornithobacterium rhinotracheale* (0.06-1 µg/mL) (Varga et al. 2001) and *Mycoplasma synoviae* and MG (0.0125-0.1 µg/mL) and lower than the MICs for *Clostridium perfringens* strains isolated from commercial broiler farms (Watkins et al. 1997) and *Actinobacillus suis* and *Pasteurella multocida* isolated clinically from swine (DeRosa et al. 2000). This clearly demonstrates that administration of tilmicosin at the recommended dosage is effective for control of respiratory disease in several animal species (Moore et al. 1996, Christodoulopoulos et al. 2002) due to its prolonged stay in lung tissues at therapeutic concentrations (Papich & Riviere 2001). In rats infected with *Mycoplasma pulmonis*, tilmicosin concentration in lungs were higher than in serum at all times and were higher than non-infected rats (Modric et al. 1999). This results were seen also in lung tissues of chickens, swine and cattle (Scorneaux & Shryock 1998 a, b). The apparent volume of distribution of tilmicosin was large (>1 L/kg) indicating a large tissue distribution. These findings are in agreement with those reported in cows, sheep, goats and swine (Ziv et al. 1995, Ramadan 1997, Modric et al. 1998, Shen et al. 2005).

Residue levels of tilmicosin phosphate (tilmicoral®) after oral administration of 25 mg/kg body weight were highest in lung and lowest in muscle during and after treatment (Table 3) suggesting that lung is the target tissue for tilmicosin in broiler chickens. These results further revealed that, tilmicosin was absorbed and distributed rapidly in chicken's body. Tilmicosin characterized pharmacokinetically by rapid absorption, long elimination half-time, rapid and extensive penetration from blood into tissues. In addition, the apparent distribution volume is large (>1.0 L/kg). Warren et al. (1997) found that tilmicosin was detected in lung and air sac tissues within 6 h after dosing in drinking water, and after 24 h, the concentration in air sac exceeded that in lung. Tilmicosin residue was depleted completely from muscle and heart after four days, while from liver and kidney after five days and more slowly from lung (six days). Therefore, the recommended withdrawal time is six days. These results were similar to those obtained by Zhang et al. (2004).

The MIC of tilmicosin and tylosin against MG field strain was 0.054 and 0.319 µg/mL, respectively. This result confirm that of Jordan & Horrocks (1996) who recorded that tilmicosin had a lower MIC against several tested strains of MG than that of tylosin

at both initial reading (at pH 7.0) and final reading at 14 days of incubation.

Regarding the *in vivo* evaluation of the efficacy of tilmicosin and tylosin against MG, Table 4 showed the mean clinical score in MG infected untreated and infected treated groups. The mean clinical score between MG infected untreated and infected treated groups was significantly ($p<0.05$) different during the whole study course. Chickens in tilmicosin and tylosin treated groups showed a gradual and significant ($p<0.05$) decrease in mean clinical score to reach their lowest values (1.015) and (1.055) in tilmicosin and tylosin treated chickens, respectively during 33-42 days of age. This finding is in agreement with Jordan et al. 1999 who recorded that, turkey poults who received tilmicosin at dosage of 150 mg/L had a less clinical respiratory and nervous signs as well as a smaller clinical score (of respiratory and nervous signs) than those treated by tylosin at dosage of 500 mg/L in drinking water for 72 h. These data were also consistent with Stipkovits et al. (1977). They found that tylosin significantly decreased the clinical symptoms in MG infected chickens and turkey. The obtained clinical score in the current study is in agreement with Abd El-Ghany (2009) who recorded that chickens treated with tilmicosin showed a gradual and significant ($p<0.05$) decrease in mean clinical score to reach their lowest values (1.01) during 33-42 days of age. Ziv (1980) also found that when tylosin tartrate is given in drinking water at dose of 500-700 mg/L, a good antimycoplasmal activity was obtained.

The first mortalities was recorded at 22 days of age and was associated with appearance of clinical signs. There was no significant ($p<0.05$) difference in mortality rate between tilmicosin and tylosin treated groups but a significant difference ($p<0.05$) between infected untreated and treated chickens was found at all study intervals. Gradual decline in mortality rate was observed in treated groups to reach the least values (1.10%) and (2.25%) in tilmicosin and tylosin treated chickens, respectively at the last interval of the study (33-42 days of age). These data are in agreement with Jordan et al. (1999) who recorded that turkey poults who received tilmicosin at dosage of 75 and 150 mg/L had a less mortality than those treated by tylosin at dosage of 500 mg/L in drinking water for 72 h. Tilmicosin treatment at concentration of 0.125, 0.25 or 0.5 g/L significantly reduced mortality rate in MG-treated chickens compared with infected untreated group (Jordan & Horrocks 1996, Abd El-Ghany 2009).

The MG-infected untreated chickens showed significant ($p<0.05$) increases in the mean air sac lesion score in comparison with treated ones. Moreover; there was a significant ($p<0.05$) difference between tilmicosin treated and tylosin treated birds. Chickens treated with (tilmicoral®) showed a lower mean air-sac lesion score than tylosin medicated ones. Tilmicosin at 300-500 g/ton prevented the development of air-saculitis caused by MG infection (Shryock et al. 1994). In addition, the air saculitis of MG-inoculated and tilmicosin medicated birds were less than that for the inoculated and unmedicated ones (Jordan & Horrocks 1996, Abd El-Ghany 2009). Furthermore, tilmicosin treatment at different concentrations for 5 days significantly diminished the respiratory signs and air sac and peritonitis lesions caused by MG (Kempf et al. 1997, Charleston et al. 1998, Jordan et al. 1999, Abd El-Ghany 2009).

There were significant ($p<0.05$) differences in average body weights between infected untreated chickens and treated ones at different week intervals of study. Although there were no significant ($p<0.05$) differences between tilmicosin treated and tylosin treated group, but tilmicosin treated birds showed the best average body weights. Regarding the results of cumulative feed conversion, tilmicosin treated chickens showed the best feed conversion (1.85) followed by tylosin treated ones (1.97) while that of infected untreated birds was 2.87. Similar results were obtained by Kempf et al. (1997) who recorded that tilmicosin treatment at 50-300 mg/L significantly decreased growth losses. Additionally, Jordan et al. (1999) proved that the mean body weight gain of poults surviving to the end of experiment was significantly greater in the uninfected and MG-infected and tilmicosin treated poults than the only MG-infected ones. Moreover, similar results were also obtained by Abd El-Ghany (2009).

In conclusion; Timicoral® (tilmicosine phosphate) is a very effective drug for control of *Mycoplasma gallisepticum* infection in broiler chickens due to its rapid absorption, long elimination half-life, rapid and extensive penetration from blood into tissues especially lungs and air sacs. Additionally, tilmicoral® had a short withdrawal time. Moreover, its superior efficacy (*in vitro* and *in vivo*) over many drugs.

Acknowledgments

The authors would like to thank Prof. Dr. Ashraf Elkomy (pharmacology Department, Faculty of Veterinary Medicine, Benha University, Egypt) for his final revision of the manuscript. We also thank Dr. Sawsan Elbasiony, (Department of Poultry Diseases, Faculty of Veterinary Medicine, Benha University, Egypt) for her help and guide in assessment of severity of clinical signs, lesion scores and cumulative feed conversion.

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