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Comparative pharmacokinetics of metronidazole in healthy and *Trichomonas gallinae* infected pigeons (*Columba livia domestica*)

Q1 Q2 M. A. Tabari[a], B. Poźniak[b], M. R. Yousefi[c], M. R. Roudaki Sarvandani[d] and M. Giorgi[e,f]

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ABSTRACT

1. This study investigated the pharmacokinetics of metronidazole after intravenous (i.v.) and oral administration to healthy and experimentally *Trichomonas gallinae*-infected pigeons, and determined the *in vitro* antiprotozoal activity of metronidazole against *T. gallinae*.
2. Twelve pigeons which were experimentally infected to *T. gallinae* and twelve healthy pigeons received metronidazole at the dose of 25 mg/kg by oral or i.v. administration. Serial blood sampling was used for pharmacokinetic analysis. The metronidazole minimum lethal concentration (MLC) and the concentration killing 50% of the trophozoites (LC_{50}) in the culture media were determined.
3. *In vitro* data showed that the 24 h LC_{50} and MLC of metronidazole were 0.31 and 25 µg/ml, respectively. *In vivo* results showed no statistical differences between pharmacokinetics in infected and non-infected pigeons for both routes of administrations. The area under the curve was statistically higher after the i.v. administration in both infected and healthy pigeons. The mean oral bioavailability was similar in the infected (83.8%) and the healthy (81.5%) birds.
4. In conclusion, the pharmacokinetics of metronidazole in pigeons was not affected by experimentally-induced trichomoniasis. Despite *in vitro* susceptibility testing, which showed probable resistance of the isolated *T. gallinae* to metronidazole, five-day oral treatment of infected pigeons with 25 mg/kg metronidazole twice a day resulted in total eradication of trophozoites recovered in crop lavage of infected birds.

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Introduction

Avian trichomoniasis, caused by the flagellated protozoan *Trichomonas gallinae*, commonly manifests as a caseous lesion within the anterior region of the digestive tract. The lesions vary from mild, causing subclinical infections, to severe fatal inflammation which causes obstruction of the oesophageal lumen, leading to death due to starvation. In the acute severe form of trichomoniasis, various internal organs, including lungs, air sacs, myocardium and liver, may be affected (Gerhold et al. 2008).

Outbreaks of trichomoniasis have resulted in mass mortality in bird populations (Robinson et al. 2010; Lawson et al. 2011; Amin et al. 2014). *T. gallinae* infections cause significant economic impacts on commercial pigeon producers (*Columba livia domestica*) and those raising quarry birds (Stockdale et al. 2015). In addition, this disease has been diagnosed in domestic turkeys and chickens. Pathologic changes reported include masses and necrotic ulcers in the crop and oesophagus, accompanied by catarrhal enteritis in some cases (Taylor et al. 2007).

In the treatment of avian trichomoniasis, nitroimidazoles are the drugs of choice (Munoz et al. 1998). The most commonly used representative of these chemotherapeutics is metronidazole (1–2 hydroxyethyl-2-methyl-5-nitroimidazole) which is effective against a wide range of protozoal and anaerobic bacterial pathogens. The pharmacokinetics of metronidazole have been well characterised in horses (Steinman et al. 2000), camels, sheep, goats (Ali et al. 2003), hens (Cybulski et al. 1996), turkeys (Świtała et al. 2016) and some reptilian species (Kolmstetter et al. 1998; Bodri et al. 2006). Metronidazole is well absorbed after oral administration in many species, has a large volume of distribution and reaches high concentrations in tissues, including the central nervous system, placenta and bone, as well as in the peritoneal fluid and inflamed tissues (Steinman et al. 2000; Świtała et al. 2016;). The use of metronidazole in the food producing animals is banned in most countries (Davis et al. 2009). However, it is still of great importance for the treatment of diseases caused by susceptible organisms in the non-food producing animals. Despite the widespread use of metronidazole for the treatment of trichomoniasis in pigeons, studies on its pharmacokinetics in pigeons are scarce in the scientific literature. Only a single abstract in conference materials pertaining to this topic is available (Switala et al. 2009). Moreover, it is not known whether the disease condition (trichomoniasis) may affect the pharmacokinetics of the drug used to treat it.

The present study investigated the pharmacokinetics of metronidazole after intravenous (i.v.) and oral administrations in healthy and experimentally *Trichomonas*-infected pigeons, and ~~to~~ determine the *in vitro* antiprotozoal activity of metronidazole against *T. gallinae*.

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Material and methods

Parasites

Five pigeons with suspected trichomoniasis were purchased from local racing pigeon breeders in Babol, Mazandaran, Iran. In order to confirm *T. gallinae* as the pathogenic causative agent of the lesions, samples were taken from caseous lesions in the oropharyngeal cavity, using the wet mount method, and examined under a light microscope (100 and 400× magnification). Parasite cultures were prepared by immersing oral swabs in trypticase/yeast extract/maltose (TYM) medium supplemented with 10% foetal calf serum (Sigma-Aldrich Chemie Gmbh Munich, Germany), and incubated at 37°C. Isolates were sub-cultured every 48 h when the parasites showed normal morphology and more than 95% mobility. To gain axenic cultures, initial subcultures were supplemented with 120 international units streptomycin and penicillin (Rooyandarou, Tehran, Iran) (Amin et al. 2010).

In vitro sensitivity determination

To check the susceptibility of the isolated *T. gallinae* the cultured trophozoites were exposed to different concentrations of metronidazole in the culture medium. For this purpose, a stock solution was prepared by dissolving 2 mg of metronidazole (98%, Alborzdaru, Tehran, Iran) in 10 ml of TYM medium. To ~~improve dissolution,~~ the drug-containing medium was mechanically shaken at 37°C for 8 h, ~~which completely dissolved the drug~~. A volume of 100 µl of culture medium, containing approximately 1×10^4 parasites, was pipetted into each well of 300 µl sterile multi well plates. Then the pre-diluted metronidazole solution was added to the wells, with each well containing a two-fold dilution of the previous concentration. The final range of metronidazole concentrations was 100 to 0.39 µg/ml. The plates were incubated at 37°C, and every 12 h (at time points of 12, 24, 36, and 48 h) the number of dead trophozoites in the medium was counted using the trypan blue exclusion assay (Tabari et al. 2017). All experiments were run in triplicate. The minimum lethal concentration (MLC) was defined as the lowest concentration of metronidazole in the culture medium at which no motile trophozoites were observed, which was later confirmed by the absence of growth in 96 h cultivations. Additionally, to assess the concentration required for killing 50% of the trophozoites (LC_{50}), the E_{max} model was fitted to the experimental data using the nonlinear least-squares fitting with generalised reduced gradient method (Solver, Microsoft Excel). LC_{50} was calculated based on the standard Hill function:

$$E = \frac{E_{max}}{1 + \left(\frac{LC_{50}}{C}\right)^{nHill}}$$

where: E was killing effect (% of control), E_{max} was the maximal effect (fixed to 100%) and nHill was the Hill exponent.

Animals

Twenty-four male, clinically healthy pigeons of approximately 450 g weight were purchased from a local breeder in Behshahr, Mazandaran, Iran. The birds were tested by the wet mount method (Youssefi et al. 2017) to be free of *T. gallinae*, identified individually and placed in cages in a room with average temperature of 25 ± 1°C and water and feed (containing no antibiotic or antiparasitic) were provided *ad libitum*.

Experimental infection with T. gallinae

After 1 week of acclimatisation, the birds were randomly divided into two groups (n = 12). Pigeons in group one were experimentally infected with *T. gallinae* by intra-ingluvial inoculation with 0.5 ml of culture medium containing approximately 5×10^4 live trophozoites. Trichomoniasis infection was confirmed seven days post-inoculation by the presence of caseous lesions and microscopic observation of trophozoites in the birds' crop lavages. The study was conducted in accordance to animal welfare law and approved by the Institutional Ethics Committee for Animal Care and Use of the Amol University of Special Modern Technologies (approval number 99/712/4).

Study design

For the pharmacokinetic study, infected pigeons were subdivided into two groups (groups 1A and 1B; n = 6 in each group), and uninfected/healthy control pigeons were divided into two groups (groups 2A and 2B, n = 6 in each group). Group A animals (whether infected or not) received metronidazole i.v. at the dose of 25 mg/kg (volume of 2.25 ml per bird) and group B animals received the drug by gavage ~~in to~~ the crop at a dose of 25 mg/kg (volume of 2.25 ml per bird). All birds were fasted for 12 h before drug administration. Metronidazole (5 mg/ml, Metris, Claris Lifesciences, Gujarat, India) was administered i.v. *via* the right wing vein to birds in the groups 1A and 2A. For the oral dose, a single metronidazole 250 mg tablet (Cosar Pharmaceutical Company, Tehran, Iran) was crushed and mixed in 50 ml tap water to make an aqueous suspension which was administered orally *via* a soft tube to the crop of pigeons in groups 1B and 2B. Blood samples (0.5 ml) were collected from the left wing vein using a heparinised syringe and a 23 gauge needle at 0.125, 0.25, 0.5, 1, 2, 4, 6, 12, and 24 h for each bird. Plasma was separated by centrifugation, and samples were stored at −80° C until analysis.

Metronidazole analysis

Plasma metronidazole concentrations were determined by high performance liquid chromatography (HPLC) with a UV detector following the method described by Świtała et al. (2016). The HPLC system ~~consisted of~~ a guard column (ODS 4 mm 3.0 mm I.D, security guard, Phenomenex, Torrance, CA, USA) and a C18 column (Prodigy ODS 250 4.6 mm, 5 mm, Phenomenex, Torrance, CA, USA). The mobile phase consisted of ammonium acetate 0.05 M adjusted to pH 4.3 and acetonitrile (70:30). The flow rate of the mobile phase was 1.0 ml/min, and the drug was detected at UV wavelength of 320 nm. The limit of detection, calculated as a 3:1 signal to noise ratio, was 0.01 µg/ml, and the limit of quantitation, based on the 10:1 signal to noise ratio, was 0.04 µg/ml. The mean percentage recovery of metronidazole in plasma samples was more than 85%. The intra-day

and inter-day precision was tested using independently spiked blank plasma samples at the tolerance level and were determined as 2.0 and 3.9%, respectively. The extraction of metronidazole from plasma samples (0.5 ml) was performed in a 20% solution of trichloroacetic acid in methanol (0.25 ml), followed by centrifugation at 19 000 × *g* for 15 min. The clear supernatant was collected and 20 µl was injected to the HPLC system. Metronidazole concentration was calculated based on calibration curves prepared by spiking drug-free plasma as the analytical standard (Sigma, Germany), and the procedure was the same as described above. Calibration curves were prepared for metronidazole in the range of 0.05–30 µg/ml. The assay was linear over this range (r^2 = 0.99988).

Pharmacokinetic and statistical analysis

The concentration of metronidazole *vs* time was pharmacokinetically analysed using a non-compartment approach (ThothPro™ 4.3 software, Gdansk, Poland). The C_{max} was the observed peak plasma concentration and T_{max} was the time at peak plasma concentration. The elimination half-life ($t_{1/2\lambda z}$) was calculated using linear least squares regression analysis of the concentration-time curve, and the area under the curve (AUC) was calculated by the linear-up log-down rule to the final concentration-time point (Ct). From these values, the apparent volume of distribution (V_{ss} = dose × AUMC/AUC^2), mean residence time (MRT = AUMC/AUC) and clearance (CL = dose/AUC) were determined. The relative bioavailability (F) was calculated for each group using the following equation:

$$(\%)F_{(\text{OralInfested/noninfested})} = \text{individualAUC}_{(\text{OralInfested/noninfested})} / \text{averageAUC}_{(\text{IVInfested/noninfested})} \times 100$$

Limit values, R^2 value and AUCrest%, were set as >0.85 and <20%, respectively.

Data were found to be normally distributed (Kolmogorov-Smirnov test). The unpaired Student's t-test was used to verify statistically significant differences in pharmacokinetic estimates between groups using GraphPad Software (La Jolla, CA, USA). The pharmacokinetic parameters are presented as means ± SD and T_{max} (categorical variable) expressed as median and range. The differences in this latter parameter were analysed using the unpaired t-test with Welch's correction. In all experiments, differences were considered significant if $P < 0.05$.

Results

In vitro MLC and LC_{50} of metronidazole

The MLC and LC_{50} values of metronidazole on *T. gallinae* are shown in Table 1. After 12 h of incubation, the MLC was 50 µg/ml and LC_{50} was 1.34 µg/ml. At 24 and 36 h time points, metronidazole had MLC values of 25 and 6.25 µg/ml, respectively. The 24 h and 36 h LC_{50} values for metronidazole were 0.31, and 0.22 µg/ml, respectively. After 48 h at the metronidazole concentration of 1.5 µg/ml, no motile trophozoites were observed in the media, which indicated the MLC value for this timepoint. However, based on the mortality rates and fitted model, the 48 h LC_{50} could not be calculated for metronidazole. Figure 1 shows lethal activity curves of metronidazole on *T. gallinae* at different time points.

Table 1. *In vitro* minimum lethal concentration (MLC) and 50% lethal concentration (LC_{50}) values of metronidazole against *Trichomonas gallinae* at 12, 24, 36, and 48 h time points.

Metronidazole Concentration (µg/ml)		
Time (h)	MLC	LC_{50}
12	50	1.34 ± 0.28
24	25	0.31 ± 0.08
36	6.25	0.22 ± 0.03
48	1.5	n.d.

n.d.: not detected by the fitted model since all of the tested concentrations of metronidazole at 48 h time period led to higher than 50% mortality in *Trichomonas gallinae*.

Pharmacokinetics

Figures 2 and 3 show the plasma concentration–time profile of metronidazole in healthy and *Trichomonas*-infected pigeons after i.v. and oral administration, respectively. After both routes of administration, metronidazole was quantifiable from the first time point to 24 h.

The pharmacokinetic parameters are summarised in Tables 2 and 3. No statistical differences were found between infected and non-infected groups after i.v. and oral administrations. The AUC was found to be statistically higher after i.v. as compared to oral administration, in both infected and healthy pigeons. The mean oral F% was similar for the infected (83.8%) and the healthy (81.5%) birds.

Discussion

Despite the high importance of metronidazole in the treatment of trichomoniasis in pigeons, the available data on pharmacokinetics of this drug in pigeons have been limited to one brief conference abstract (Switala et al. 2009). The intention of this work was to fill this gap as well as to answer whether clinical trichomoniasis affects the absorption, distribution and elimination of metronidazole in pigeons. Additionally, for better interpretation of the achieved concentrations and pharmacokinetic profiles, the study assessed the *in vitro* killing effect of metronidazole against the field strain of *T. gallinae*.

In the present study, i.v. administration of 25 mg/kg metronidazole to healthy pigeons resulted in a volume of distribution (Vd) of 1.68 l/kg, and CL of 0.21 ml/g/h. In the *T. gallinae*-infected pigeons, after i.v. administration of the same dose of metronidazole, a Vd value of 2.03 l/kg and CL of 0.26 ml/g/h were obtained. No significant difference was noted between Vd and CL in healthy and *T. gallinae*-infected pigeons.

In the study of Switala et al. (2009), values of 0.61 l/kg, and 0.13 ml/g/h were reported for Vd and CL, respectively. The reported Vd of metronidazole in turkeys with average weight of 1.4 kg was 0.99 l/kg, which was reduced to 0.63 l/kg in turkeys with weight of 10.7 kg. In the low weight turkeys, Vd was higher than in heavier birds (Świtała et al. 2016). In the present study, the metronidazole Vd in pigeons with 450 g average weight (1.68 l/kg) was higher than reported in turkeys. The reported CL in 1.4 kg turkeys was 3.64 l/kg, which was higher than the obtained CL in pigeons. The higher Vd

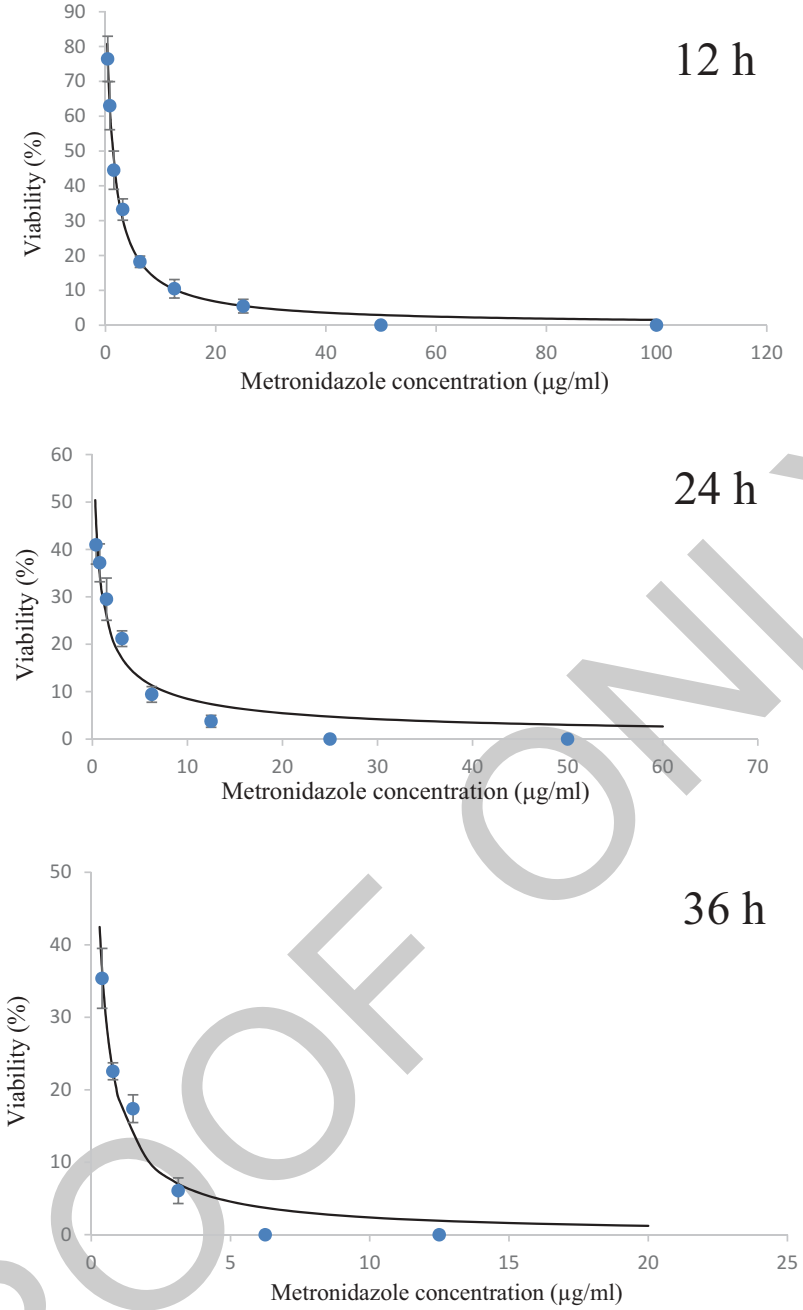

Figure 1. Concentration-viability curves of metronidazole *in vitro* lethal activity on *Trichomonas gallinae* at time points of 12, 24, and 36 h.

in pigeons probably is secondary to lower CL in pigeons in comparison to turkeys, as a decrease in CL is involved in Vd alterations (Toutain and Bousquet-Mélou 2004). A characteristic which may affect the pharmacokinetics of drugs in these birds is the flying ability of pigeons. In flying birds, the ratio of the muscle myofibrils with respect to the body mass and the heart capacity is significantly increased (Bishop 2005). These variables may be responsible for the higher Vd of metronidazole in pigeons, especially racing pigeons.

In the present study, the oral administration of metronidazole at a dose of 25 mg/kg to healthy pigeons resulted in a C_{max} of 14.82 µg/ml, T_{max} of 2 h and bioavailability of 81.51%. Switala et al. (2009) administered metronidazole orally to pigeons at a dose of 50 mg/kg and obtained a mean C_{max} of 57.4 µg/ml with the same T_{max} of 2 h. The reported bioavailability of 109.3% suggested complete absorption in their study. In another study on turkeys with an average weight of 1.4 kg receiving the same dose of metronidazole, the reported values for C_{max}, T_{max}, and bioavailability were 13.8 µg/ml, 3 h, and 95.6%, respectively (Świtała et al. 2016). In crossbreed laying hens, a C_{max} of 31.9 µg/ml, T_{max} of 2 h and bioavailability of 78.4% were reported after oral administration of 30 mg/kg metronidazole (Cybulski et al. 1996). The comparison of these parameters of absorption suggested that metronidazole is well absorbed from the gastrointestinal tract in avian species; however, high variability may be expected, even within one species. The $AUC_{(0-\infty)}$ after oral administration of metronidazole in pigeons was 93.15 mg/h/l which was lower in comparison to the $AUC_{(0-\infty)}$ (111.5 mg/h/l) previously reported for turkeys (Świtała et al. 2016). This difference may have been due to the lower bioavailability of metronidazole in pigeons, probably as a result of species variation or to a difference in the formulations administered. It is known that, in turkeys, even up to 25% of metronidazole may be hydroxylated in the liver (Świtała et al. 2016). It is not known how efficient the hepatic metabolism of metronidazole is in pigeons, therefore it is

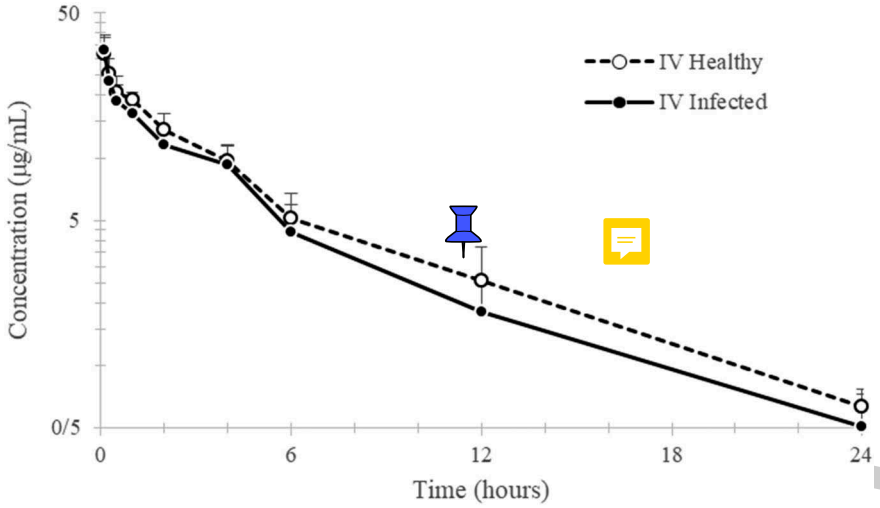

Figure 2. Semilog plasma metronidazole concentrations Vs time in healthy and *Trichomonas galliane*-infected pigeons after i.v. administration at the dose of 25 mg/kg (n = 6 in each group). Vertical bars represent the standard deviation.

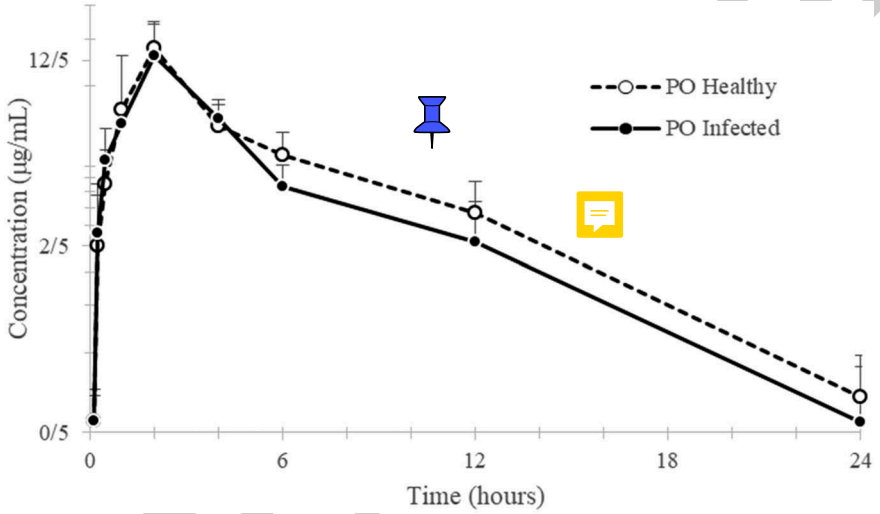

Figure 3. Semilog plasma metronidazole concentrations Vs time in healthy and *Trichomonas gallinae*-infected pigeons after oral administration at the dose of 25 mg/kg (n = 6 in each group). Vertical bars represent the standard deviation.

Table 2. Mean (±SD) pharmacokinetic parameters of metronidazole after intravenous and oral administration at a dosage of 25 mg/kg b.w. in *Trichomos gallinae*-infected pigeons (n = 6).

Parameter	Unit	Intravenous	Oral
$AUC_{(0-\infty)}$	mg/h/l	100.19 ± 18.79	83.32 ± 6.56*
$AUC_{(0-t)}$	mg/h/l	104.15 ± 20.57	87.31 ± 8.25*
$MRT_{(0-t)}$	h	4.90 ± 0.56	6.86 ± 0.80
K_{el}	1/h	0.13 ± 0.015	0.13 ± 0.021
$t_{1/2\lambda}$	h	5.54 ± 0.67	5.32 ± 0.85
C_{max}	µg/ml	-	13.15 ± 3.83
t_{max} §	h	-	2 (2–4)
CL	ml/g/h	0.26 ± 0.051	-
V_{area}	l/kg	2.03 ± 0.32	-
V_{ss}	l/kg	1.24 ± 0.13	-
F	%	-	83.84 ± 7.93
MAT	h	-	2.08 ± 1.24

$AUC_{(0-\infty)}$ = area under the curve from zero to the last, $AUC_{(0-t)}$ = area under the curve up to the last measurable concentration, $MRT_{(0-t)}$ = mean residence time, Kel = elimination rate constant, $t_{1/2\lambda}$ = elimination half-life, C_{max} = the maximum concentration, t_{max} = time at maximum concentration, CL = clearance,V_{area} = volume of distribution, V_{ss} = volume of distribution at steady-state, F = bioavailability, MAT = mean absorption time. § Median value (range)

* Significant difference between the groups, P < 0.05

Table 3. Mean (±SD) pharmacokinetic parameters of metronidazole after intravenous and oral administration at a dosage of 25 mg/kg b.w. in healthy pigeons (n = 6).

Parameter	Unit	Intravenous	Oral
$AUC_{(0-\infty)}$	mg/h/l	118.03 ± 15.57	93.15 ± 15.73*
$AUC_{(0-t)}$	mg/h/l	122.87 ± 15.72	100.16 ± 18.31*
$MRT_{(0-t)}$	h	5.27 ± 0.66	7.38 ± 0.55*
K_{el}	1/h	0.13 ± 0.012	0.12 ± 0.03
$t_{1/2\lambda}$	h	5.40 ± 0.53	6.16 ± 1.35
C_{max}	µg/ml	-	14.82 ± 3.75
t_{max} §	h	-	2 (1–2)
CL	ml/g/h	0.21 ± 0.03	-
V_{area}	l/kg	1.68 ± 0.34	-
V_{ss}	l/kg	1.12 ± 0.03	-
F	%	-	81.51 ± 14.90
MAT	h	-	2.81 ± 1.42

$AUC_{(0-\infty)}$ = area under the curve from zero to the last, $AUC_{(0-t)}$ = area under the curve up to the last measurable concentration, $MRT_{(0-t)}$ = mean residence time, Kel = elimination rate constant, $t_{1/2\lambda}$ = elimination half-life, C_{max} = the maximum concentration, t_{max} = time at maximum concentration, CL = clearance,V_{area} = volume of distribution, V_{ss} = volume of distribution at steady-state, F = bioavailability, MAT = mean absorption time. § Median value (range)

* Significant difference between the groups, P < 0.05

difficult to tell whether nearly 20% of the administered dose was lost in the systemic circulation due to elimination by the liver during the first-pass effect, or simply remained in the gastrointestinal tract. If the latter is the case, lower bioavailability could actually help in targeting the pathogens located in the digestive system.

In pigeons, the $t_{1/2\lambda}$ and MRT after 25 mg/kg oral administration of metronidazole were 6.16, and 7.38 h, respectively. In the work by Switala et al. (2009), the MRT of metronidazole in pigeons after oral administration of 50 mg/kg was 6.7 h (calculated based on the MRT of the i.v. administration and the mean absorption time, MAT). No estimate of $t_{1/2\lambda}$ was provided (Switala et al. 2009). In turkeys which received metronidazole at the dose of 25 mg/kg, the reported $t_{1/2\lambda}$ and MRT were 3.75, and 6.72 h, respectively. Higher values for $t_{1/2\lambda}$ and MRT in pigeons suggested slightly slower elimination of metronidazole in pigeons compared to turkeys. It was difficult to tell whether this was caused by the differences in the efficacy of metabolism or the perfusion of the eliminating organs. Although there was clear evidence that the elimination of metronidazole depended on haemodynamic parameters *e.g.* cardiac output (Grabowski et al. 2017), the species-specific degree of hepatic biotransformation may have affected the overall CL of the drug. In many species metronidazole is transformed to metabolites including hydroxy-metronidazole (El-Nahas and El-Ashmawy 2004). Since no metronidazole metabolites were measured in this study, it was difficult to identify the precise underlying mechanisms responsible for interspecies differences.

Studies have reported alterations in the pharmacokinetics of drugs in diseased animals, including during parasitic infections (Haritova et al. 2013; Kandeel 2015). However, the present study found no significant difference in metronidazole pharmacokinetics between healthy and *Trichomonas gallinae*-infected pigeons. This was in-line with the results of a study on metronidazole pharmacokinetics in healthy and amoebiasis-infected human volunteers who demonstrated no significant differences and no need to change the dose of metronidazole in the infected patients (Ashiq et al. 2011).

Metronidazole is effective in the control and treatment of avian trichomoniasis; however, there are several reports of nitroimidazole-resistant strains of *T. gallinae* (Lumeij and Zwijnenberg 1990; Munoz et al. 1998; Rouffaer et al. 2014; Tabari et al. 2017). Under *in vitro* conditions, a 24 h MLC of 15.6 µg/ml was set as the cut-off to determine the resistance of *T. gallinae* strains to nitroimidazoles (Rouffaer et al. 2014). As the 24 h MLC in the present study was 25 µg/ml, the strain used in this study was probably resistant to metronidazole. Nitroimidazole-resistant strains of *T. gallinae* are more frequent in racing than in wild pigeons. Prophylactic use of subtherapeutic doses of nitroimidazoles, particularly in racing pigeons, is one of the contributing factors for the development of resistance in *T. gallinae* strains (Rouffaer et al. 2014). In the present study, *T. gallinae* isolates were recovered from local racing pigeons. Probably the pigeons which have been used in this study were infected with metronidazole resistant or reduced susceptibility strains, but five-day oral treatment of infected pigeons with metronidazole at the dose of 25 mg/kg twice a day after the pharmacokinetic study resulted in total eradication of trophozoites recovered in the crop lavage of infected birds (data not shown). In common with the present study, Franssen and Lumeij (1992) reported that, under *in vitro* conditions, six out of eight *T. gallinae* isolates recovered from racing pigeons in the Netherlands showed resistance to nitroimidazoles including ronidazole, carnidazole, and metronidazole, even though increasing the administered *in vivo* doses resulted in the elimination of trichomoniasis infection in the affected birds.

The discrepancy which has been observed between *in vitro* and *in vivo* results in the present study was probably due to the hepatic metabolism of metronidazole and its hydroxy metabolite. It has been a long time since the report of higher activity of hydroxy metabolite compared to the parent compound against some strains of *Gardnerella vaginalis* in human medicine (Easmon et al. 1982). In addition, a synergistic effect has been reported to exist between hydroxy metronidazole and metronidazole against *Bacteroides* spp. under *in vitro* conditions (Pendland et al. 1994). However, to date, no report on the activity of metronidazole metabolites on the susceptible veterinary pathogens and their probable role in the treatment of infected animals has been published. Based on previous reports and the findings in the present study, it can be suggested that *in vitro* metronidazole susceptibility tests need re-evaluation and the possible role of active metabolites needs ~~taking in to~~ consideration.

In conclusion, the pharmacokinetics of metronidazole in pigeons is not affected by experimentally-induced trichomoniasis. Further studies on metabolites of metronidazole in pigeons are needed for better understanding of pharmacokinetics and transformation of this drug in this bird species and possible antitrichomonal activity of the metabolites.

Disclosure statement

The authors declare that they have no conflict of interest.

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