Pharmacokinetic profile of enrofloxacin and its metabolite ciprofloxacin in Asian house geckos (*Hemidactylus frenatus*) after single-dose oral administration of enrofloxacin

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ABSTRACT

The pharmacokinetics of enrofloxacin and its active metabolite ciprofloxacin were determined following oral administration in 21 Asian house geckos (*Hemidactylus frenatus*) at a dose of 10 mg/kg. Changes in enrofloxacin and ciprofloxacin plasma concentrations were quantified at regular intervals over 72 h (1, 2, 6, 12, 24, 48, and 72 h). Samples were analysed by high-pressure liquid chromatography (HPLC) and the enrofloxacin pharmacokinetic data underwent a two-compartment analysis. Due to the limited ciprofloxacin plasma concentrations above the lower limit of quantification (LLOQ), the ciprofloxacin data underwent non-compartment analysis and the half-life was determined by the Lineweaver-Burke plot and analysis. The enrofloxacin and ciprofloxacin mean half-lives ($t_{1/2}$) were 0.95 h (α) / 24.36 h (β), and 11.06 h respectively, area under the curve (AUC_{0-24h}) were 60.56 and 3.14 µg/mL*h, respectively, maximum concentrations (C_{max}) were 12.31 and 0.24 µg/mL, respectively, and time required to reach the C_{max} (T_{max}) were 1 and 2 h respectively. Enrofloxacin was minimally converted to the active metabolite ciprofloxacin, with ciprofloxacin concentrations contributing only 4.91% of the total fluoroquinolone concentrations (AUC_{0-24h}). Based on the pharmacokinetic indices when using susceptibility breakpoints when determined at mammalian body temperature it is predicted that single oral administration of enrofloxacin (10 mg/kg) would result in plasma concentrations effective against susceptible bacterial species inhibited by an enrofloxacin MIC $\leq$ 0.5 µg/mL in vitro, but additional studies will be required to determine its efficacy in vivo.

1. Introduction

Reptile populations are threatened by invasive species, habitat destruction, climate change, pollution and infectious diseases (Gibbons et al., 2000; Böhm et al., 2013). It is estimated that 34% of reptile species are threatened (IUCN, 2019). Threatened species occur across all orders of reptilians, however, smaller species such as skinks and geckos within squamata are overrepresented (IUCN, 2019). To prevent extinction of threatened reptiles, conservation breeding programs have been implemented globally. Captive management of reptiles is not, however, without risk. Reptiles are often difficult to maintain in captivity requiring species-specific enclosures, diets, lighting, temperature gradients and humidity. Failure to maintain best practice captive husbandry can lead to maladaptation and immunosuppression,

and increase disease susceptibility, emergence and transmission (Jacobson, 1993).

Examples of conservation breeding programs threatened by disease are those for the Christmas Island endemic Lister's geckos (*Lepidodactylus listeri*) and blue-tailed skinks (*Cryptoblepharus egeriae*) (Rose et al., 2017). These species are extinct in the wild on Christmas Island, but are sustained in captive breeding programs. In 2014 an outbreak of a biofilm-forming *Enterococcus* sp. (proposed *Enterococcus lacertideformus*. nov.) caused disease in the captive blue-tailed skinks and Lister's geckos (Rose et al., 2017), and likely occurred as a direct spillover from invasive Asian house (*Hemidactylus frenatus*) and mute (*Gehyra mutilata*) geckos. Microscopically, bacteria formed colonies surrounded by a biofilm-like matrix replacing normal tissue. Infection appeared to start in the head, as the disease progressed, it became

Table 1
Summary of the pharmacokinetic indices of enrofloxacin studies in reptilians.

Species	Route	Method	Ambient temperature (°C)	Dose (mg/kg)	C_{max} (μg/mL)	T_{max} (h)	$t_{1/2}$ (h)	AUC_{0-t}	$AUC_{0-\infty}$	MRT_{0-t} (h)	$MRT_{0-\infty}$ (h)	Reference
Asian house gecko	**PO**	**HPLC**	**24-27**	**10**	**12.31**	**1**	**0.95 (α) 24.36 (β)**	**79.66**	**86.16**	**n/a**	**21.60**	**This study**
Central bearded dragon	IM (ear)	HPLC	24-26	10	11.92	1	21.43	n/a	163.67	27.65	n/a	(Salvadori et al., 2017)
	IM (del)				9.44	2	20.17	n/a	146.04	25.71	n/a	
Green iguana	IM	HPLC	n/a	5	2.03	1	26	n/a	n/a	n/a	n/a	(Maxwell and Jacobson, 1997)
Savannah monitor	IM	HPLC	27	10	12.47	6	56	n/a	n/a	n/a	n/a	(Hungerford et al., 1997)
Red-eared slider	PO[NF]	HPLC	23-29	10	3.44	5	32.84	53.22	59.91	29.48	n/a	(James et al., 2003)
	IM			5	6.28	2.08	17.64	65.05	75.07	23.25	n/a	
Yellow-bellied slider	IC	HPLC	24-26	10	10.36	2.61	47.6	n/a	308.77	57	n/a	(Giorgi et al., 2013)
Loggerhead sea turtle	PO[NF]	HPLC & MBA	25-29	10	4.1	20	37.8	77	261	68	n/a	(Jacobson et al., 2005)
				20	21.3	26	54.4	481	1799	89	n/a	
Gopher tortoise	IM	HPLC	29-31	5	2.4	1	23.1	56.7	n/a	27.6	n/a	(Prezant et al., 1994)
Indian star tortoise	IM	HPLC	26-30	5	3.59	0.5	5.1	n/a	19.9	n/a	7	(Raphael et al., 1994)
Hermann's tortoise	IC	HPLC	30-33	10	8.61	2.19	37	66.39	102.12	n/a	33.13	(Salvadori and Vito, 2015)
Burmese python	IM	HPLC	30	5	1.66	5.75	6.37	22.17	n/a	n/a	n/a	(Young et al., 1997)
Pit viper	IM	HPLC	27-29	10	4.81	4.5	27.91	118	120	30.4	33.2	(Waxman et al., 2014)
South American rattlesnake	IM	HPLC	27-29	10	5.49	7.61	20.20	161.64	163.14	28.04	29.16	(Waxman et al., 2015)
Saltwater crocodile	IM	HPLC	24-32	5	8.9	0.65	19.02	74.38	133.23	10.2	28.2	(Martelli et al., 2009)
American alligator	PO	HPLC	27	5	0.5	55	77.73	37.31	201.02	139.58	n/a	(Helmick et al., 2004)
	IV			5	1.41	n/a	21.05	119.10	n/a	77.04	n/a	

PO, per os; IM, intramuscular injection; IC, intracardiac injection; IV, intravenous injection; α, alpha; β, beta; C_{max}, maximum plasma concentration; T_{max}, time of maximum plasma concentration; $t_{1/2}$, terminal half-life; AUC_{0-t}, area under the concentration-time curve from time 0 to last quantifiable time; $AUC_{0-\infty}$, area under the concentration-time curve from time 0 to infinity; MRT_{0-t}, mean residence time from time 0 to last quantifiable time; $MRT_{0-\infty}$, mean residence time from time 0 to infinity; n/a, not available; ear, early blood collection; del, delayed blood collection; MBA, microbiological assay.
[NF], not fasted.

systemic and the animal died. An unusual feature of this disease was the minimal or complete absence of a host immune response. Although the outbreak was effectively managed through quarantine, a significant threat to the breeding stock remained (Rose et al., 2017). Treatment was not considered because the organism had not been morphologically or genetically characterised, and there was no data on the pharmacokinetic profile of any antimicrobials in reptiles (less than 10 g in bodyweight).

Enrofloxacin, a fluoroquinolone, is a bactericidal, broad-spectrum antimicrobial that has therapeutic activity against both aerobic gram-negative and gram-positive bacteria (Schroder, 1989). This antimicrobial also inhibits biofilm formation in some bacteria (Yang et al., 2017). Enrofloxacin is commonly used in reptile medicine because of its favourable pharmacokinetic profile and therapeutic index (Salvadori and Vito, 2015), and formation of its active metabolite ciprofloxacin, contributes to the antimicrobial efficacy (Walker et al., 2000).

A small number of pharmacokinetic studies have been undertaken in reptiles (Table 1) and predominantly in crocodilians, chelonians, and snakes. Only three studies have been published in lizards (Hungerford et al., 1997; Maxwell and Jacobson, 2008; Salvadori et al., 2017) and none in smaller species of skinks and geckos. Based on these studies, significant variations in rates of enrofloxacin metabolism, conversion of enrofloxacin to ciprofloxacin, and elimination of enrofloxacin have been reported. As a result, it is difficult to accurately predict the pharmacokinetic profiles of enrofloxacin in unstudied reptiles. These limitations highlight the importance of conducting species-specific pharmacokinetic and pharmacodynamic (PK/PD) studies to identify effective dosing regimens.

The aims of this study were to determine the pharmacokinetic profile of enrofloxacin and ciprofloxacin following oral administration of enrofloxacin in Asian house geckos used as a surrogate for the critically endangered Lister's gecko and blue-tailed skink which are of similar mass. These findings will inform evidenced-based therapeutic protocols that will have important implications for health and welfare of other species of small lizards.

2. Materials and methods

2.1. Animal ethics

The use of wild reptiles was approved by The University of Sydney Animal Ethics Committee, protocol 2018/1380 on 16 July 2018.

2.2. Animals and housing

Twenty-four outwardly healthy adult geckos (10 females, 14 males), ranging in weight from 3.0 to 4.5 g (mean 3.4 g) were used in this study. Geckos were classified as healthy if they had a body condition score of 3 or greater (range 1 to 5), as determined by the amount of muscling on the tail and the dorsal pelvis, were active, and had no physical deformities or lesions. The geckos were captured within 200 m of the Christmas Island Immigration Detention Centre (-10.472023, 105.578303), where *E. lactertideformus*infected geckos have not been observed. The geckos were individually housed in PenPal terrariums (30 cm x 18 cm x 18 cm) (Living World® PenPals) in a biosecure facility and acclimatised for a minimum period of one week prior to study commencement. Animals were kept at ambient temperatures (24 to 26˚C), with relative humidity ranging between 80% and 85%, and were exposed to a 12 h photoperiod. Animals were fed three days per week a mixture of termites and crickets and fasted for three days prior to drug administration and throughout the study period. Animals were monitored daily during the acclimatisation and treatment period.

2.3. Experimental design

The geckos were randomly allocated into eight treatment groups consisting of three animals. Each gecko (excluding baseline) was weighed and then received a single-dose 10 mg/kg oral administration of enrofloxacin (Enrotril® 25 mg/mL, Troy Laboratories Australia Pty Ltd). A dose of 10 mg/kg was selected as it is the highest limit of the suggested dose range of enrofloxacin for lizards (Allen et al., 1993; Hungerford et al., 1997). Oral administration of enrofloxacin was performed using a 29G insulin syringe with a blunt needle tip. The needle was used to open the mouth and create a space between the lower and upper rows of teeth to allow direct administration of enrofloxacin into the back of the oral cavity. Any enrofloxacin not swallowed was redrawn and administered as above to minimise any loss of volume. A treatment group was euthanised at time zero (baseline analysis), and at 1, 2, 6, 12, 24, 48 and 72 h after dosing. Prior to euthanasia, geckos were given a subcutaneous injection of the sedative alfaxalone (Alfaxan, Jurox Animal Health) (5 mg/kg). Once unresponsive, lizards were decapitated and whole blood (40 to 80 μL) from the carotid artery was collected into capillary tubes containing lithium heparin (Fisher Scientific, Pennsylvania, USA). No coagulation of whole blood was observed. Tubes were placed on ice. Within 10 min of collection plasma was separated by centrifugation (2,000 x *g*) and frozen and protected from light. Samples were stored at -20˚C until transported to the Australian mainland then stored at -80˚C until analysis. Enrofloxacin plasma concentration quantification occurred within four weeks of blood collection. Plasma samples were heat sterilised at 60˚C to satisfy quarantine regulations (WHO, 2014). Known concentrations of enrofloxacin and its active metabolite ciprofloxacin were added to unmedicated plasma from the common brushtail possum(Trichosurus vulpecula) (n = 1) and analysed before and after heat inactivation to verify that enrofloxacin and ciprofloxacin concentrations were heat stable.

2.4. Instrumentation and chromatographic conditions

Enrofloxacin and ciprofloxacin concentrations in plasma were quantified by high-pressure liquid chromatography (HPLC) by the Veterinary Pharmacology Unit, Sydney School of Veterinary Science, The University of Sydney. The HPLC system consisted of Shimadzu LC-20AT delivery unit, DGU-20A3 HT degassing solvent delivery unit, SIL-20A auto injector unit, SIL-20A auto injector, RF-20A fluorescence detector and CTO-20A column oven. Shimadzu LC solution software (Kyoto, Japan) was used for chromatographic control, data collection and processing.

Quantification of enrofloxacin and ciprofloxacin was modified from previous validated protocols (Griffith et al., 2010; Black et al., 2014). Optimal chromatographic separation was performed with a Synergy, 4 μm, Max-RP80A (150 × 4.6 mm) (Phenomenex, Lane Cove, NSW, Australia) with a 1 mm optic-guard C-18 pre-column (Choice Analytical, Thornleigh, NSW, Australia) at ambient temperature (26˚C). The isocratic mobile phase used consisted of a mixture of buffer containing 1% of tetrabutylammonium hydrogen sulfate, 5 mM sodium dodecyl sulfate, and 0.2% triethylamine (Sigma-Aldrich, Castle Hill, NSW, Australia), and HPLC grade acetonitrile (60:30, v/v) (Thermo Fisher Scientific, Scoresby, VIC, Australia). The flow rate was 1 mL/min and fluorescence detection occurred at an excitation wavelength of 280 nm and an emission wavelength of 450 nm. Retention times of enrofloxacin, ciprofloxacin and marbofloxacin as the internal standard (IS) were 5.85, 5.44 and 3.85 min, respectively.

2.5. Sample processing

Stock solutions of the analytical standards enrofloxacin (purity ≥ 98%), ciprofloxacin (purity ≥ 98%), and marbofloxacin (purity ≥ 97%) (Sigma-Aldrich, Castle Hill, NSW, Australia) were prepared in

acetonitrile at the concentration of 500 μg/mL. Nine standard concentrations of both enrofloxacin and ciprofloxacin, ranging from 0.039 to 10 μg/mL were prepared in water (purified deionised water [MilliQ] in place of plasma as additional plasma for construction of the standard curves was not available). Ten microlitres of tetrabutylammonium hydrogen sulfate (10%), and 20 μL of acetonitrile (containing 0.0625 μg/mL IS) were added to 10 μL of the plasma samples (or 10 μL of water for the standards), which were then vortexed and centrifuged at 14,000 x *g* for 10 min. Twenty microlitres of the supernatant were transferred to microcentrifuge tubes and 180 μL of the mobile phase was added. A total volume of 5 μL per sample was injected into the chromatographic system.

2.6. Standard curve validation

Due to the very low volume of gecko plasma available, in order to confirm standard concentrations prepared in water were accurate, 1 μg/mL of enrofloxacin and ciprofloxacin were added into the gecko's pooled plasma and were compared with the detected drugs' concentration when 1 μg/mL of each drug were also added to water.

2.7. Quantification

Plasma concentrations of enrofloxacin and its active metabolite ciprofloxacin, were simultaneously quantified. Both enrofloxacin and ciprofloxacin were measured via standard curves (0.039 to 10 μg/mL) where a weighting factor $(1/ \times^2)$ was used to establish regression of the curve. The theoretical limit of the lower limit of detection (LLOD) and lower limit of quantification (LLOQ) were estimated by 3.3 × y-intercepts from the regression lines (σ) / mean slope of calibration curves (S) and 3 × LLOD, respectively (ICH, 2015). Intra-assay precision and accuracy for high (10 μg/mL), middle (0.625 μg/mL), and low (0.039 μg/mL) quality controls were calculated. Precision was calculated using the coefficient of variation [CV = (standard deviation / mean value) × 100], and accuracy, expressed as bias was determined by a percentage difference between estimated value and the nominal value [Bias = (estimated value – nominal value) / nominal value × 100].

2.8. Pharmacokinetic analysis

The mean and standard deviation drug concentration at each timepoint consisted of the observed concentrations of three animals. The enrofloxacin data were fitted to a non-compartment analysis, a one-compartment model and a two-compartment model, the best fit was the two-compartment model as the semi-log curve demonstrated two distinct slope gradients (Fig. 1 c) and had the lowest Akaike Information Criterion (AIC) (Olofsen and Dahan, 2013). Calculations for the two-compartment model can be found in (Riviere, 2011a) and were calculated by the PKSolver program (Zhang et al., 2010). The maximum concentration (C_{max}) of enrofloxacin in plasma, and the time required to reach the C_{max} (T_{max}) were obtained directly from the measured concentrations. The area under the enrofloxacin concentration-time curve (AUC_{0-t}) was also calculated manually by the log-linear trapezoidal method (Chiou, 1978). The ciprofloxacin semi-log curve appeared linear over 24 hours (Fig. 1 d), however, the half-life was calculated from the Lineweaver-Burke equation $t_{1/2} = (0.693 \times (km + C))/Vmax$ (Riviere, 2011b). The AUC, area under the moment curve (AUMC) and mean residence time (MRT) were also calculated for ciprofloxacin over 24 hours.

The PK/PD index AUC/minimum inhibitory concentration (MIC) was calculated by dividing the combined enrofloxacin and ciprofloxacin AUC_{0-24h} by the MIC of enrofloxacin to inhibit susceptible streptococci isolates when grown at mammalian temperatures as documented as susceptible breakpoints in CLSI document VET08 Table 2D (CLSI, 2018). The data for *Streptococci* spp. were used as the MIC values

for enrofloxacin were not available for *Enterococci* spp. and the genus *Streptococcus* is most closely related to the *Enterococcus* genus of bacteria. The ratio of enrofloxacin to ciprofloxacin for each timepoint up to and including t = 24 h were calculated by dividing the average concentration of enrofloxacin by that of ciprofloxacin.

2.9. Plasma protein binding

A preliminary estimation of the plasma protein binding (PPB) of enrofloxacin and ciprofloxacin in gecko plasma at concentrations of 0.72 and 0.35 μg/mL, respectively was determined in duplicate by the modified ultrafiltration method (Dow, 2006). This involved 150 μL of pooled gecko plasma treated with enrofloxacin and ciprofloxacin and then incubated in a water bath at 26°C for 30 min. Ten μL of plasma was removed for determination of the total enrofloxacin and ciprofloxacin concentrations (Drug$_{total}$), and the remaining plasma was transferred to the reservoir of the Centrifree (Merk Millipore, Macquarie Park, Australia) with a membrane molecular weight cut-off of 30 kDa. The ultrafiltrate device was centrifuged at 1,500 x *g* for five mi at 26°C. After centrifugation, the filtrate was used to determine the free enrofloxacin and ciprofloxacin concentrations (Drug$_{free}$). Both the (Drug$_{total}$) and (Drug$_{free}$) fractions were analysed by HPLC as described previously. The PPB of enrofloxacin and ciprofloxacin in gecko plasma was determined by the following formula: PPB % = 100 − [(Drug$_{free}$/Drug$_{total}$) × 100]. *In vitro* testing was used to determine the degree of non-specific binding to the ultrafiltrate device membrane, using phosphate buffer (pH 7.4) as a control.

3. Results

3.1. Experimental animals

All geckos remained healthy during the acclimatisation and study periods.

3.2. Preliminary heat stability study

A negligible difference in the concentrations of enrofloxacin and ciprofloxacin were detected between the heat-treated and non-heat-treated plasma samples (± 0.1%) (Data not shown).

3.3. Accuracy and precision

For the standard curve validation in plasma, the accuracy and precision of enrofloxacin and ciprofloxacin were < 15% and < 5%, respectively. Recovery of enrofloxacin and ciprofloxacin from pooled plasma samples were 94% and 102%, respectively, when compared to the control prepared in water. There was a negligible difference of the recovered drug and metabolite concentrations between plasma samples and the control.

For the three standard concentrations of 0.039, 0.625 and 10 μg/mL prepared in water, the average intra-assay accuracy for enrofloxacin and ciprofloxacin varied between 85.03% and 110.89%, and 97.80% and 110.09% respectively. The average intra-assay precision for enrofloxacin and ciprofloxacin were 4.56% ± 0.00, 5.29% ± 0.03, 2.44% ± 0.26 and 4.58% ± 0.00, 3.95% ± 0.02, 1.70% ± 0.17 at 0.039, 0.625 and 10 μg/mL respectively. The LLOD and LLOQ were 0.84 ng/mL and 2.53 ng/mL, and 2.30 ng/mL and 6.91 ng/mL, for enrofloxacin and ciprofloxacin, respectively.

3.4. Pharmacokinetic profile

The plasma concentrations of enrofloxacin and its active metabolite ciprofloxacin are shown (Table 2), results presented as mean and standard error (SE). The changes in enrofloxacin and ciprofloxacin plasma concentrations vs time curves following oral administration are
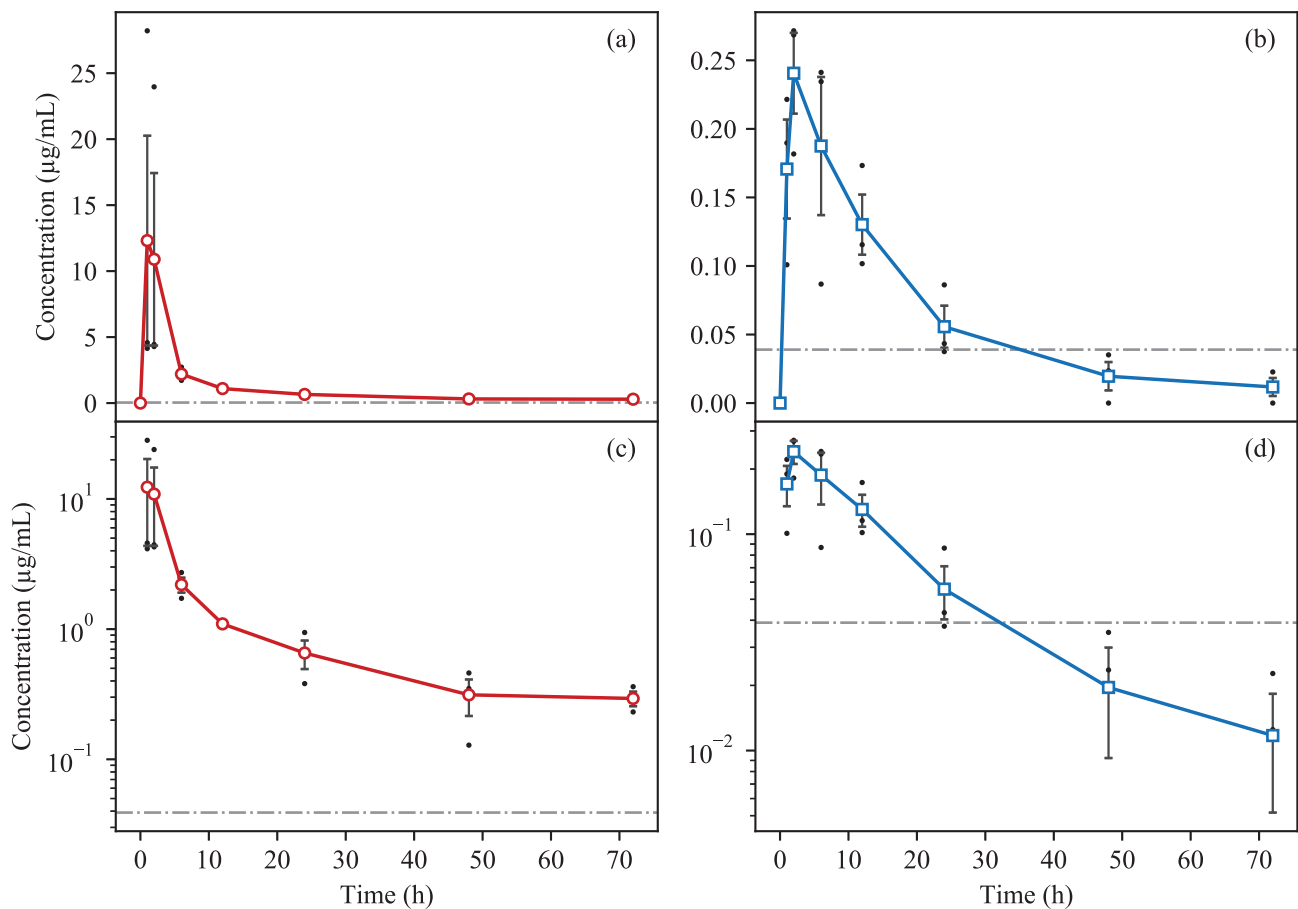
Fig. 1. Plasma concentrations of enrofloxacin and metabolite ciprofloxacin in Asian house geckos following oral administration of enrofloxacin at 10 mg/kg. (a) Linear plot of mean enrofloxacin concentrations (± SE). (B) Linear plot of mean ciprofloxacin concentrations (± SE). (c) Semi-logarithmic plot of mean enrofloxacin concentrations (± SE). (d) Semi-logarithmic plot of mean ciprofloxacin concentrations (± SE). Red data points, enrofloxacin; blue data points, ciprofloxacin; black data points, observed concentrations for each gecko; broken line, lower limit of quantification (LLOQ).

Table 2
Plasma concentrations after oral administration of enrofloxacin (10 mg/kg) in Asian house geckos, observations of three animals at each timepoint.

Time (h)	Enrofloxacin (µg/mL) Mean	SE	Ciprofloxacin (µg/mL) Mean	SE	E:C ratio Mean
1	12.31	7.95	0.17	0.04	72.13
2	10.90	6.53	0.24	0.03	45.29
6	2.20	0.29	0.19	0.05	11.72
12	1.10	0.03	0.13	0.02	8.43
24	0.66	0.16	0.06	0.02	11.77
48	0.31	0.10	0.02*	0.01	15.99
72	0.29	0.04	0.01*	0.01	25.01

SE, standard error of the mean.
* plasma concentrations below the limit of quantification

shown (Fig. 1). The pharmacokinetic indices for enrofloxacin and ci-profloxacin are also shown (Table 3). Plasma concentrations of enro-floxacin were above the LLOQ until 72 h. Plasma concentrations for ciprofloxacin were detected at concentrations higher than the LLOQ for all timepoints up to and including 24 h (Table 2). The C_{max} of cipro-floxacin (0.24 µg/mL) was 1.95% that of enrofloxacin (12.31 µg/mL). Ciprofloxacin comprised 4.91% of the total fluoroquinolone AUC_{0-24h}.

3.5. Plasma protein binding

The average PPB for enrofloxacin and ciprofloxacin in the geckos' plasma were 51.33% and 27.92%, respectively. Non-specific binding of enrofloxacin and ciprofloxacin to the microcentrifugation tube or filter

were 6.16% and 6.26%, respectively.

4. Discussion

The PK/PD indice such as AUC/MIC has been used to guide some antimicrobial dosing regimens. Fluoroquinolones are considered con-centration-dependant antibiotics (Turnidge, 1999), whereby such an-tibiotics are considered efficacious based on the AUC to MIC ratio (Toutain et al., 2017). Therefore, ideal dosing regimens maximise drug concentrations to achieve effective antimicrobial therapy (Wright et al., 2000). There are challenges associated with determining antibiotic dosage rates in reptiles because published MIC susceptibility and re-sistant breakpoints, as documented in CLSI VET08, are usually de-termined for bacteria incubated at 35 to 37°C, the body temperature of most mammals. No MIC studies have been published that inhibit *En-terococci* spp. or well-studied and closely related *Streptococci* spp. grown at the preferred optimum temperature zone of reptiles, however, MIC antimicrobial susceptibility tests have been performed at 28, 22 and 18-19°C in fish for other bacterial species (as reviewed in Smith et al., 2002). Based on these studies, the MIC calculated at temperatures less than mammalian body temperature can be lower, higher, or identical to those calculated at mammalian body temperatures depending on the antibiotic, but no studies on enrofloxacin's MIC to inhibit bacterial species at less than mammalian body temperature have been reported. Therefore, in this study, interpretations were limited to mammalian susceptibility breakpoints for *Streptococci* spp. taken from CLSI docu-ment VET08 Table 2D (CLSI, 2018).

The AUC/MIC ratio is the best PK/PD index of clinical outcome for

Table 3

Mean pharmacokinetic indices for enrofloxacin and the metabolite ciprofloxacin in Asian house geckos after single-dose oral administration of 10 mg/kg enrofloxacin. PK indices for enrofloxacin determined using a two-compartment model; $t_{1/2}$ of ciprofloxacin determined by the Lineweaver-Burke equation/plot (Riviere, 2011b). Blank cells denote not applicable.

Indices	Units	Enrofloxacin	Ciprofloxacin
C_{max}	μg/mL	12.31	0.24
T_{max} at the highest Cmax timepoint	h	1	2
$t_{1/2}$	h		11.06
k_{10}	1/h	0.33	
k_{12}	1/h	0.37	
k_{21}	1/h	0.063	
$t_{1/2}$ α	1/h	0.95	
$t_{1/2}$ β	1/h	24.36	
AUC_{0-24h}	μg/mL*h	60.56 (59.28)	3.14
$AUMC_{0-24h}$	μg/mL*h²	1303.87	30.13
MRT_{0-24h}	h	16.48	9.63
AUC_{0-72h}	μg/mL*h	79.66 (77.67)	
$AUC_{0-\infty}$	μg/mL*h	86.16	
$AUMC_{0-\infty}$	μg/mL*h²	1861.17	
$MRT_{0-\infty}$	h	21.60	
V/F	L/kg	0.35	
CL/F	L/kg/h	0.12	
V2/F	L/kg	2.04	
CL2/F	L/kg/h	0.13	

C_{max}, maximum drug concentration; T_{max}, time of maximum plasma concentration; $t_{1/2}$, terminal half-life; k_{10}, elimination rate constant from central component; k_{12}, inter-compartment rate constant from central to peripheral compartment; k_{21}, inter-compartment rate constant from peripheral to central compartment; $t_{1/2}$ α, distributive half-life; $t_{1/2}$ β, elimination half-life; AUC_{0-24h}, area under the concentration-time curve from time 0 to 24 h; $AUMC_{0-24h}$, area under the first moment curve from time 0 to 24 h; MRT_{0-24h}, mean residence time from 0 to 24 h; AUC_{0-72h}, area under the concentration-time curve from time 0 to 72 h; $AUC_{0-\infty}$, area under the concentration-time curve from time 0 to infinity; $AUMC_{0-\infty}$, area under the first moment curve from time 0 to infinity; $MRT_{0-\infty}$, mean residence time from 0 to infinity; V/F, apparent volume of distribution during terminal phase; CL/F, apparent total clearance of the drug from plasma; V2/F, volume of distribution for central compartment; CL2/F, clearance between compartments.
The bracketed values were calculated manually by the logarithmic trapezoidal method as this method is recommended for post-peak plasma data (Chiou, 1978). SE not provided as observations from a subset of animals (n = 1 – 3) at each timepoint (timepoint variability provided in Fig. 1 and Table 2) were used to construct the PK curve from which these indices were calculated.

enrofloxacin to inhibit a bacterial pathogen in patients (Toutain et al., 2017). Therefore, using a MIC of 0.5 μg/mL, the combined enrofloxacin and ciprofloxacin AUC_{0-24h} [63.70] / MIC [0.5] ratio is 127.40. It is recommended that for the fluoroquinolones a minimum AUC/MIC ratio of 120 to 125 are required for fluoroquinolones to achieve clinical success in critically ill people (Levison and Levison, 2009). Consequently, susceptible *Streptococci* spp. isolates would be considered susceptible to enrofloxacin (susceptible < 0.5 μg/mL, intermediate 1-2 μg/mL, resistant > 4 μg/mL) (CLSI, 2018).

In addition to the AUC/MIC ratio, multiple other host and pathogen factors can impact the efficacy of enrofloxacin. For drugs that have a high percentage of PPB, the AUC/MIC may be overestimated (Levison and Levison, 2009). In reptiles, this is the first time enrofloxacin and ciprofloxacin PPB has been estimated. Based on these findings, enrofloxacin PPB estimate is similar to that in calves (46%) (Davis et al., 2007) and therefore, PPB may have some impact on the AUC/MIC ratio in this species. These findings must be considered preliminary as due to the limited plasma, only one concentration of enrofloxacin (0.72 μg/mL) was undertaken when it is recommended that drug PPB should be performed at low, medium and high drug concentrations (Dow, 2006). Additionally, for most accurate results it is recommended that determination of PPB should be done with fresh plasma (Riviere and Buur, 2011), however, only frozen plasma was

used here. Once the enrofloxacin and ciprofloxacin unbound fraction have been confirmed in future studies, the fAUC/MIC would be a more accurate indice than AUC/MIC (Toutain et al., 2017).

As this pathogen produces a thick biofilm (Rose et al., 2017) this may also impact the efficacy of enrofloxacin to inhibit *E. lacertideformis*. As an example, biofilm producing *Staphylococcus aureus* require an antimicrobial MIC up to 100 times higher than those that do not produce biofilms (Girard et al., 2010; Di Domenico et al., 2018). Additionally, factors associated with the infection site and host immune status all affect the antibiotic's MIC to inhibit the pathogen (Giguere and Dowling, 2013).

In many mammalian species enrofloxacin is bio-transformed to the active metabolite ciprofloxacin via hepatic de-ethylation (Küng et al., 1993). The rate of hepatic metabolism varies significantly in different species including reptiles, and in some species conversion of enrofloxacin to ciprofloxacin is limited (Fitzgerald and Vera, 2006; Dimitrova et al., 2007; Ogino and Arai, 2007). Limited conversion rates may be associated with poor expression of the enzyme responsible for biotransformation (enzyme CYP450 3A) in some reptiles (Ertl et al., 1998; Vaccaro et al., 2003), or their slow metabolism rates when kept at temperatures below 37°C. In this study the mean plasma concentrations of ciprofloxacin were minimally increased above the LLOQ, and fell below it by 48 h, only contributing 4.91% of the total AUC_{0-24h} for enrofloxacin and ciprofloxacin. The low concentrations of ciprofloxacin may be a function of a decreased metabolic rate associated with low body temperature or be the result of a poorly developed metabolic pathway for converting enrofloxacin to ciprofloxacin. Therefore, ciprofloxacin may not contribute to the *in-vivo* efficacy of enrofloxacin at 25°C in geckos or does so minimally.

The validation of enrofloxacin and ciprofloxacin in water in place of plasma was necessary due to the inability to obtain adequate volumes of gecko plasma. The re-analysis of enrofloxacin using a single sample of gecko plasma was undertaken and shown to be very similar in plasma and water, however, future studies should use the target species' plasma for assay validation.

The time of maximum plasma concentration of enrofloxacin was at the 1 h timepoint, however, the true Tmax may occur prior to, or after this timepoint. Therefore, additional sampling timepoints between 0 and 2 hours would be necessary to adequately assess the true Cmax. Though, the observed Tmax does indicate rapid oral absorption. In contrast, delayed absorption was observed in red-eared sliders (5 h) (James et al., 2003) and loggerhead sea turtles (20 h) (Jacobson et al., 2005). Reasons for this marked variation are not known but may have to do with the fact that the turtles were not fasted and the presence of food in the stomach may have delayed absorption, or due to differences in species' metabolic rates.

Fig. 1 c demonstrates two distinct curve gradients for enrofloxacin therefore, a two-compartment model was used to analyse this data. The enrofloxacin half-life α is approximately 1 h, however the half-life β is approximately 24 h suggesting that the drug has a longer duration in peripheral compartments. Enrofloxacin half-life at the same administration route and dosage used in this study was 32.84 hours in red-eared sliders (James et al., 2003) and 37.8 hours in loggerhead sea turtles (Jacobson et al., 2005).

When delivering a small volume of medication to a small animal orally, it is difficult to ensure all medication is consumed. This may explain the variability of plasma concentrations observed between replicates at some timepoints. It is likely, however, with repeated dosing, which would be required to achieve therapeutic drug concentrations, the impact of variation in dosage due to the challenges of oral administration would be minimised. An alternate approach to oral delivery would be administering the drug via a feeding tube into the oesophagus. This method, however, would be stressful, require adjustments to compensate for increased dead-space in the delivery system, and cause abrasions to the oral mucosa, and so was not used in this study. Parenteral injection of enrofloxacin would result in consistent drug

administration. Parenteral delivery, however, is not viable in small species as enrofloxacin causes pain, local tissue swelling and necrosis at the injection site (Prezant et al., 1994; Young et al., 1997; Perry and Mitchell, 2019). Variability may have also been introduced because it was necessary to use a sampling method where an individual animal represented each sampling timepoint. However, similar methods have been used successfully in other studies when the study animal was small or rare, there was a need to minimise stress and discomfort or blood volumes (Sanchez-Migallon Guzman et al., 2010; Innis et al., 2012; Musser et al., 2013; Kinney et al., 2014; Cerreta et al., 2019).

5. Conclusion

This study shows that single oral administration of enrofloxacin at 10 mg/kg in Asian house geckos achieves plasma concentrations that, assuming mammalian susceptibility breakpoints may be effective at treating *E. lacertideformus* and comparable bacterial pathogens with fluoroquinolone MICs $\leq$ 0.5 μg/mL. Given that similar pharmacokinetic parameters would be expected in other small reptiles, including the critically endangered reptiles threatened by *E. lacertideformus* on Christmas Island, these findings provide baseline data that can be used as a basis for the treatment of other small lizard species. However, the impact of PPB and other factors on reptile tissue concentrations of enrofloxacin are not known. Additionally, as *E. lacertideformus* forms a biofilm, and an immune response was absent from infected hosts, the only means to ultimately determine the true efficacy of enrofloxacin would be to conduct an experimental therapeutic trial in infected lizards.

Ethical statement

The use of wild reptiles was approved by The University of Sydney Animal Ethics Committee, protocol 2018/1380 on 16 July 2018.

This statement has been included in the methodology of this original research article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.vas.2020.100116.

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