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Pharmacokinetics of tylvalosin in healthy and experimentally *mycoplasma gallisepticum* infected broiler chickens

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Abstract : A study of tylvalosin pharmacokinetics was conducted in healthy and *Mycoplasma gallisepticum*-infected broiler chickens. Tylvalosin was administered intravenously and orally at a dose rate of (25 mg/kg b.wt.) to determine its concentrations in blood as well as its kinetic disposition. The serum concentration - time curve indicated a two compartment open model. Following intravenous injection, the mean elimination half-lives ($t_{1/2\beta}$) of (6.666±0.285 and 3.048±0.232 h) before and after infection, respectively. The apparent volume of distribution ($V_{d(area)}$) of tylvalosin was (3.802±0.148 and 0.657±0.367 L/Kg) with body clearance CL_{β} (0.953±0.040 and 1.976±o.743. L/kg/h) with mean of MRT was (9.314±0.407 and 1.739±0.779 h) in healthy and *M. gallisepticum* -infected chickens, respectively. Following oral administration, Tylvalosin was absorbed with ($t_{½ka}$) of (0.963±0.045 and 0.958±0.207 h) with peak serum concentration of (1.226±0.041 and 0.0760±0.024 µg/ml) at ($t_{max)}$ of (1.723±0.04 and 1.310±0.055 h) and eliminated with ($t_{½\beta}$) of (3.504±0.49 and 3.862±0.103 h) in healthy and infected chickens. The systemic bioavailability of Tylvalosin (F%) following oral administration was (48.39±0.001 and 72.96±0.003 %) in healthy and infected birds respectively. It is to conclude that serum and tissues Tylvalosin concentration following twice-daily dose of (25 mg/Kg b. wt./day) were suitable to maintain its therapeutic regimen for treatment of mycoplasma infection in broiler chickens, in addition mycoplasma infection significantly decrease and/or consumed serum concentration, increased elimination rate so it is recommended that dose rate is adjusted in case of mycoplasma infection.
Keywords: Tylvalosin; Disposition kinetics; Healthy; Broiler chickens; *Mycoplasma gallisepticum*.

Introduction

Avian mycoplasmosis causes considerable economical losses to the poultry industry, especially in chickens and turkeys, all over the world. *Mycoplasma gallisepticum* is responsible for chronic respiratory disease in chickens and for infectious sinusitis in turkeys. In broilers, it causes a reduction in weight gain, a decrease in feed conversion efficiency, an increased mortality rate, and increased condemnations at slaughter. In breeders and layers, the disease may cause a drop in egg production and an increase in embryo mortality **(Ley [1]).** Vertical transmission of *M. gallisepticum* has been documented and may result in infected progeny flocks **(Bradbury [2]).** *M. gallisepticum* can also be transmitted horizontally by contaminated fomites and workers **(Ley [1]).** Wild birds may also play a role in *M. gallisepticum* transmission. In recent years, certain strains of *M. gallisepticum* have caused disease in house finches (Carpodacus mexicanus) and infected similar songbirds in the United States **(Ley et al.,[3], Luttrell et al.,[4], Mikaelian et al.,[5]; Farmer et al.,[6]).**

Macrolide- antibiotics are commonly used against avian and animal mycoplasmosis due to their good distribution in most tissue particularly the respiratory tissues **(Giguere, [7])**. Tylvalosin is a new macrolide and derived from The fermentation of Tylosin results in the acetylation of the highly active 16-member lactone ring. These structure changes confer benefits related to rapid absorption, **(Huang et al.,[8])**. **WHO, [9]** mention that Tylvalosin (formerly acetyl isovaleryl tylosin) is derived from fermentation of factor A Tylosin with *Streptomyces thermotolerans*, the chemical formula of Tylvalosin (Acetyl isovaleryl tylosin) is $C_{53}H_{87}NO_{19}$ while the chemical nomenclature is: (4*R*,5*S*,6*S*,7*R*,9*R*,11*E*,13*E*,15*R*,16*R*)-15-{[6-deoxy-2,3-di-*O*-methyl -β-D-allopyranosyl) oxy] methyl} -6-({3,6-dideoxy -4-*O*-[2,6-dideoxy-3-*C*-methy l-4-*O*-(3-methylbutanoyl)-α-L-*ribo*-hexopyranosyl] -3-(dimethylamino) -β-D- glucopyranosyl} oxy) -16-ethy l-5,9,13- trimethyl-2, 10-dioxo-7- (2oxoethyl) oxacyclohexadeca-11,13-dien-4-yl acetate].

Tylvalosin tartrate is a white to light yellow powder and is not described in any Pharmacopoeia. Manufacture is via fermentation, using a genetically modified strain of *Streptomyces thermotolerans*. Tylvalosin is isolated from the fermentation broth and the tartrate salt is formed with the final material being spray dried. No organic solvents are employed during the manufacturing process, **(European Medicines Agency, [10]).**

Similarity, to the that of other macrolides, Tylvalosin has antibacterial activity against gram positive and some gram. Negative bacteria and mycoplasma species. Tylvalosin is recently used for treatment and meta-phylaxis of mycoplasmosis in poultry due to *M. gallisepticum* and *M. synovi*. **(EMA[11]; Forrester et al.[12])** as well as diseases associated with *clostridium perfringens* in chicken's replacement pullets & Turkeys, in addition to its anti-inflammatory property **(Zhao et al,[13])**. Few studies on the pharmacokinetics (PK) aspects of Tylvalosin in chickens were performed following oral administration in the healthy birds. **(Cerda et al.[14]; Radi, [15]; Abo– El– Ela et al.,[16])** but not in diseased ones. Thus, this study was performed to investigate the disposition kinetics of tylvalosin in clinically healthy and experimentally *Mycoplasma gallisepticum* infected broiler chickens after its intravenous and oral administration.

Materials and Methods

Drug:

Avilosin^{Tm} is manufactured by ECO Animal health, London, UK. It is dispended as water soluble powder. Each 1 g contains 625 mg tylvalosin tartrate. It is administrated in a dose of (25 mg/kg b.wt.) according to manufacturer.

Mycoplasma gallisepticum:

The strain was obtained from Microbiology Department in the Faculty of Veterinary Medicine, Cairo University. The experimental infection was performed by intranasal administration of 10^6 *Mycoplasma* Units/ml **(Gharaibeh and Hailat, [17]).**

Animals and drug administration:

Twenty-four (24) broiler chicks was used in this study and classified into two equal groups (12) chicks in each one; Group (1) was apparently healthy and treated with Avilosin™ orally and intravenously, Group (2) was experimentally infected with *Mycoplasma gallisepticum* and treated with Avilosin™ orally and intravenously after appearing of clinical signs which appear after 6-8 days after experimental infection. Avilosin™ was administrated at a dose of (25 mg/kg b.wt.) according to manufacturer.

Blood samples:

Avilosin™ was administrated as single dose by i.v. route at a dose of 25 mg/kg b.wt. Blood samples were collected immediately before and at 0.25, 0.5, 1, 2, 4 ,6 ,8 ,12 and 24 hours following administration. After 10 days, Avilosin™ was administrated orally and blood samples were collected immediately before and at 0.5, 1, 2, 4 ,6 ,8 ,12, 24 hours following administration. The birds were re-administrated orally with tylvalosin with a daily dose of 25mg/Kg b.wt./day for 4 successive days, blood samples were collected daily before day administration. All collected samples were kept as frozen until analysis for determination of tylvalosin concentration in variant blood.

Analytical methods:

Serum concentrations of tylvalosin were determined using a high performance liquid chromatographic (HPLC) method. Sample analysis, solutions and HPLC conditions were carried out according to **(Lina,[18]).** 0.5 ml of serum was added to 3 ml of Acetonitrile in centrifuge and was mixed for 1 min by vortex, samples was centrifuged at 3000 rpm for 20 min, then the supernatant was transferred to other centrifuge tube and was evaporated under nitrogen flow to dryness, then 150 µl of mobile phase and 400 µl of Hexane was added to dry sample and mixed for 1 min by vortex, samples were centrifuged at 3000 rpm for 20 min, the supernatant was discarded and 50 µl was injected to HPLC.

Pharmacokinetic analysis:

The pharmacokinetic parameters were calculated by **PK Solver**: An add-in program for Microsoft Excel, version 2 **(Zhang et al.,[13]).** The pharmacokinetic parameters were derived by the usual technique from the time course of the studied antibiotic concentration in serum described by **(Baggot,[19]).** The non-compartmental pharmacokinetic parameters, volume of distribution at steady state ($Vd_{(ss)}$), body clearance ($Cl_{(B)}$) and the mean residence time (MRT) were calculated according to standard methods using statistical moment theory. The area under the blood concentration (Cpt) time (t) curve to infinity (AUC), and the area under the first moment curve (AUMC = Cpt × t2) were calculated from the first to the last blood and bronchial sample by using the trapezoidal rule, and an estimate of the residual area under the curve was obtained from Cp(t) / β, where Cp(t) is the last measured blood concentration of tylvalosin, and β the overall elimination rate constant. An estimate of the residual part of the AUMC curve was obtained from Cp(t) × t / β. The MRT, corrected for the duration of the infusion to give a corresponding value for a bolus injection, was calculated from AUC and AUMC (MRT = AUMC / AUC - T1/2), where T is the infusion time.

Statistical analysis:

The data were statistically analyzed using SPSS 14[20] software package (SAS, Cary, NC, USA). by using analysis of variance (ANOVA). The data were calculated as mean ± standard error.

Results

The calibration curves of tylvalosin were linear over the range of 0.039 – 10 µg/mL, and the correlation coefficients (r^2) were $>$0.999. The calculated limit of quantification was 0.039 µg/ml. The level of tylvalosin recovery calculated by comparing the peak height ratios for the serum samples and aqueous samples ranged from 81 – 83-7%. The inter-assay CV were 4%, 5%, and 3% while the intra-assay CV were 3%, 4% and 7% respectively, for tylvalosin concentrations of 20, 40 and 60 µg/ml. Following intravenous injection of tylvalosin (25 mg/kg body weight), its concentration decreased in a bi-exponential manner that could be described in a two-compartment open model. The mean serum concentration-time profiles of tylvalosin (25 mg/kg b.wt.) after a single IV administration in healthy and *Mycoplasma gallisepticum*-infected broiler chickens are shown in table 1 and the PK parameters in healthy and *Mycoplasma gallisepticum*-infected broiler chicken are shown in Table 2. In the healthy chickens, the serum concentration of tylvalosin was 3.89 µg/mL 15 min post-administration. This concentration declined gradually to 0.19 µg/mL at 24 h post-administration. Compared to the healthy group, the mean serum concentration of tylvalosin was significantly lower in *Mycoplasma gallisepticum*-infected broiler chickens at all times after infection and not detectable after 8 hours. Tylvalosin was slowly eliminated from the healthy group with ($t_{1/2\beta}$) of (6.66 h) compared to (3.04 h) for the infected group chickens. Following oral administration of tylvalosin (25 mg/kg), the mean peak concentration of tylvalosin in serum (C_{max}) of (1.22 and 0.76 µg/ml) and (T_{max}) was reached in (1.72 and 1.31 h) in healthy and *M. gallisepticum* -infected chickens, respectively. The mean serum concentration-time profiles of tylvalosin (25 mg/kg b.wt.) after single oral administration in healthy and *Mycoplasma gallisepticum*-infected broiler chickens are shown in table 1 and the PK parameters in healthy and *Mycoplasma gallisepticum*-infected broiler chicken are shown in Table 3. The mean elimination half-life ($t_{1/2\beta}$) after oral administration was (3.504 and 3.826 h) and a body clearance of (1.910 and 3.187 L/Kg/h). Absolute bioavailability (F) of tylvalosin after oral administration was 48.39 and 72.96 % while the protein binding was (67.3 and 61.3 %) in *Mycoplasma gallisepticum*-infected broiler chickens compared to the healthy chickens.

Table (1) Serum concentration of tylvalosin (25 mg/kg bw) in healthy and *Mycoplasma gallisepticum*-infected broiler chickens after intravenous and single and repeated oral administration.

Time	Intravenous administration		Oral administration	
	Healthy broiler chickens	*Mycoplasma gallisepticum*-infected broiler chickens	Healthy broiler chickens	*Mycoplasma gallisepticum*-infected broiler chickens
0.25	3.899±0.052	3.221±0.074*	-	-
0.5	3.001±0.044	0.970±0.041**	0.426±0.007	0.478±0.006**
1	2.507±0.053	0.675±0.005**	1.503±0.064	0.835±0.015**
2	2.132±0.032	0.590±0.002**	1.107±0.004	0.646±0.010**
4	1.845±0.029	0.412±0.020**	0.841±0.017	0.456±0.009**
6	1.451±0.033	0.192±0.014**	0.686±0.009	0.366±0.005*
8	1.202±0.024	0.098±0.003*	0.552±0.018	0.250±0.008*
12	0.678±0.094	ND	0.308±0.029	ND
24	0.195±0.020	ND	0.150±0.011	ND
48	⇨**Repeated doses for Oral administration**		0.273±0.009	0.198±0.006
72			0.185±0.009	0.146±0.006
96			0.312±0.003	0.308±0.005

***P≤ 0.05**
****P≤ 0.01**

Table (2) Pharmacokinetics (PK) parameters of tylvalosin (25 mg/kg bw) in healthy and *Mycoplasma gallisepticum*-infected broiler chickens after intravenous (IV) administration.

Pharmacokinetic Parameters	Unit	Healthy broiler chickens	*Mycoplasma gallisepticum*-infected broiler chickens
C_{P0}	µg.ml^{-1}	6.658±0.241	6.797±0.205
A	µg.ml^{-1}	3.976±0.214	5.912±0.197**
Alpha	h^{-1}	4.622±0.225	3.830±0.564
$t_{1/2\alpha}$	H	0.153±0.008	0.057±0.017**
B	µg.ml^{-1}	2.682±0.030	0.885±0.007**
B	h^{-1}	0.105±0.004	0.232±0.016**
$t_{1/2\beta}$	H	6.666±0.285	3.048±0.232**
K_{el}	h^{-1}	0.252±0.012	2.884±0.304**
k_{12}	h^{-1}	2.554±0.170	1.535±0.189**
k_{21}	h^{-1}	1.920±0.051	0.407±0.068**
$Vd_{(area)}$	L/Kg	3.802±0.148	0.657±0.367**
V_C	L/Kg	4.938±0.074	5.066±2.746
V_{dss}	L/Kg	8.740±0.102	5.724±3.116
CL_B	L/Kg/h	0.953±0.040	1.976±0.743
AUC_{0-t}	µg.ml/h	24.423±0.837	5.733±0.149**
AUMC	µg.ml/h	252.426±22.025	18.848±2.340**
MRT	H	9.314±0.407	1.739±0.779**

Data are presented as the mean ± SE. *$p < 0.05$, **$P \leq 0.01$, Vd(ss): volume of distribution at steady-state, $Vd_{(area)}$: volume of distribution based on the terminal phase, V_C: volume of concentration, AUC_{0-t}: area under the plasma concentration-time curve, F: bioavailability, C_0: initial concentration, Cl_B: total body clearance, $t_{1/2\beta}$: elimination half-life, MRT: mean residence time.

Table (3) Pharmacokinetics (PK) parameters of tylvalosin (25 mg/kg bw) in healthy and *Mycoplasma gallisepticum*-infected broiler chickens after oral administration.

Pharmacokinetic Parameters	Unit	Healthy broiler chickens	*Mycoplasma gallisepticum*-infected broiler chickens
A	**µg/ml**	21.173±1.297	13.72±1.530**
Alpha	**h^{-1}**	0.729±0.032	0.820±0.113
$t_{1/2\alpha}$	**H**	0.963±0.045	0.958±0.207
B	**µg/ml**	0.882±0.070	0.553±0.113*
Beta	**h^{-1}**	0.075±0.005	0.086±0.031
$t_{1/2\beta}$	**H**	3.504±0.049	3.826±0.103
Ka	**H**	0.799±0.030	1.302±0.172**
$t_{1/2}$Ka	**H**	0.875±0.034	0.563±0.060**
K_{el}	**µg.h/ml**	0.200±0.007	0.165±0.019
k_{12}	**h^{-1}**	0.327±0.015	0.6±0.0571
k_{21}	**h^{-1}**	0.278±0.026	0.365±0.119
$Vd_{(area)}$	**L/Kg**	9.543±0.119	14.473±2.776**
V_C	**L/Kg**	11.837±1.150	37.463±5.541**
CL_B	**L/Kg/h**	1.910±0.077	3.187±0.0701*
T_{max}	**H**	1.723±0.041	1.310±0.055**
C_{max}	**µg/ml**	1.226±0.024	0.760±0.024**
AUC_{0-t}	**µg.ml/h**	11.138±0.282	3.770±0.078**
AUMC	**µg.ml/h**	170.729±20.276	78.203±43.571
MRT	**H**	12.678±1.028	14.800±3.685
Bioavailability	**%**	48.39±0.001	72.96±0.003*

Data are presented as the mean ± SE. *$p < 0.05$, **$P \leq 0.01$, C_{max}: maximal concentration, t_{max}: when the maximal serum concentration is reached, Vd(ss): volume of distribution at steady-state, $Vd_{(area)}$: volume of distribution based on the terminal phase, V_C: volume of concentration, AUC_{0-t}: aea under the plasma concentration-time curve, F: bioavailability, Cl_B: total body clearance, $t_{1/2\beta}$: elimination half-life, MRT: mean residence time.

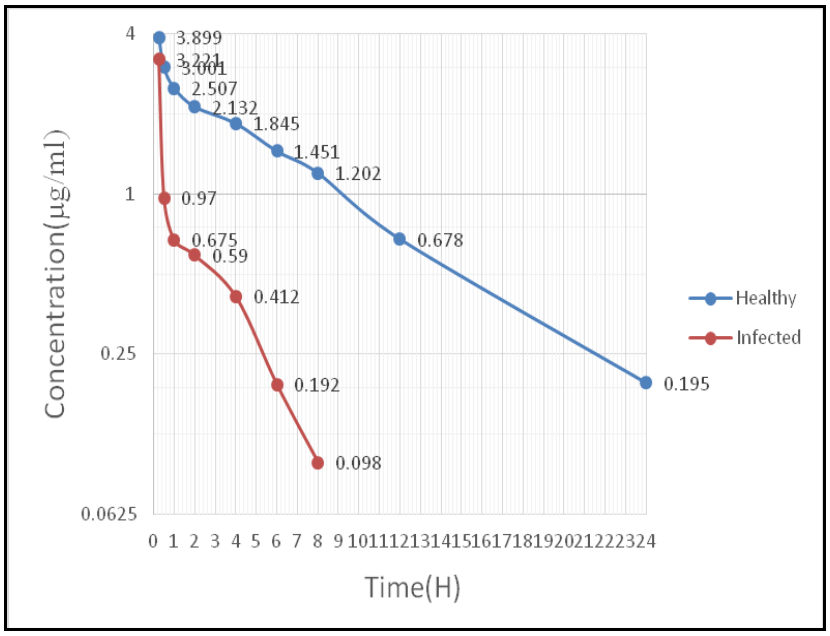

Fig (1): Tylvalosin concentration in serum after intravenous administration of 25mg/kg B.W in healthy and experimentally infected chickens with *Mycoplasma gallisepticum.*

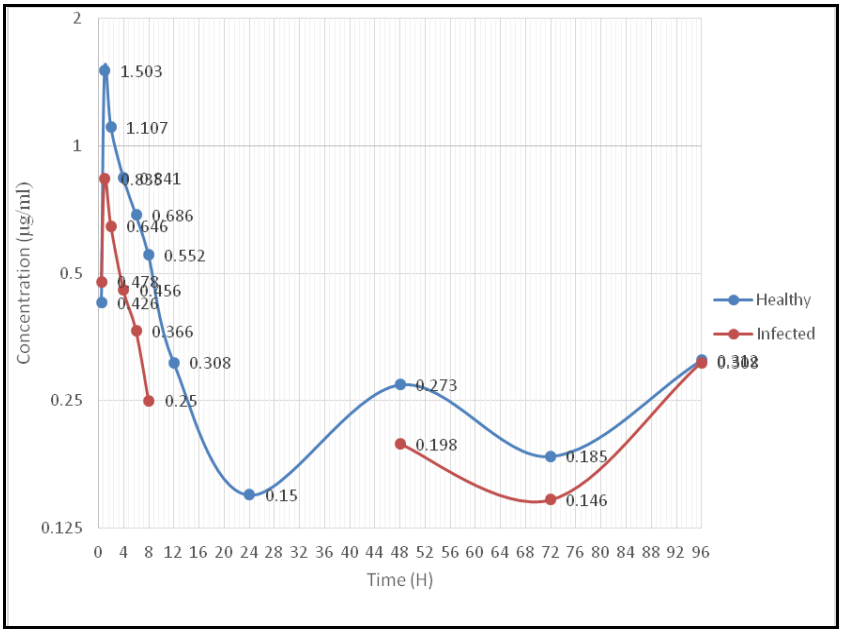

Fig (2): Tylvalosin concentration in serum after oral administration of 25mg/kg B.W in healthy and experimentally infected chickens with *Mycoplasma gallisepticum*

Discussion

As macrolides, including Tylvalosin are commonly used to treat CRD, a possible positive effect of Tylvalosin to cure the *Mycoplasma gallisepticum* infection in broiler chickens, can be put forward. To identify the disposition of Tylvalosin in serum broiler chickens, a pharmacokinetic behavior of the drug in healthy and experimentally *Mycoplasma gallisepticum* infected broiler chickens, was performed following its single IV and single and repeated oral administration…

The present work demonstrated that Tylvalosin blood concentration data were best fitted to fallow a two compartment open model after single dosing of (25 mg Kg/b.wt.), IV. In healthy and infected chickens.

Similar findings were recently reported in healthy broiler chickens by **(Baggot and Gingerich, [21]; Abo El-Ela et al., [16]; Radi [15]).**

The observed distribution half-life ($t_{½\alpha}$) values of Tylvalosin following IV. administration (0.153 h) in healthy chickens and (0.057 h) in infected ones indicates that infected birds distribute the drug faster than healthy ones. In contrast, Tylvalosin is showed a shorter distributed half-life ($t_{½\alpha}$) following single IV dose in turkeys (0.076 h) **(Radi, [15])**. In addition, Tylvalosin was distributed in the central compartment with a value of distribution (Vc) of (3.80 L/kg) and volume of steady state (V_{dss}) of (8.74 L/kg). This indicated that the peripheral compartment is the major compartment for Tylvalosin distribution at steady state, our results of (V_{dss}) Was higher in healthy broilers that determined in healthy turkeys (V_{dss} = 1.155 L/Kg). These values are higher than those values previously repeated for Tylosin in broiler chickens **(Kowalski et al., [22])**. These findings are consisted with those reported by **(Stuart et al., [23])** who found that the differences in kinetic parameters is referred the changes in chemical structure of Tylvalosin than Tylosin.

On the other hand, the results showed that the distribution values are higher and clearance rate faster (as shown by shorter elimination half-life), in *mycoplasma gallisepticum* infected chickens as compared with healthy ones. ($t_{1/2\beta}$ = 3.04 h & CL_B= 1.97 l/kg and $t_{1/2\beta}$ = 6.66 h & CL_B= 0.953 l/kg respectively), this may attribute to a higher penetration of the Tylvalosin to diseased tissues **(Baggot, [24]; Kosters et al.,[25]; Atef et al., [26])**. Furthermore, inflammation decreased the protein bending tendency due to hyperproteinemia lead to lowering of serum concentration of administrated drugs **(Baggot, [24]; Riviere [27])**. The recorded low value of protein bending tendency of Tylvalosin in infected birds (61.3%) compared with healthy ones (76.3%) and findings of **Baggot,[24]; Riviere[27]**.

Following a single oral administration of Tylvalosin at dose of 25mg/ kg b.wt., the drug is detected in serum 30 mints post-administration (0.426 mg/ ml & 0.478 mg/ml in healthy and mycoplasma infected broiler chickens. The concentration was continued to increase to reach maximum concentration. (Cmax) of (1.226 mg/ml and 0.760 mg/ml) at (t_{max}) (1.72 and 1.31 h) in healthy and infected ones, respectively. Tylvalosin persisted in lower concentration in infected birds than healthy ones for 8 hours and 24 hours, respectively. These concentrations were higher than MICs for Tylvalosin (MIC 50 and MIC 90 of 0.016 and 0.06 mg/ml) respectively **(Forrester et al., [12])**. The result of Cmax. In healthy broilers chickens is consisted with that reported by **Creda et al.,[14]** which was (1.64 mg/ml) and lower than value recorded by **Abo- EL- Ela et al.,[16]** which was (3.27mg/ml).

In addition, the drug was rapidly absorbed in healthy birds and widely distributed to tissues indicated by low absorption rate constant (Ka = 0.799 $^{-}$h) and absorption half- life ($t_{½Ka}$= 0.876 h) Those values were higher than those values obtained by **Creda et al., [14]** which was (0.175 h) and **Abo- El- Ela et al., [16]** which was (0.69 h) in broiler chickens. These variations in the absorption profile of the drug were interpreted by **Creda et al., [14]** due to species variations as well as the absorption may be decreased as consequence of presented food and due to the possible interactions between Macrolide antibiotics and intestinal Lactobacillus flora in the crop **(Devriese and Dutta[28]).**

In the present investigation, the absorption rate of Tylvalosin given orally in infected chickens was slower than the healthy ones. As indicated by its high value of ($t_{½Ka}$) which was (0.875 h) and higher value of (Cmax) in healthy chickens (1.226 mg/ml). In addition, the higher values of distribution of Tylvalosin in infected birds, recorded have led to lower serum concentration of Tylvalosin in these infected birds as well as the higher rate of drug consuming by bacterial infection. These findings were attributed to the phenomena previously reported and discussed by **(Baggot,[24]; Kosters et al.,[25]; Atef et al.,[6] and Riviere,[27]).**

Tylvalosin is eliminated with elimination half-life ($t_{½\beta}$) of (3.504 and 3.862 h) and total body clearance (cl_{β}) of (1.910 and 3.187 L/Kg/h) in healthy and infected broiler chickens. This indicate the faster clearance of the drug in infected birds than the healthy ones are closed with the persistence the drug till 8 hours over MICs in infected birds comparable with 24 hours in healthy ones.

On the other hand, the value of ($t_{½\beta}$) recorded in healthy birds following single oral administration of the drug (25 mg/Kg b.wt.) is higher than those reported by **(Creda et al., [14])** which was (1.61 h) and **(Abo-El-Ela et al., [16])** which was (2.42 h) in broiler chickens and (0.467h) in turkeys **(Radi, [15]).** The minor differences may have attributed to physiological and anatomical differences between used species **(Riviere,[27]).**

The systemic bioavailability of Tylvalosin after oral administration was (48.39 and 72.96 %) in health and infected broiler chickens respectively. The lower values of systemic bioavailability in healthy chickens than those recorded for infected ones is another indication pattern of the higher volume of tissue distribution of the drug in infected birds than healthy ones, and observed with our findings in contrast lower value of systemic bioavailability of Tylvalosin have been recorded by **(Radi,[15])** in normal Turkeys after oral administration of the drug (25 mg/Kg b.wt.).

Conclusion

It is to conclude that serum and tissues Tylvalosin concentration following twice-daily dose of (25 mg/Kg b. wt./day) were suitable to maintain its therapeutic regimen for treatment of mycoplasma infection in broiler chickens, in addition mycoplasma infection significantly decrease and/or consumed serum concentration, increased elimination rate so it is recommended that dose rate is adjusted in case of mycoplasma infection.

Refreances:

1. Ley, D.H. (2008): Mycoplasma gallisepticum infection. In Y.M. Saif, A.M. Fadly, J.R. Glission, L.R. McDougald, L.K. Nolan & D.E. Swayne (Eds.). Diseases of Poultry 12th edn (pp. 807-834). Ames, IA: Blackwell Publishing
2. Bradbury, J.M. (2001): Avian mycoplasmas. In F. Jordan, M. Pattison, D. Alexander & T. Faragher (Eds.). Poultry Diseases 5th edn (pp. 178-193). London: W.B. Saunders
3. Ley, D.H.; Berkhoff, J.E. and McLaren, J.M. (1996): Mycoplasma gallisepticum isolated from house finches (Carpodacus mexicanus) with conjunctivitis. Avian Diseases, 40, 480-483
4. Luttrell, M.P.; Stallknecht, D.E.; Kleven, S.H.; Kavanaugh, D.M.; Corn, J.L. and Fischer, J.R. (2001): Mycoplasma gallisepticum in house finches (Carpodacus mexicanus) and other wild birds associated with poultry production facilities. Avian Diseases, 45, 321-329
5. Mikaelian, I.; Ley, D.H.; Claveau, R.; Lemieux, M. and Berube, J.P. (2001): Mycoplasmosis in evening and pine grosbeaks with conjunctivitis in Quebec. Journal of Wildlife Diseases, 37, 826-830
6. Farmer, K.L.; Hill, G.E. and Roberts, S.R. (2005): Susceptibility of wild songbirds to the house finch strain of Mycoplasma gallisepticum. Journal of Wildlife Diseases, 41, 317-325
7. Giguere, S. (2013): Macrolides, azalides and ketolides, antimicrobial therapy in Veterinary Medicine, Black well publishing, Oxford, pp. 191-205
8. Huang, G.; Okabe, M.; Kahar, P.; Tsunekawa, H., and Park, Y.(2001): Optimization of Tylosin feeding rate profile in production of acetylisovalerylTylosin (AIV) from Tylosin by *Streptomyces thermotolerans* YN554. *J. Biosci. Bioeng*., (91), 504-508
9. World Health Organization, (2007): WHO Drug Information, Vol. 21, NO. 1, PP 80
10. European Medicines Agency, (2009): Scientific discussion for the assessment of aivilosin, PP:1,4,9,10,16,17,30,31
11. EMA. (2004): Aivlosin, Summary of Product Characteristics. European Medicines Agency;. Available from: http://www.ema.europa.eu/docs /en.GB/document_library/EPAR-Product Information /veterinary /000083/WC500061063 .pdf
12. Forrester, C.A.; Bradbury, J.M.; Dare, C.M.; Domangue, R.J.; Windsor, H.; Tasker, J.B., and Mockett., A. P. A., (2011): Mycoplasma gallisepticum in pheasants and the efficacy of Tylvalosin to treat the disease, Avian Pathology, 40:6, P: 581-586
13. Zhang, Y.; Huo, M.; Jianping Zhou, J. and Xie, S. (2010): Department of Pharmaceutics, China Pharmaceutical University, No.24, Tongjiaxiang, 210009, Nanjing, China
14. Cerda, R.O.; Petruccelli, M.; Piscopo, M.; Origlia, J. and landoni, M. (2009): Impact of the type of catheter on the absorption of Tylvalosin (acetyl valeryl tylosin) administered orally to broiler chickens, J. Vet. Pharmacol. Therap., 33, 202-203
15. Radi, A. M. (2016): Pharmacokinetic and bioavailability of Tylvalosin after oral, intramuscular and intravenous administration in turkys, Inter. J., of Pharmacy and Pharmaceutical Science, Vol. 8, Issue 2, 140-144

16. Abo El-Ela, F.I.; El-Banna, H.A.; El-Dean, M.B.; El-Gendy, A.A. and Tohamy, M.A. (2015): Pharmacokinetics of Tylvalosin alone or in combination with Vitamin E in broiler chickens. Asian, J. of animal and Veterinary advances 10: 556-566
17. Gharaibeh, S. and Hailat, A. (2011): Mycoplasma gallisepticum expermimental infection and tissue distribution in chickens, sparrow and pigeon, Avian path., 40:4, 349-354
18. Lina, L. (2008). Pharmacokinetics of Acetylisovaleryltylosin Tartrate in Laying Hens, Master dissertation, Huazhong Agricultural University College of Veterinary Medicine, China
19. Baggot, J.D. (1978): Some aspects of clinical pharmacokinetics in veterinary medicine. Journal of Veterinary Pharmacology and Therapeutic., 1: 5-18
20. SSPS 18, PASW Statistics 18, Release 18.0.0 (jul 30,2009), (SAS, Cary, NC, USA).
21. Baggot J.D. and Gingerich, D.A. (1976): Pharmacokinetic interpretation of Erythromycin and Tylosin activity in serum after intravenous administration of a single dose to cows. Res. Vet. Sci., 21 :318 – 323
22. Kowalski, C.; Rolinski, z.; zan, X. and wawron, w. (2002): pharmacokinetics of Tylosin in broiler chickens. Pol. J. Vet. Sci., 27 – 30
23. Stuart, A.D.; Brown T.D.K.; Imerie, G.; Taskes J.b.; and Moketl A.P.A. (2007): Intracellular accumulation and trans-epithelial transport of airlosion, tylosion and tilmicosin. Pig J, 60: 26 – 35
24. Baggot, J.D. (1980): Distribution of antimicrobial agents in normal and diseased animals. Journal of the American Veterenary Medical Association, 176: 1086 – 1090
25. Kosters, J.; Sabrantzki, S.; and Jakopy, J.R. (1984): Pharmacokinetic of Gentamicin in healing pigeons with salmonellosis, Prackitsche, Tieraizt, 65: (8), 673 – 676
26. Atef, M.; Youssef, S. A. H.; Amer, AM. M. and El- Banna H. A. H. (1992): influence of E.coli infection on the Disposition Kinetic of nalidixic acid in broiler chicken. Deutche Tierarztliche. Wachenschrift, 99: 140 – 143
27. Riviere, J. E. (2009): Absorption, Distribution, Metabolism and Elimination in Veterinary and Therapeutics. 9tu ed. Wiley – Blackwell, Ames, IA; P. 47 – 74
28. Devriese L. A. and Dutta G. N. Effects of erythromycin-inactivating *Lactobacillus* crop flora on blood levels of erythromycin given orally to chicks. J. Vet. Pharmacol. Ther. 1984;7:49-53

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